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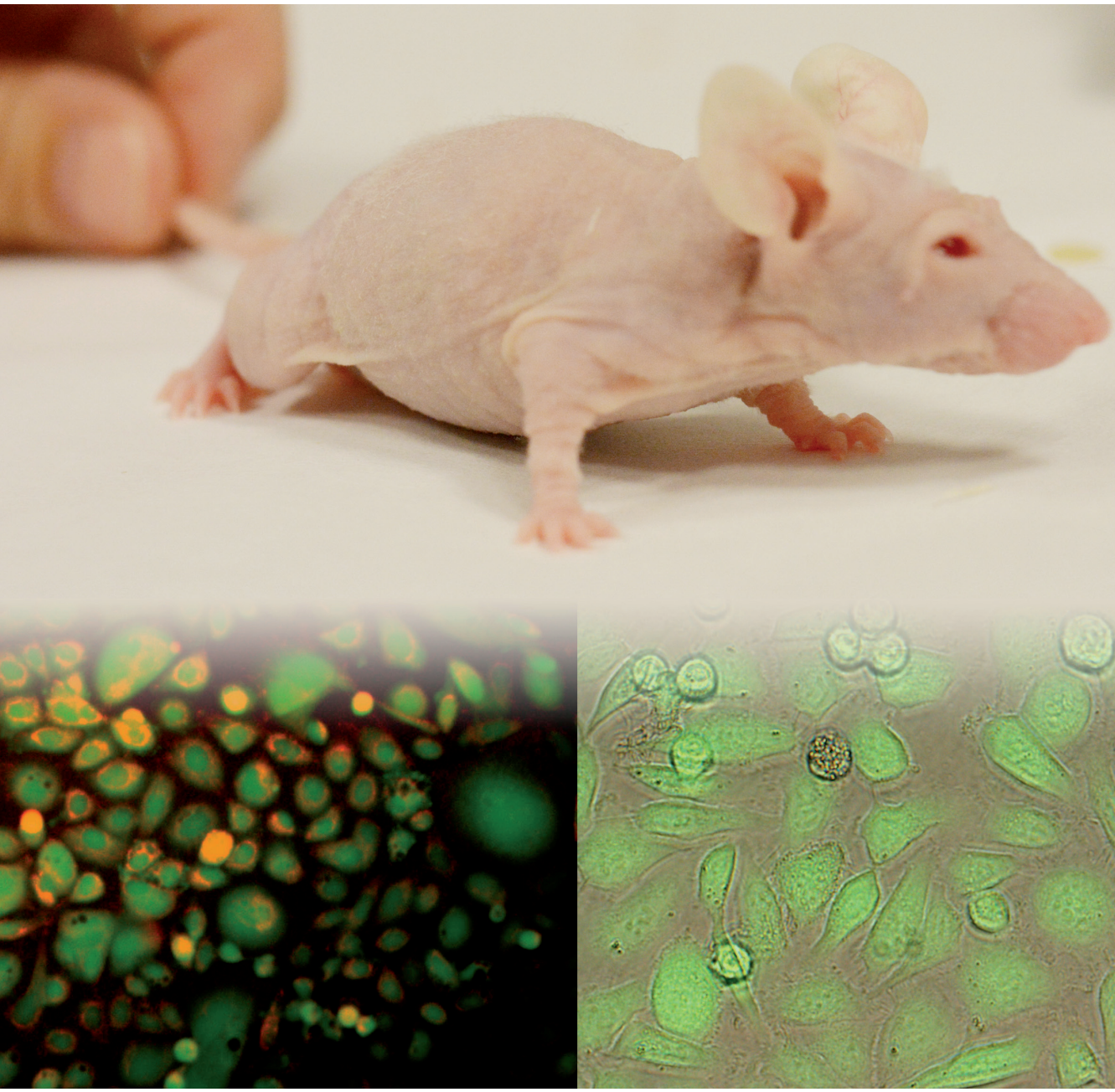

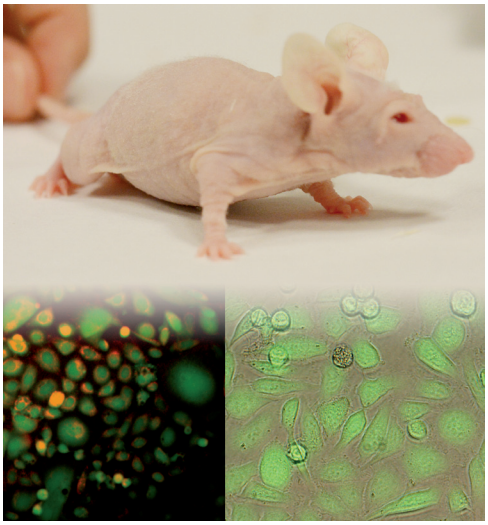

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The Journal of Metallomics and Nanotechnologies is a new educational and scientific journal which is associated with emerging metallomics and nanotechnologies as a new field of research. This journal is freely available and distributable via website with minimal advertising support. The objective of articles, which is comming quarterly, is application of nano-scale techniques for biologic, chemical and biotechnological research or specific nanobiotechnologic applications.

Cover:

Upper part - Photograph of nude *nu/nu* mouse. Nude mice possess mutation in *FOXN1* gene that causes a deteriorated or absent thymus, resulting in inhibited immune system. Hence, they are valuable model for xenogeneic transplantaion of tumor cells (Photo was captured in Laboratory of cell nanobiology and nanomedicine, author: Hana Polanska). Lower part - The PC-3 cells established from a grade 4 androgen independent and unresponsive prostate adenocarcinoma from 62-year old Caucasian male and derived from metastatic site in bone, stained using fluorescein diacetate, which is a cell-permeant esterase substrate showing viable cells. (Photo was captured in Laboratory of metallomics and nanotechnologies, author: Amitava Moulick).

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Dear readers,

the second issue of „Journal of Metallomics and Nanotechnologies“ in 2015 is generally dedicated to substantial metals-proteins relations in cancer progression and to the promising methods utilized in basic research in the cancer diagnostics and management. Current issue is also partially dedicated to the activity of Czech local organization named League against cancer Prague, which actively supports the cancer research and prevention programmes in the Czech Republic.

I am pleased to announce that our international editorial board has been enlarged by Prof. Martin Pumera from Nanyang Technological University, Singapore. Martin belongs to 0.08% of top Scientists in Chemistry having more than 370 publications and H-index up to 48 according to the Web of Science.

We are really glad that JMN slowly gains the worldwide interest, which is demonstrated by a number of internet connections from outside of the Czech Republic. We are also pleased that authors from abroad have started to publish in JMN. The third coming number of the volume in 2015 will be dedicated to a biophysical characterization of nanomaterials or bio-nanomaterials, especially by optical methods.

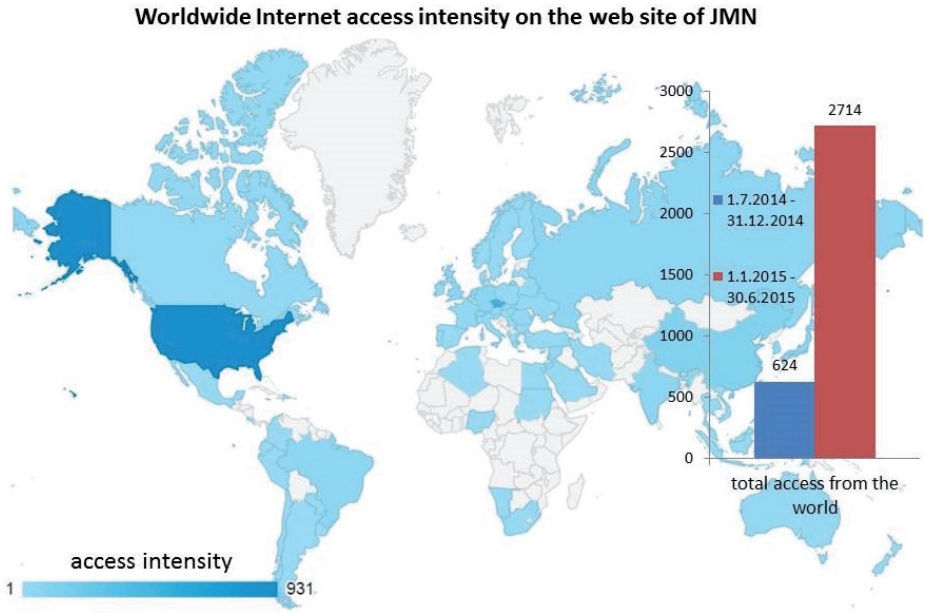

Figure 1: Worldwide internet access intensity on the web site of JMN

Ondrej Zitka
Chief Editor

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The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

Review

Laboratory Report

REVIEW

Molecular Targeting of Human Papillomavirus oncogene expression using RNA interference Technology

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Cervical cancer is a killer disease that claims innumerable lives of women all over the world. Human Papilloma Virus (HPV) serotypes 16 and 18 have been implicated as the causal organism of the disease. Initiation of sexual intercourse at an early age, multiple sex partners, poor hygiene etc are regarded as risk factors that are also associated with progression of HPV infection. Two genes in HPV encode the oncoproteins E6 and E7. These proteins have been found to be in high content in cervical cancer positive cell lines. Attempts have been made to selectively inhibit the expression of these two oncogenes by molecular biological techniques. Of these RNA interference (RNAi) deserves special mention. RNAi occurs naturally in mammalian cells through which expression of a particular gene can be knocked down with high precision. Different authors from time to time have targeted to knock out these E6 and E7 proteins as a preventive measure of cervical cancer. The present review portrays the state of the art evaluation of retrospective as well as recent literature on these challenging techniques.

Keywords: cervical cancer; RNAi; antisense RNA; ribozyme; siRNA; shRNA

1. Introduction

Cervical cancer is the second most common cancer in women throughout the world. Human Papilloma Virus (HPV) infection is the most important risk factor for cervical intraepithelial neoplasia and invasive cervical cancer [1,2, 3]. Cervical cancer occurs early during the productive period of a woman's life. HPV serotypes 16 and 18 account for nearly 76.7% cervical cancer. Other factors like high parity, early initiation of sexual intercourse, multiple sex partners, low socioeconomic status, poor hygiene etc. have been found to be associated with progression from HPV infection to pathological consequences of cancer. [4].

Physical ablation of HPV induced lesions like cryo therapy, photo therapy, laser cone biopsy and localized radiotherapy are effective to some extent in treating lesions. Removal of afflicted tissue is accomplished by removal of keratinocytes harboring HPV. These are the conventional approaches for treatment of cervical cancer. Prophylactic vaccines are available and should be administered prior to viral exposure. Initiatives for vaccination have been undertaken by the governments and also non-governmental agencies. Attempts are being made to develop therapeutic vaccines that would treat prevailing HPV infection. Use of immunomodulators like interferon and imiquimod induce cytokine production to increase host immune response against HPV infection [5]. Many antioxidants and poly herbal formulations are also in use. Molecular targeting of HPV gene expression using RNA interference technology includes use of antisense oligonucleotides, ribozymes, short interfering RNAs (siRNA), short hairpin RNA (shRNA) etc. The present review is a cri-

tique of RNAi approaches used for prevention of cervical cancer.

RNA interference (RNAi) occurs naturally in mammalian cells, through which expression of a particular gene can be knocked down with high specificity and selectivity [6]. Pre-clinical studies confirm that RNAi techniques can be used to silence cancer related targets [7]. *In vivo* studies have also shown favorable outcomes by RNAi targeting of components critical for tumor cell growth, metastasis, angiogenesis and chemo resistance.

2. Anti sense RNA therapy for cervical cancer

HPV has been implicated as the causal agent in cervical cancer. Two genes in HPV encode the oncoproteins E6 and E7. E6 can form complexes and also can degrade p53. E7 protein binds to the Rb protein preventing it from binding to its normal substrate E2F-1 protein. Attempts have been made to selectively inhibit the expression of these two oncogenes by antisense RNA. Steele observed[8] that a plasmid expressing antisense RNA of HPV18 decreased the growth rate of human cervical cancer cell line HeLa. Antisense E6 and E7 oligonucleotides have been shown to inhibit the growth of HPV positive cancer cells [9]. However when the oligonucleotides were withdrawn after 3 days the remaining cells in the culture recovered and grew as before. To explore the potential of an adenoviral antisense RNA transcript for gene therapy of cervical cancer, Hamada et al. [10] introduced the antisense RNA transcript of E6 and E7 genes of HPV 16 into cervical cancer cells via a recombinant adenoviral vector. They observed that the growth of infected cells was greatly suppressed as evidenced by decrease in cell counts. In an ex vivo study in nude mice tumorigenicity was completely inhibited. It was suggested by the authors that transfection of cervical cancer cells with HPV16 E6/E7 antisense RNA is a potential novel approach for treatment of cervical cancer. Inhibition of E6 and E7 by complementary antisense transcripts led to reduced growth rates, loss of transformed phenotype of cervical and oral carcinoma cell lines and inhibition of tumor formation exhibited in an animal model.

3. Ribozyme mediated therapy in cervical cancer

Ribozymes have several advantages over antisense RNA. They are capable of catalytic activity and do not require the presence of an auxiliary enzyme like RNase H. More over one molecule of ribozyme can bind and cleave many molecules of mRNA [5]. Chen et al. [11] showed that ribozymes can be used for effective cleavage of HPV transcripts and can also inhibit E6/E7 mediated immortalization [12]. Zheng et al [13] demonstrated that a hammerhead ribozyme Rz 170 can specifically target HPV 16 E6/E7 transcripts and was effective in inhibiting cell growth and promoting apoptosis. Rao et al [14] demonstrated that anti HPV 16 E6 ribozyme when transfected into CaSki cell increased the sensitivity to cisplatin, the chemotherapeutic agent used to treat cervical cancer. The increase of sensitivity to cisplatin in these cells may be associated with increased expression of p53, Bax protein and decreasing expression of Cmyc, Bcl-2 proteins.

4. shRNA mediated treatment for cervical cancer therapy:

HPV is known to play an important role in the pathological manifestation of the disease. Its early genes E6 and E7 can activate several intracellular signal pathways for carcinogenesis. *In vitro* knock down of E6 and E7 can reduce the survival ability of cervical cancer cell lines. Chen et al [11] studied the effect of lentiviral shRNA against E6/E7 on the tumor growth of xenografted cervical cancer model in mice. The growth of HeLa cells transduced with lentiviral shRNA against E6/E7 was compared to controls, *in vitro. In vivo* the RAG-/- mice, which were deficient in T and B lymphocytes were used to establish the HeLa xenografted model. HeLa cells transduced with lentiviral shRNA against E6/E7 grew slower than those with control vector. Lentiviral shRNA significantly reduced the size of xenografted tumors compared with controls. According to these authors RNAi could be used for the treatment of cervical cancer by intra tumor injection.

Gu et al. [15] reported inhibition of cervical cancer cell growth *in vitro* and *in vivo* with

lentiviral vector delivered short hairpin RNA targeting HPV E6 and E7 oncogenes.

RNAi holds great promise for the treatment of cervical cancer but delivery remains a key issue. Lentiviral vectors are widely used for stable transfer of short hairpin RNA (shRNA) in to cells and are expected to deliver a stable and durable interference. Gu et al. have shown that lentiviral delivered shRNAs directed against HPV E6/E7 oncogenes are effective for less than three weeks. This short lived RNAi was not due to the loss of the vector in the host cells but was more likely to be related to shRNA expression or RNAi machinery itself. Using this vector to carry two copies of the same shRNA or two shRNAs targeting at different genes (HPVE6 and VEGF) was more effective at silencing the gene targets and inhibiting cell or tumor growth than their single shRNA counterparts. These results indicate that a multi shRNA strategy is a more attractive approach than single shRNA for developing RNAi treatment for cervical cancer.

5. siRNA mediated approaches for cervical cancer therapy

A major advancement in RNA interference methods in recent years have been specifically aimed at HPV oncogenes E6 and E7. It has been established that siRNA can induce selective silencing of exogenous viral genes in mammalian cells. Moreover, the silencing process does not interfere with the recovery of cellular regulatory systems previously inhibited by viral gene expression. It is conjectured that since E6 and E7 genes are absent in normal cells, RNAi based therapies would not affect them.

Yoshinouchi et al [16] targeted a gene specific therapy for HPV related cancer. They studied the effects of E6 siRNA on the expression of E6 and E7 oncogenes and on the cell growth of HPV16 related cervical cancer cells. Using SiHa cell line these authors showed that E6 siRNA decreased the levels of mRNA encoding E6 as well as E7 proteins and induced nuclear accumulation of p53 which is the most important target of E6. It was found that E6 siRNA suppressed monolayer and anchorage-independent growth of SiHa cells. This cervical cancer cell line treated with E6 siRNA formed tumors in NOD/SCID mice that were significantly smaller than in those treated with control siRNA. It was suggested that HPV E6 siRNA could be a candidate for gene specific therapy for HPV related cervical cancer.

Koivusalo et al [17] observed that HPV18 positive HeLa cervical cancer cells when transfected with short interfering RNA (siRNA) molecules targeting HPV18 E6 mRNA before treatment with chemotherapy compounds showed nuclear accumulation of p53. But the effect was transient despite continuously suppressed HPV mRNA levels. According to these authors activating p53 by degrading E6mRNA may either increase or decrease the chemosensitivity of cervical cancer cells depending upon the drug used for chemotherapy.

Chang et al. [18] developed nine siRNAs against either the E6 or E7 genes of HPV-16 or HPV-18 in several combinations, yielding siRNAs targeting 16E6, 16E7, 18E6 and 18E7. HPV siRNAs significantly reduced cell growth, colony formation and apoptosis in HPV positive CaSki (HPV-16) or HeLa(HPV-18) cell lines, and had no effect in HPV negative C33A cells. These authors also found that intratumoral injection of the siRNAs reduced tumor growth in BALB/c nude mice. According to these authors siRNA treatment has potential as adjuvant therapy for cervical cancer.

Palanichamy et al [19] observed silencing of integrated HPV-16 oncogenes by siRNA mediated heterochromatinization. These authors have screened multiple siRNAs homologous to one of the NF-1 binding sites in the HPV-16 enhancer and identified one siRNA which causes specific transcriptional gene silencing of the HPV-16 oncogenes E6 and E7 when transfected into HPV-16 positive cell lines SiHa and CaSki. This phenomenon was found to be specific to the HPV-16 enhancer and showed no effect on HPV-18 enhancer. Gene silencing was found to be associated with heretochromatinization of the target region of the enhancer. However no DNA methylation was reported by these authors during the time period studied. These authors proposed that this siRNA causes simultaneous silencing of E6 as well as E7 oncogenes by an epigenetic mechanism for HPV-16 positive cervical and other epithelial cancers.

Dutta et al. [20] proposed a dendrosome based delivery of siRNA against E6 and E7 oncogenes in vitro in cervical cancer. Since siRNAs are large molecules and polyanionic in nature, they do not freely cross the cell membrane. Different types of synthetic vectors have been investigated for gene silencing. Cationic lipids, liposomes, cationic polymers, cationic dendrimers and cationic cell penetrating peptides have been used by different authors for delivery of siRNA from time to time. Rapid enzymatic degradation, limited permeability across the cell membrane and substantial liver and renal clearance restricted the therapeutic applications of siRNA in vivo. According to Dutta et al. [20] the dendrosomes hold potential for the delivery of siRNA in vivo.

Eaton et al. [21] studied the efficacy of TRAIL (Tumor Necrosis Factor related apoptosis inducing ligand) treatment against HPV16 injected SiHa cells undergoing senescence following siRNA knock down of E6/E7 genes. They observed that E6/E7 siRNA induces senescence rather than apoptosis in SiHa cells. These findings are significant for combinatorial strategies for cancer therapy because the induction of senescence can preclude apoptosis rendering cells to be recalcitrant to TRAIL treatment.

Yang et al. [22] evaluated the efficiency of chitosan based HPV 16 E7 siRNA delivery and also studied the chitosan /HPV 16 E7 siRNA complex to induce apoptosis in HPV 16 positive CaSki cells. Chitosan is derived from the biopolymer chitin and is biologically safe, nontoxic, biodegradable and biocompatible. Chitosan/HPV16 E7 siRNA nanoparticles were delivered to CaSki cells and induced apoptosis. According to these authors, the delivery of nanoparticles *in vivo* may serve as a promising therapy for cervical cancer.

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor constitutively active and aberrantly expressed in cervical cancer. A study by Shukla et al. [23] performed on HPV-16 positive cervical cancer cell lines (SiHa and CaSki) and primary tumor tissues revealed a positive correlation of STAT3 with expression of HPV16 E6 and E7 oncoproteins and a negative association with levels of p53 and pRB. STAT3 specific siRNA targeting showed accumulation of p53 and pRb in cervical cancer cell lines. These authors have found a positive correlation of active STAT3 with E6 and E7 and an inverse relation with p53 and pRB pools. Specific targeting of STAT3 expression in cervical cancer cell lines have been performed earlier using recombinant adenoviral dominant negative STAT3 or STAT3 specific siRNA. Specific silencing of E6/E7 using specific siRNA also results in similar growth inhibition of cervical cancer cells, loss of transformed phenotype, induced apoptosis and replicative senescence and inhibited tumor formation in animal models. Delivery of E6/E7 siRNA into nude mice has shown significant reduction in the number of tumor nodules and retarded tumor growth of HPV16 positive cells in NOD/SCID mice. According to these authors the loss of expression of oncoproteins is not only the direct and sole cause of p53 and pRB accumulation in cervical cancer cells. However, leads obtained from this study provide a strong rationale for developing novel STAT3 based approaches for therapeutic interventions against HPV infections to control cervical cancer.

6. Conclusion

Molecular targeting of HPV oncogene expression using RNAi technology has tremendous potential for therapeutic use. The challenge might be overcome by application of different delivery systems of siRNA to specifically target the E6 and E7 oncogenes.

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.

References

1. Schiffman, M.; Castle, P.E.; Jeronimo, J.; Rodriguez, A.C.; Wacholder, S. Human papillomavirus and cervical cancer. Lancet 2007, 370, 890-907.
2. Kaarthigeyan, K. Cervical cancer in india and hpv vaccination. Indian J Med Paediatr Oncol 2012, 33, 7-12.
3. Jimenez A.M.J., K.C., Chudobova D., Ruttkay-Nedecky B., Vesely R., Adam V. and Kizek R. Regulation of human oncogenes' expression by human papillomavirus (hpv) 16 e6 protein Journal of Metallomics and Nanotechnologies 2015, 2.
4. Raychaudhuri, S.; Mandal, S. Socio-demographic and behavioural risk factors for cervical cancer and knowledge, attitude and practice in rural and urban areas of north bengal, india. Asian Pac J Cancer Prev 2012, 13, 1093-1096.
5. Bharti, A.C.; Shukla, S.; Mahata, S.; Hedau, S.; Das, B.C. Anti-human papillomavirus therapeutics: Facts & future. Indian J Med Res 2009, 130, 296-310.
6. Oh, Y.K.; Park, T.G. Sirna delivery systems for cancer treatment. Adv Drug Deliv Rev 2009, 61, 850-862.
7. Rao, D.D.; Vorhies, J.S.; Senzer, N.; Nemunaitis, J. Sirna vs. Shrna: Similarities and differences. Adv Drug Deliv Rev 2009, 61, 746-759.
8. Steele, C.; Sacks, P.G.; Adler-Storthz, K.; Shillitoe, E.J. Effect on cancer cells of plasmids that express antisense rna of human papillomavirus type 18. Cancer Res 1992, 52, 4706-4711.
9. Steele, C.; Cowsert, L.M.; Shillitoe, E.J. Effects of human papillomavirus type 18-specific antisense oligonucleotides on the transformed phenotype of human carcinoma cell lines. Cancer Res 1993, 53, 2330-2337.
10. Hamada, K.; Sakaue, M.; Alemany, R.; Zhang, W.W.; Horio, Y.; Roth, J.A.; Mitchell, M.F. Adenovirus-mediated transfer of hpv 16 e6/e7 antisense rna to human cervical cancer cells. Gynecol Oncol 1996, 63, 219-227.
11. Jiezhong Chen, N.M., Wenyi Gu. Intra-tumor injection of lentiviral-vector delivered shrna targeting human papillomavirus e6 and e7 oncogenes reduces tumor growth in a xenograft cervical cancer model in mice. Journal of Solid Tumors 2012, 2.
12. Alvarez-Salas, L.M.; Cullinan, A.E.; Siwkowski, A.; Hampel, A.; DiPaolo, J.A. Inhibition of hpv-16 e6/e7 immortalization of normal keratinocytes by hairpin ribozymes. Proc Natl Acad Sci U S A 1998, 95, 1189-1194.
13. Zheng, Y.; Zhang, J.; Rao, Z. Ribozyme targeting hpv16 e6e7 transcripts in cervical cancer cells suppresses cell growth and sensitizes cells to chemotherapy and radiotherapy. Cancer Biol Ther 2004, 3, 1129-1134; discussion 1135-1126.
14. Rao, Z.; Gao, J.; Zhang, B.; Yang, B.; Zhang, J. Cisplatin sensitivity and mechanisms of anti-hpv16 e6-ribozyme on cervical carcinoma caski cell line. The Chinese-German Journal of Clinical Oncology 2012, 11, 237-242.
15. Gu, W.; Putral, L.; Hengst, K.; Minto, K.; Saunders, N.A.; Leggatt, G.; McMillan, N.A. Inhibition of cervical cancer cell growth in vitro and in vivo with lentiviral-vector delivered short hairpin rna targeting human papillomavirus e6 and e7 oncogenes. Cancer Gene Ther 2006, 13, 1023-1032.
16. Yoshinouchi, M.; Yamada, T.; Kizaki, M.; Fen, J.; Koseki, T.; Ikeda, Y.; Nishihara, T.; Yamato, K. In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by e6 sirna. Mol Ther 2003, 8, 762-768.
17. Koivusalo, R.; Krausz, E.; Helenius, H.; Hietanen, S. Chemotherapy compounds in cervical cancer cells primed by reconstitution of p53 function after short interfering rna-mediated degradation of human papillomavirus 18 e6 mrna: Opposite effect of sirna in combination with different drugs. Mol Pharmacol 2005, 68, 372-382.
18. Chang, J.T.; Kuo, T.F.; Chen, Y.J.; Chiu, C.C.; Lu, Y.C.; Li, H.F.; Shen, C.R.; Cheng, A.J. Highly potent and specific sirnas against e6 or e7 genes of hpv16- or hpv18-infected cervical cancers. Cancer Gene Ther 2010, 17, 827-836.
19. Palanichamy, J.K.; Mehndiratta, M.; Bhagat, M.; Ramalingam, P.; Das, B.; Das, P.; Sinha, S.; Chattopadhyay, P. Silencing of integrated human papillomavirus-16 oncogenes by small interfering rna-mediated heterochromatization. Mol Cancer Ther 2010, 9, 2114-2122.
20. Dutta, T.; Burgess, M.; McMillan, N.A.; Parekh, H.S. Dendrosome-based delivery of sirna against e6 and e7 oncogenes in cervical cancer. Nanomedicine 2010, 6, 463-470.
21. Eaton, S.; Wiktor, P.; Thirstrup, D.; Lake, D.; Nagaraj, V.J. Efficacy of trail treatment against hpv16 infected cervical cancer cells undergoing senescence following sirna knockdown of e6/e7 genes. Biochem Biophys Res Commun 2011, 405, 1-6.
22. Yang, J.; Li, S.; Guo, F.; Zhang, W.; Wang, Y.; Pan, Y. Induction of apoptosis by chitosan/hpv16 e7 sirna complexes in cervical cancer cells. Mol Med Rep 2013, 7, 998-1002.
23. Shukla, S.; Mahata, S.; Shishodia, G.; Pandey, A.; Tyagi, A.; Vishnoi, K.; Basir, S.F.; Das, B.C.; Bharti, A.C. Functional regulatory role of stat3 in hpv16-mediated cervical carcinogenesis. PLoS One 2013, 8, e67849.

Biomarkers of Zn status associated to colorectal cancer pathogenesis

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Colorectal cancer (CRC) is the third most frequent malignant disease in developed countries. A large number of studies have been undertaken to identify potential risk factors for cancer, amongst which the association with trace elements, such as zinc, found naturally in the environment, and whose human exposure derives from a variety of sources. Significant alterations in Zn(II) levels in tissues have previously been reported in patients with various forms of cancer. Moreover, low plasma Zn(II) levels have been observed in patients with cancer of the colon, bronchus or digestive system. In this review, we focus largely on the association between zinc noted above and risk of CRC. Intervention plan in this type of cancer needs to consider nutritional responses towards anti-cancer drugs based on their biological and genetic characteristics, furthermore, a possible association with zinc in cancer treatment also requires attention. The analysis of Zn biomarkers levels could provide new biological insights applied in prevention, molecular diagnosis, prognosis and treatment of CRC.

Keywords: zinc; colorectal cancer; nutritional assessment; trace elements, cancer disease

1. Introduction

Cancer is a leading cause of death in both more and less economically developed countries, particularly in less ones, in which about 82% of the world's population resides [1]. Colorectal cancer (CRC) is the third most frequent malignant disease [2]. Over the last decade, a whole range of new technologies have been introduced in clinical practice to diagnose and treat the disease, with therapeutic modalities extending to advanced stages of the disease. Nevertheless, prevention undoubtedly remains the key to reducing morbidity and mortality [3]. Variation in international incidence rates [2] suggests that CRC aetiology is influenced by modifiable lifestyle factors, such as diet [4–7].

Beyond their general effects on health, micronutrients in patients with cancer have unique implications because of their potential direct effects on existing cancers, and effects on factors that may influence carcinogenesis, such as immunity and interactions with treatment [8]. Zinc is an essential trace element that participates as cofactor in a large number of intermediary metabolism proteins, in hormone secretion pathways and in different mechanisms of immune defence [9]. Zinc is known to be an essential component in DNA-binding zinc fingers proteins, as well as in copper/zinc superoxide

dismutase and in several proteins involved in DNA repair mechanisms. Thus, zinc plays an important role in transcription factors function and, antioxidant defence. Dietary deficiencies in zinc can contribute to single- and double-strand DNA breaks and DNA oxidative modifications that increase the risk for cancer development [10]. It is well known that carcinogenesis is a multistep process in which genetic sequence alterations helped by environmental factors, such as oxidative stress and antioxidant status [11], stimulating the selection and proliferation of malignant clones, eventually leading to the development of a detectable tumor. Furthermore, significant alterations in Zn(II) levels in tissues have previously been reported in patients with various forms of cancer. Moreover, low plasma Zn(II) levels have been observed in patients with cancer of the colon, bronchus or digestive system [12].

Because CRC treatment plan needs to consider nutritional responses towards anti-cancer drugs based on their biological and genetic characteristics, a possible association with zinc in cancer treatment also requires attention. Therefore, in this paper, we analyse the physiological and biological implication of Zn on CRC to provide screening, treatment, and prevention strategies. The analysis of Zn biomarkers levels could provide new biological insights that could be applied in prevention, molecular diagnosis, prognosis and treatment of CRC.

2. Zinc concentration in CRC tissues

As previously reported, carcinogenesis is a sequential process where genetic alterations aided by environmental factors favouring the selection and proliferation of malignant cells, eventually leading to the development of a tumor [13]. Imbalances in Zn(II) levels in tissues have been reported in patients with various types of cancer [14–16]. Nevertheless, some studies have suggested that the Cu(I)/Zn(II) ratio would be an indicator of the extent and prognosis of carcinomas [6–19].There is a significant increase in mean Cu level and Cu/Zn ratio in human colon neoplasm compared with normal colon mucosa. Tissue zinc level in human colon neoplasm, including adenoma and adenocarcinoma, was significantly lower than in the normal colon mucosa, studying cell lines HCT 116 and HT-29, and NIH3T3 (mouse embryonic fibroblast cell line) [20], being in accordance with other authors that observed Zn(II) level decreases related to tumor development in colon tissues [11], and with Gupta et al. [21] that demonstrated lower serum zinc in patients with CRC regarding only in advanced stages. The exact mechanism responsible for the alterations in trace element levels in patients with CRC cancer is largely unclear and requires further evaluation.

Zinc has been reported to inhibit the growth of malignant colonocytes via post-translational regulation of expression of adenomatous polyposis coli protein, extracellular signal-regulated kinase (ERK)-dependent activation of cell cycle inhibitor p21 and disruption of cell-cell communication as well as microtubule activity [22]. Rudolf et al. [23] showed that increased external zinc concentrations inhibit cell growth of three different colon cancer cell lines representing different stages of colon cancer: HCT-116, HT-29 and SW620 cells and induce their death, proving to be the most sensitive to externally added zinc and this sensitivity was at least partly due to increased levels of intracellular free zinc and the inability to overexpress metallothionein. The variability of responses to zinc in colon cancer at different stages as modelled *in vitro* suggests that zinc-induced cell death despite common underlying mechanism(s) might have a variable nature. Since intracellular zinc management is in particular in colonic cells quite complex and involves various zinc-specific transporters and binders that ascertain stable intracellular zinc environment.

3. Zinc action in oxidative stress and inflammation during CRC

Because Cu(I) and Zn(II) are cofactors of superoxide dismutase, which is an antioxidant enzyme, alterations of the concentrations of these metal ions may be related to neoplasm and malignancy [24,25]. Reactive oxygen species (ROS), such as superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are potentially

harmful by-products of normal cellular metabolism that directly affect cellular functions. ROS is generated by all aerobic organisms and it seems to be indispensable for signal transduction pathways that regulate cell growth and reduction-oxidation (redox) status. However, overproduction of these highly reactive oxygen metabolites can initiate lethal chain reactions, which involve oxidation and damage to structures that are crucial for cellular integrity and survival [26]. Many pathological factors including reactive oxygen species are involved in the process of CRC initiation and progression. It is known that excessive ROS are formed in chronic diseases being particularly susceptible to its attack, which leads in turn to carcinogenesis, but the precise mechanism underlying oxidative stress in cancer cells and molecular pathogenesis of CRC remains to be understood [20].

A special position among metals is occupied by the redox inert metal zinc. Zn is an essential component of numerous proteins involved in the defence against oxidative stress. Zn(II) is a cofactor for superoxide dismutase, an antioxidant enzyme, so that changes in the concentrations of these metal ions can be associated with neoplasia and malignancy. The mechanism by which tissue levels and serum Zn(II) decrease in various cancerous tissues and how this contributes to carcinogenesis is still unknown. The inhibitory effects of zinc on the antioxidant defence system of the colon and histoarchitecture during colon carcinogenesis induced animal models (1,2 dimethylhydrazine), finding that zinc has a beneficial effect during initiation of key events leading to the development of experimentally induced carcinogenesis [27] . Gopčević et al. [11] showed an increased oxidative stress when different stages of CRC were considered, which was accompanied with an unbalance of antioxidant defence. SOD activity as a first line of defence against ROS was depleted in all tumor stages, while total peroxidase activity was being induced, which suggests that peroxide was the most abundant ROS produced during CRC.

By the other hand, chronic inflammation, such as inflammatory bowel disease, is associated with increased risk of colon cancer [28–30]. The quantification of serum levels of IL-8, MMP-9 and CRP appears to be a reliable indicator of inflammation-related processes during the malignant stage of colorectal carcinogenesis, since these molecules are constantly increasing in blood of patients with CRC to promote tumor growth and invasion [28]. Therefore, TNF-α, IL-6, and markers of oxidative stress, cysteine (CySS) and F2-isoprostanes, were chosen by different authors as potential biomarkers because their association with CRC and their susceptibility to be modulated by antioxidants [29]. They found that an antioxidant micronutrient cocktail can substantially decrease circulating biomarkers of inflammation (TNF-α) and oxidative stress (CySS) in sporadic CRC patients.

4. Zinc dependent proteins, gene expression and CRC

Catalytically, zinc acts as the critical electrophile in many hydrolases [31] and structurally, zinc stabilizes many protein domains, for example, "zinc-finger" proteins [32]. Genome analysis studies have revealed thousands of potential zinc-binding protein sequences [33], however, only a small percentage of them have been structurally characterized [34]. Therefore, it is of substantial interest to develop computational structure prediction methods that are able to generate three-dimensional structural models of zinc-binding proteins from their sequences with accuracy in terms of both overall topology and atomic details around zinc-binding site.

In the last years, efforts have been led to the identification of zinc-sensitive genes in response to zinc deficient diet. DNA array analysis of mammalian genes identified in the small intestine, thymus and liver cells respond with altered expression level changes in the state of zinc [35,36]. Some of the identified genes encode proteins involved in intestinal fluid secretion, signal transduction pathways that control immune response, growth and energy metabolism, suggesting that regulation can contribute to the development of symptoms of zinc deficiency in mammals. To date, no blood biomarkers with high sensitivity or specificity for potentially curable early stage CRC have been validated for clinical use, even though numerous reports

have demonstrated that CRC is associated with changes in the blood proteome [37,38]. Studies in cell line human colon adenocarcinoma (HT-29) genes were identified zinc sensitive under conditions of a zinc deficiency[39].

DNA methylation was recently shown to be more frequent than genetic changes in CRC40. In addition to being frequent, aberrant DNA methylation has been shown to be an early event in tumorigenesis[41]. Genes with promoter DNA hypermethylation have been detected in various body fluids from cancer patients, including bile, faeces, plasma and urine, indicating that methylation of biomarkers may be useful for non- or minimally invasive cancer diagnostics [42,43]. ZNF331 and ZSCAN18 are recently reported to be hypermethylated in CRC, being highly expressed after epigenetic treatment in CRC cell lines, and confirming that the reduced expression of these genes most likely is caused by aberrant promoter methylation [44]. Promoter DNA methylation is commonly associated with reduced or lost gene expression, and aberrant promoter methylation may be one mechanism used by cancer cells to silence specific genes, thereby providing them with a growth advantage. The frequent and specific methylation of these genes in CRC makes them promising biomarkers for detection of this malignancy.

As previously described, zinc controls the normal development of the cells, tissues, and organs via zinc-containing proteins that orchestrate cell genesis, differentiation and viability [45]. Many transcriptional factors contain zinc finger motifs. Zinc finger is able to form a complex with DNA based on the interactions between α-helix of a zinc finger and DNA-specific bases. The function of the zinc fingers consists especially in the recognition of DNA and the activation of transcriptional processes [46]. Zinc finger proteins as ZNF148, a Kruppel-type zinc finger transcription factor, may play a significant role in the regulation of cell growth, apoptosis, and carcinogenesis [47]. Physiologically, ZNF148 protein potentiates the induction of the cyclin-dependent kinase inhibitor p21(waf1) transcription and leads to growth arrest in cultured colon cancer cells [48]. O'Reilly et al. [49], recently demonstrated that zinc finger proteins ZNF346, ZNF638, ZNF700 and ZNF768 are suitable for use as capture antigens in a blood-based biomarker assay for CRC. A multi-marker ZNF autoantibody assay provides a potential tool for improving cancer detection, and could be used for cancer screening as well as diagnosis, monitoring of cancer progression and therapeutic interventions. In a study developed by Yan et al. [50], it has been demonstrated that the expression of zinc-finger protein X-linked in CRC tissues was significantly higher than that in corresponding normal tissues. The associations between protein expression of ZFX and clinical-pathological parameters showed that ZFX expression was significantly associated with tumor differentiation, size and invasion, lymph node metastasis and distant metastasis, demonstrating that ZFX expression may be associated with the progress of CRC and suggested that ZFX has the potential value to be an effective prognostic predictor for CRC patients.

By the other hand, metallothioneins (MT) are a family of low molecular weight proteins that share significant sequence homology, and are involved in zinc and redox metabolism [51], as well as in many aspects of cancer biology. The human genome contains at least 11 functional MT genes that may be divided into four subgroups (MT1-4). There are several MT1 isoforms each encoded by its own gene and along with MT2A are ubiquitously expressed. Given their stress-inducible nature and their capacity to chelate toxic metals and electrophiles, many studies have proposed MT expression to confer resistance to many toxic drugs [52]. Free zinc ions exist in the picomolar range and may be considered negligible due to tight regulation by zinc transporters, MTs, and organelle sequestration [53]. Intracellular zinc pools consist mainly of tightly bound, unexchangeable zinc bound to proteins, and of the exchangeable, loosely bound zinc termed the „labile" pool, which is complexed to low molecular weight ligands and MTs[54].

The antiapoptotic, antioxidant, proliferative, and angiogenic effects of MT-I+II has resulted in increased focus on their role in oncogenesis, tumor progression, therapy response, and pa-

tient prognosis. Many studies have reported increased expression of MT-I+II mRNA and protein in various human cancers, where MT-I+II expression is sometimes correlated to higher tumor grade/stage, chemotherapy/radiation resistance, and poor prognosis. However, MT-I+II are downregulated in other types of tumors as CRC where MT-I+II is either inversely correlated or unrelated to mortality. Large discrepancies exist between different tumor types, and no distinct and reliable association exists between MT-I+II expression in tumor tissues and prognosis and therapy resistance. Furthermore, a parallel has been drawn between MT-I+II expression as a potential marker for prognosis, and MT-I+II's role as oncogenic factors [55]. MT is silenced during CRC progression, mainly through epigenetic mechanisms, and this loss is associated with poor survival. MT1G reexpression in CRC may be a viable strategy to sensitize tumor cells to chemotherapy, and that it may be brought about by HDACi. Zinc supplementation to chemotherapy regimens was able to resensitize chemoresistant tumor cells independently of MT induction and should be considered in future clinical studies [56].

Besides, considerable evidence has implicated matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, in the degradation of extracellular matrix during the metastatic process. Most MMPs are secreted as inactive zymogens and are activated extracellularly. Over expression of MMP-1, -2, -3. -7, -9, -13, and MT1-MMP have been demonstrated in human CRC [57]. The degree of over expression of some MMPs has been noted to correlate with stage of disease and/or prognosis. An unresolved debate has centred on whether MMPs are produced by the stromal cells surrounding a tumor or by the CRC cells themselves. MMP-7 is produced abundantly by CRC cells. The presence of a mutation in the APC gene results in nuclear accumulation of the beta-Catenin/TCF complex, which serves as a transcriptional factor that upregulates MMP-7 expression. Increased expression of MMP-3 in CRC correlates with low levels of microsatellite instability and poor prognosis. Recent studies, demonstrated MMP3 protein expression in the lamina propria itself seems to be highly specific for the detection of tumorous transition in cases of sporadic colorectal tumors [58. Increased levels of MMP-9 (produced primarily by inflammatory cells) have early been demonstrated in the transition from colon adenoma to adenocarcinoma. In contrast to other MMPs, overexpression of MMP-12 is associated with increased survival in CRC, presumably as a result of an inhibitory effect on angiogenesis [57]. Recently, there is a study that demonstrated that Nur77 (an orphan member of the nuclear receptor superfamily) could promote the invasion and metastasis of CRC cells through regulation of MMP-9 signalling. These observations provide a possible recent strategy for potentially treating or preventing the metastasis of CRC through targeting of Nur77 [59]. MMPs may have a crucial role not only in the invasive process of CRC, but also in the progression conditions and lesions to CRC. MMPs could constitute effective independent prognostic markers in CRC. Their determination might be useful to identify patients at higher risk for progression to cancer.

5. Conclusions

Low plasma Zn(II) levels have been observed in patients with cancer of the colon, but the mechanism by which serum and tissue Zn(II) levels decrease in cancerous tissue, and how this contributes to carcinogenesis is still being studied . Some studies have suggested that the increased Cu(I)/Zn(II) ratio would be an indicator of the extent and prognosis of carcinomas. The inhibitory effects of zinc on the antioxidant defence system during colon carcinogenesis in animals suggest that zinc has a beneficial effect during initiation of key events leading to the development of induced carcinogenesis.

CRC tumor invasion and metastasis, is a highly complicated multi-step phenomenon. In the complex event of tumor progression, tumor cells interact with basement membrane and extracellular matrix components. A large number of zinc binding proteins are involved in the degradation of extracellular matrix, but also in cancer invasion and metastasis. Therefore, by the analysis of Zn biomarkers levels it could be provided new biological insights that could

be applied in prevention, molecular diagnosis, prognosis and treatment of CRC. Early molecular detection of the CRC may augment the accuracy of diagnosis.

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Conflicts of Interest

For each author listed on this manuscript, there is no personal or financial support or author involvement with an organization with financial interest in the subject matter and no conflict of interest exists. The authors declare that they have no competing interests.

References

1. Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A. Global cancer statistics, 2012: Global Cancer Statistics, 2012. Cancer Journal for Clinicians 2015, 65(2), 87-108.
2. American Cancer Society. Global Cancer Facts & Figures 3rd Edition. Atlanta. American Cancer Society 2015.
3. Zavoral M., Suchanek S., Zavada F., Dusek L., Muzik J., Seifert B., Premysl F. Colorectal cancer screening in Europe. World Journal of Gastroenterology, 2009, 15(47), 5907-15.
4. Chun Y.J., Sohn S.-K., Song H.K., Lee S.M., Youn Y.H., Lee S., ParkH. Associations of colorectal cancer incidence with nutrient and food group intakes in korean adults: a case-control study. Clinical Nutrition Research 2015, 4(2), 110-23.
5. Azizi H., Asadollahi K., Davtalab Esmaeili E., Mirzapoor M. Iranian dietary patterns and risk of colorectal cancer. Health Promotion Perspectives 2015, 5(1), 72-80.
6. Romaguera D., Ward H., Wark P.A., Vergnaud A.-C., Peeters P.H., van Gils C.H., et al. Pre-diagnostic concordance with the WCRF/AICR guidelines and survival in European colorectal cancer patients: a cohort study. BMC Medicine 2015, 13(1), 107.
7. Vrieling A., Kampman E. The role of body mass index, physical activity, and diet in colorectal cancer recurrence and survival: a review of the literature. The American Journal of Clinical Nutrition 2010, 92(3), 471-90.
8. Giovannucci E., Chan A.T. Role of Vitamin and Mineral Supplementation and Aspirin Use in Cancer Survivors. Journal of Clinical Oncology 2010, 28(26), 4081-5.
9. Vallee B.L., Auld D.S. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 1990, 29(24), 5647-59.
10. Ho E. Zinc deficiency, DNA damage and cancer risk. The Journal of Nutritional Biochemistry 2004, 15(10), 572-8.
11. Gopčević K.R., Rovčanin B.R., Tatić S.B., Krivokapić Z.V., Gajić M.M., Dragutinović V.V. Activity of Superoxide Dismutase, Catalase, Glutathione Peroxidase, and Glutathione Reductase in Different Stages of Colorectal Carcinoma. Digestive Diseases and Sciences2013, 58(9), 2646-52.
12. Mazdak H, Yazdekhasti F, Movahedian A, Mirkheshti N, Shafieian M. The comparative study of serum iron, copper, and zinc levels between bladder cancer patients and a control group. International Urology and Nephrology2010, 42(1), 89-93.
13. Klaunig J.E., Kamendulis L.M. The role of oxidative stress in carcinogenesis. The Annual Review of Pharmacology and Toxicology 2004, 44, 239-67.
14. Christudoss P., Selvakumar R., Pulimood A.B., Fleming J.J., Mathew G. Tissue zinc levels in precancerous tissue in the gastrointestinal tract of azoxymethane (AOM)-treated rats. Experimental and Toxicologic Pathology2008, 59(5), 313-8.
15. Kuo H.W., Chen S.F., Wu C.C., Chen D.R., Lee J.H. Serum and tissue trace elements in patients with breast cancer in Taiwan. Biological Trace Element Research 2002, 89(1), 1-11.
16. Gupta S.K., Singh S.P., Shukla V.K. Copper, zinc, and Cu/Zn ratio in carcinoma of the gallbladder. Journal of Surgical Oncology2005, 91(3), 204-8.
17. Gupta S.K., Shukla V.K., Vaidya M.P., Roy S.K., Gupta S. Serum and tissue trace elements in colorectal cancerJournal of Surgical Oncology 1993,52(3), 172-5.
18. Arslan M., Demir H., Arslan H., Gokalp A.S., Demir C. Trace elements, heavy metals and other biochemical parameters in malignant glioma patients. Asian Pacific Journal of Cancer Prevention 2011, 12(2), 447-51.
19. Oyama T., Kawamoto T., Matsuno K., Osaki T., Matsumoto A., Isse T., Isse T., Nakata S., Ozaki S., Sugaya M., Yasuda M., Yamashita T., Takenoyama M., Sugio K., Yasumoto K. A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. Anticancer Research 2003, 23(1B), 605-12.
20. Kim E.S., Lim C.S., Chun H.J., Keum B., Seo Y.S., Kim Y.S., Jeen Y.T., Lee H.S., Um S.H., Kim C.D., Ryu H.S., Cho B.R. Detection of Cu(I) and Zn(II) ions in colon tissues by multi-photon microscopy: novel marker of antioxidant status of colon neoplasm. Journal of Clinical Pathology 2012, 65(10), 882-7.
21. Gupta S.K., Shukla V.K., Vaidya M.P., Roy S.K., Gupta S. Serum and tissue trace elements in colorectal cancer. Journal of Surgical Oncology 1993, 52(3), 172-5.
22. Fraker P.J., Haas S.M., Luecke R.W. Effect of zinc deficiency on the immune response of the young adult A/J mouseJournal of Nutrition 1977, 107(10), 1889-95.
23. Rudolf. Diverse sensitivity of cells representing various stages of colon carcinogenesis to

increased extracellular zinc: Implications for zinc chemoprevention. Oncology Reports 2011, 25(3),769-80.
24. Christudoss P., Selvakumar R., Pulimood A.B., Fleming J.J., Mathew G. Protective Role of Aspirin, Vitamin C, and Zinc and their Effects on Zinc Status in the DMH-Induced Colon Carcinoma ModelAsian Pacific Journal of Cancer Prevention 2013, 14(8), 4627-34.
25. Wang C., Vernon R., Lange O., Tyka M., Baker D. Prediction of structures of zinc-binding proteins through explicit modeling of metal coordination geometry. Protein Science 2010, 19(3), 494-506.
26. Fang J., Nakamura H., Iyer A.K. Tumor-targeted induction of oxystress for cancer therapy. Journal of Drug Targeting 2007, 15(7-8), 475-86.
27. Chadha V.D., Vaiphei K., Dhawan D.K. Zinc mediated normalization of histoarchitecture and antioxidant status offers protection against initiation of experimental carcinogenesis. Molecular and Cellular Biochemistry 2007, 304(1-2), 101-8.
28. Biasi F., Guina T., Maina M., Nano M., Falcone A., Aroasio E., Saracco G.M., Papotti M., Leonarduzzi G., Poli G. Progressive increase of matrix metalloprotease-9 and interleukin-8 serum levels during carcinogenic process in human colorectal tract. PloS One 2012, 7(7), e41839.
29. Hopkins M.H., Fedirko V., Jones D.P., Terry P.D., Bostick R.M. Antioxidant Micronutrients and Biomarkers of Oxidative Stress and Inflammation in Colorectal Adenoma Patients: Results from a Randomized, Controlled Clinical Trial. Cancer Epidemiology, Biomarkers & Prevention 2010, 19(3), 850-8.
30. Kim S., Keku T.O., Martin C., Galanko J., Woosley J.T., Schroeder J.C., Satia JA, Halabi S, Sandler RS. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. Cancer Research 2008, 68(1), 323-8.
31. McCall K.A., Huang C., Fierke C.A. Function and mechanism of zinc metalloenzymes. Journal of Nutrition 2000, 130(5S Suppl), 1437S - 46S.
32. Berg J.M., Shi Y. The galvanization of biology: a growing appreciation for the roles of zinc. Science 1996, 271(5252), 1081-5.
33. Andreini C., Banci L., Bertini I., Rosato A. Counting the zinc-proteins encoded in the human genome. Journal of Proteome Research 2006, 5(1), 196-201.
34. Berman H.M., Westbrook J., Feng Z., Gilliland G., Bhat T.N., Weissig H., Shindyalov I.N., Bourne P.E. The Protein Data Bank. Nucleic Acids Research2000, 28(1), 235-42.
35. Blanchard R.K., Moore J.B., Green C.L., Cousins R.J. Modulation of intestinal gene expression by dietary zinc status: effectiveness of cDNA arrays for expression profiling of a single nutrient deficiency. Proceedings of the National Academy of Sciences 2001, 98(24), 13507-13.
36. Moore J.B., Blanchard R.K., McCormack W.T., Cousins R.J. cDNA array analysis identifies thymic LCK as upregulated in moderate murine zinc deficiency before T-lymphocyte population changesJournal of Nutrition2001, 131(12), 3189-96.
37. Choi J.-W., Liu H., Shin D.H., Yu G.I., Hwang J.S., Kim E.S., Yun J.W. Proteomic and cytokine plasma biomarkers for predicting progression from colorectal adenoma to carcinoma in human patients. Proteomics 2013, 13(15), 2361-74.
38. Huijbers A., Velstra B., Dekker T.J.A., Mesker W.E., van der Burgt Y.E.M., Mertens B.J., Deelder AM, Tollenaar RA. Proteomic serum biomarkers and their potential application in cancer screening programsInternational Journal of Molecular Sciences. 2010, 11(11), 4175-93.
39. Kindermann B., Döring F., Pfaffl M., Daniel H. Identification of Genes Responsive to Intracellular Zinc Depletion in the Human Colon Adenocarcinoma Cell Line HT-29Journal of Nutrition 2004, 134(1), 57-62.
40. Schuebel K.E., Chen W., Cope L., Glöckner S.C., Suzuki H., Yi J.-M.,Chan T.A., Van Neste L., Van Criekinge W., van den Bosch S., van Engeland M., Ting A.H., Jair K., Yu W., Toyota M., Imai K., Ahuja N., Herman J.G., Baylin S.B. Comparing the DNA Hypermethylome with Gene Mutations in Human Colorectal Cancer. PLoS Genetics2007, 3(9), 1709-23.
41. Baylin S.B., Ohm J.E. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? Nature Reviews Cancer 2006, 6(2), 107-16.
42. Ned R.M., Melillo S., Marrone M. Fecal DNA testing for Colorectal Cancer Screening: the ColoSure test. PLoS Currents 2011, 3:RRN1220.
43. Tänzer M., Balluff B., Distler J., Hale K., Leodolter A., Röcken C., Molnar B, Schmid R, Lofton-Day C, Schuster T, Ebert MP. Performance of epigenetic markers SEPT9 and ALX4 in plasma for detection of colorectal precancerous lesions. PloS One 2010, 5(2), e9061.
44. Marie Vedeld H., Andresen K., Andrassy Eilertsen I., Nesbakken A., Seruca R., Gladhaug I.P., Thiis-Evensen E., Rognum T.O., Boberg K.M., Lind G.E. The novel colorectal cancer biomarkers CDO1, ZSCAN18 and ZNF331 are frequently methylated across gastrointestinal cancers. International Journal of Cancer 2015, 136(4), 844-53.
45. Gumulec J, Masarik M, Krizkova S, Adam V, Hubalek J, Hrabeta J, et al. Insight to physiology and pathology of zinc(II) ions and their actions in breast and prostate carcinoma. Current Medicinal Chemistry Journal 2011, 18(33), 5041-51.
46. Kizek R. Relevance of infection with human papillomavirus: The role of the p53 tumor suppressor protein and E6/E7 zinc finger protein. International Journal of Oncology2013, 43(6), 1754-62.
47. Thimmarayappa J., Sun J., Schultz L.E., Dejkhamron P., Lu C., Giallongo A., Merchant JL, Menon RK. Inhibition of growth hormone receptor gene expression by saturated fatty acids: role of Kruppel-like zinc finger factor, ZBP-89. Molecular Endocrinology 2006, 20(11), 2747-60.
48. Bai L., Merchant J.L. Transcription factor ZBP-89 cooperates with histone acetyltransferase p300 during butyrate activation of p21waf1 transcription in human cellsThe Journal of Biological Chemistry2000, 275(39), 30725-33.

49. O'Reilly J.-A., Fitzgerald J., Fitzgerald S., Kenny D., Kay E.W., O'Kennedy R., Kijanka GS. Diagnostic Potential of Zinc Finger Protein-Specific Autoantibodies and Associated Linear B-Cell Epitopes in Colorectal Cancer. PLoS ONE 2015, 10(4), e0123469.
50. Yan X., Yan L., Su Z., Zhu Q., Liu S., Jin Z.,Wang Y. Zinc-finger protein X-linked is a novel predictor of prognosis in patients with colorectal cancerInternational Journal of Clinical and Experimental Pathology 2014, 7(6), 3150-7.
51. Bell S.G., Vallee B.L. The metallothionein/thionein system: an oxidoreductive metabolic zinc link. European Journal of Chemical Biology 2009, 10(1), 55-62.
52. Eckschlager T., Adam V., Hrabeta J., Figova K., Kizek R. Metallothioneins and cancer. Current Protein and Peptide Science 2009, 10(4), 360-75.
53. Cousins R.J., Liuzzi J.P., Lichten L.A. Mammalian zinc transport, trafficking, and signals. The Journal of Biological Chemistry 2006, 281(34), 24085-9.
54. Costello L.C., Fenselau C.C., Franklin R.B. Evidence for operation of the direct zinc ligand exchange mechanism for trafficking, transport, and reactivity of zinc in mammalian cells. The Journal of Inorganic Biochemistry 2011, 105(5), 589-99.
55. Pedersen M.Ø., Larsen A., Stoltenberg M., Penkowa M. The role of metallothionein in oncogenesis and cancer prognosis. Progress in Histochemistry and Cytochemistry 2009, 44(1), 29-64.
56. Arriaga J.M., Greco A., Mordoh J., Bianchini M. Metallothionein 1G and Zinc Sensitize Human Colorectal Cancer Cells to Chemotherapy. Molecular Cancer Therapeutics, 2014, 13(5), 1369-81.
57. Zucker S., Vacirca J. Role of matrix metalloproteinases (MMPs) in colorectal cancer. Cancer And Metastasis Reviews 2004, 23(1-2), 101-17.
58. Sipos F., Germann T.M., Wichmann B., Galamb O., Spisák S., Krenács T., Tulassay Z., Molnár B., Műzes G. MMP3 and CXCL1 are potent stromal protein markers of dysplasia-carcinoma transition in sporadic colorectal cancer. European Journal of Cancer Prevention, 2014, 23(5), 336-43.
59. Wang J.R., Gan W.J., Li X.M., Zhao Y.Y., Li Y., Lu X.X., Li J.M., Wu H. Orphan nuclear receptor Nur77 promotes colorectal cancer invasion and metastasis by regulating MMP-9 and E-cadherin. Carcinogenesis, 2014, 35(11), 2474-84.

Skin carcinogenesis: the pathogenetic and therapeutic role of zinc

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The incidence of non-melanoma skin cancers and cutaneous malignant melanoma has been increasing worldwide in fair-skinned populations. Ultraviolet radiation is thought to be the main pathogenetic factor for skin cancer development. Zinc is important for skin homeostasis and cellular stress response to ultraviolet radiation. Zinc deficiency results in impaired host defences against skin carcinogenesis, and the chronic inflammation that is associated with prolonged zinc deficiency can even facilitate cutaneous malignancies. Furthermore, alterations in zinc homeostatic protein expression have been described in skin cancers and are thought to affect the growth, invasion and metastatic potential of the tumours. These findings raise the possibility that the modulation of intracellular zinc levels may be relevant to prevent and treat skin cancers.

Keywords: non-melanoma skin cancer; cutaneous malignant melanoma; ultraviolet radiation; zinc deficiency; metallothionein

1. Introduction

The increasing incidence of non-melanoma skin cancers (NMSC) and cutaneous malignant melanoma (CMM) is a significant burden on the health care system. The incidence of NMSC is approximately 100 per 100000 individuals in Europe [1]. Basal cell carcinoma (BCC) is a semi-malignant tumour that usually develops on sun-exposed skin areas. Both cumulative and intermittent high-dose ultraviolet irradiation (UVR) play a role in the formation of BCC [1]. Cutaneous squamous cell cancer (CSCC) appears to be associated with the cumulative UVR because it develops on the chronically sun damaged skin of elderly people at the site of precancerous skin lesions [1]. CSCCs rarely metastasise to regional lymph nodes, but they do so in a manner that depends on tumour depth and immune status. Cutaneous malignant melanoma (CMM) has a heterogeneous aetiology and pathogenesis, e.g., lentigo maligna melanoma is associated with chronic cumulative sun exposure, whereas other forms of CMM are associated with high-dose intermittent UVR, and there are even types of CMM that are not related to sunlight [2]. The incidence of CMM is 4-19 per 100000 individuals in Europe [3], and many patients are younger than 40 years old. Hereditary factors that affect skin pigmentation, DNA repair efficacy, and immune response play a very important role in the pathogenesis of CMM. CMM is characterised by a high propensity to metastasise and a low healing rate in metastatic cases. Surgery is the mainstay of skin cancer therapies. Topical and systemic medications are used to treat very early or advanced stages of the disease.

2. Physiological role of zinc in the skin

2.1 Epidermal homeostasis, immune surveillance, zinc signalling

Approximately 9 % of the zinc content of the body is associated with the skin, primarily with the epidermis (50–70 mg·g^{-1} dry weight)

[4,5]. The severe skin symptoms of hereditary or acquired zinc deficiency, including erythaematous rashes, scaly plaques, and ulcers at orifices and acra [6,7], and the ability of systemic or topical zinc preparations to improve hair loss, acne and several inflammatory skin conditions [8] highlight the importance of zinc in skin homeostasis. Extracellular Zn(II) is believed to enter the cell through the plasma membrane zinc importers (ZIP) and is then transported via a muffler with high Zn(II) affinity like metallothionein (MT), to the intracellular storage sites such as the endoplasmic reticulum [9,10]. The cellular level and distribution of Zn(II) is tightly controlled by zinc importers and transporters (ZnT) [11]. Half of the available zinc is localised to the cytoplasm, whereas 30–40% is localised to the nucleus, and the remainder is associated with the plasma membrane [12]. Zn(II) is required for the activity of more than 300 enzymes, for proper immune function and for the conformation of more than 2000 transcription factors that control cell proliferation, apoptosis and signalling pathways [8,13,14]. The MT/thionein pair is critical to sequester or release Zn(II) depending on the local redox state, thereby influencing the function of numerous proteins, transcription factors and enzymes involved such processes as nucleic acid and protein synthesis [15]. The keratinocytes (KC) in the basal layer of the epidermis constitutively express MT1, whereas the spinous layer is characterised by MT4 expression [16]. Epidermal melanocytes, dermal fibroblasts and endothelial cells also produce MT [16,17]. In MT-null mice, the epidermal zinc content is lower, and the stimulation of epidermal hyperplasia, e.g., by UVR is impaired [18]. MT is highly expressed in hyperproliferative epidermal KC [19]. KC differentiation is associated with the increased expression of ZIP2, which leads to increased intracellular levels of Zn(II) [20]. Moreover, ZIP2 knockdown inhibits KC differentiation [20]. Interestingly, differentiation-associated higher intracellular Zn (II) concentrations have also been observed in other cell types [21].

We found that Zn (II) might also affect reactive oxygen species (ROS)-sensitive signalling pathways [22]. We observed an upregulation of the cytoprotective and anti-inflammatory protein HMOX1 [23-25] and the downregulation of some pro-inflammatory mediators such as IL8 and PTGS2 [26,27] in cultured KC upon nontoxic Zn(II) exposure. Furthermore, the ability of Zn(II) to modulate phosphorylation signalling can explain the cell cycle regulatory role of the fluctuations of intracellular Zn(II) concentrations during cell cycle progression [21].

2.2 Cellular stress response to ultraviolet radiation: zinc for skin cancer prevention?

Solar UV exposure is one of the most important environmental factors that affect skin physiology [28,29]. UVB (290–320 nm) exposure of human skin is known to induce pathophysiological processes, such as DNA damage, oxidative stress, inflammation and photo-immunosuppression, with clinical signs of erythema (sunburn reaction), tanning, photo-aging, and skin cancers [28]. UVB causes skin cell damage both directly, by inducing the production of cyclobutane pyrimidine dimers (CPDs), and indirectly, by triggering the production of reactive species and interfering with cellular redox homeostasis. CPDs are primarily responsible for the genome mutations induced by UVR; thus, UVB is considered the main pathogenetic factor for skin cancer development [29,30]. The MT levels have been found to be elevated in the epidermis after acute UVR exposure [17]. Importantly, MT seems to significantly reduce the formation of sunburn cells and induce hyperplasia after UVB irradiation [18,19,31,32]. Accordingly, in vitro, the expression of MT has been shown to increase 24 h after UV irradiation [33]. Interestingly, we observed that the expression of the MT1E isoform is down-regulated in UVB-exposed KC 6 h after UVB irradiation [22], which is also dependent on CPD formation (not reported). These results support the modulation of zinc homeostasis by UVB as part of a cellular stress response to UVR. Furthermore, the induction of MT by zinc chloride (ZnCl2) exposure enhanced cell survival and reduced both the immediate DNA damage [33,34] and the DNA fragmentation induced by solar UVR exposure [35]. We found that the level of induced CPD was lower in ZnCl2

pre-treated cells 3 h after UVB irradiation when the translocation of MTs to the nucleus could also be demonstrated. However, similar to the results reported by Saito et al. [36], pre-treating the cells with Zn(II) for 24 h was not sufficient to improve cell survival after UVB irradiation, although the fraction of early apoptotic cells decreased. Previously, the elevation of intracellular Zn(II) levels has been demonstrated after UVB irradiation, which was proportional to the fraction of dying or dead cells and suggests that UVB-induced Zn(II) release may be an important step in the UVB-induced cell death pathways [37,38]. Furthermore, we observed that the increase in superoxide production after UVB treatment was augmented by Zn(II) pre-exposure and that the fraction of late apoptotic plus necrotic cells increased. It can be assumed that a vicious cycle of ROS-induced zinc release and zinc-driven mitochondrial ROS production is involved in this type of cell death [39] or the trans-activation of signal transduction pathways (e.g., p53) by ROS alters the mechanism of UVB-induced cell death [40]. Whether a change in the mechanism of death upon Zn(II) pre-exposure can affect the immunogenic potential of cell death [41] induced by UVB exposure, which would impact the development of skin cancers, requires further investigation. Furthermore, revealing the functions of the different MT isoforms in epidermal cells may also contribute to an understanding of the role of zinc in the UV-induced stress response.

3. Zinc and skin carcinogenesis

3.1 Alterations in the expression of zinc homeostatic proteins in skin cancers have prognostic relevance

Changes in MT expression (up- or downregulation) are a known feature of tumour progression in several types of human malignancies and may be associated with a more aggressive phenotype and therapeutic resistance, ultimately resulting in a worse prognosis [42,43]. Data also exist that suggest that the upregulation of MT expression in CMM is a significant and independent factor for reduced patient survival [44,45]. We also observed that MTI/II overexpression in melanoma cells is significantly more frequent in primary CMM with haematogenous metastases [46]. It is not known which MT isoforms are overexpressed, but it may be worth noting that MT1E and MT1G have been shown to be downregulated by hypermethylation in CMM [47,48]. Regarding NMSC, significantly higher MTI/II and MTIII expression was noted in actinic keratosis and CSCC, compared with normal skin epidermis, whereas very low levels of MTIII expression were found in BCC [49,50].

3.2 Alterations in the expression of zinc homeostatic proteins in skin cancers: cause or consequence?

The role of MT in metastasis formation remains to be confirmed, and experimental evidence for its oncogenic role is still lacking. Signalling pathways activated during tumour development and/or the altered physiology of cancer cells could trigger high MT expression in malignancies. The exploration of genome-wide transcriptional and epigenetic dysregulations induced by driver mutations has only just begun [51]. Nevertheless, skin cancers such as CSCC and CMM consist of non-differentiated/dedifferentiated cells that possess high proliferative capacity. Accordingly, the zinc content of CSCC is significantly lower than that of normal skin, which is primarily composed of differentiated KC [52]. Thus, we can assume that one reason for the high expression level of MT in cancer is cellular hyperplasia [18]. Conversely, MTI/II transcription can be induced by inflammatory cytokines (IL-6, TNF-α, interferons), hypoxia and free radicals that are present in the tumour microenvironment. Furthermore, it is possible that circulating cytokines can contribute to increased MT production in skin cancers because the increased expression and nuclear translocation of MT can be observed in the basal KC layer in non-exposed skin areas when other areas are subjected to UV exposure. This phenomenon was connected to increased IL-6 blood levels produced by neutrophils upon UV irradiation [17].

Interestingly, the alterations of zinc homeostasis may also be significant in human papilloma virus (HPV)-associated skin cancer development. The transmembrane channel-like

(TMC) proteins EVER1 (TMC6) and EVER2 (TMC8) proteins form a complex and interact with the ZnT1 protein and affect the distribution of intracellular Zn (II) [53]. Mutations in EVER1/2 cause a genodermatosis (epidermodysplasia verruciformis) that is associated with HPV related skin cancers. It has been shown that HPV oncoproteins bind to EVER and ZnT1 and block their negative regulation of transcription factors stimulated by zinc (MTF-1) or cytokines (c-Jun and Elk) [53].

3.3 Modulation of cell proliferation, invasion and the tumour microenvironment: a possible pathogenetic role of zinc

Chronic inflammation is an important pathogenetic factor in several types of malignancies, such as CSCC. Key mediators of inflammation-induced cancer include nuclear factor kappa B, reactive oxygen and nitrogen species, inflammatory cytokines, prostaglandins and specific microRNAs (miR) [54]. It has been found that prolonged zinc deficiency results in the upregulation of key inflammatory genes (S100A8, S100A9, Ptgs2, Tlr4) and an oncogenic miR signature (miR-31, miR-21) in the skin of a rat model. This finding suggests that zinc deficiency can contribute to the formation of a pro-tumorigenic inflammatory microenvironment that facilitates carcinoma development [55]. A significant upregulation of miR-21, miR-31, S100A8, S100A9, PTGS2 and TLR4 has been found in human CSCC and has been linked to tumorigenesis [56-60]. In addition, zinc deficiency is associated with impaired innate and adaptive immune functions that can contribute to cancer development [61].

A high expression level of MT in CSCC and CMM suggests that the release or sequestration of Zn(II) by MT [15] may be important for tumour progression. Many of the zinc-dependent enzymes are involved in skin homeostasis and host defence against cancer formation; however, after the cancer has been formed, they can promote the growth and invasion of malignant cells [16]. In addition, several zinc finger transcription factors are involved in oncogenic driver signalling pathways [62,63].

Finally, we have found that the expression of MTI/II in melanoma cells might play a role in the formation of an immunosuppressive tumour microenvironment, which can promote CMM progression [46].

4. Zinc in the treatment of skin cancers

4.1 Cytotoxic effect of zinc

High concentrations of zinc are cytotoxic to cancer cells. It has been reported that 20 % topical zinc sulphate can induce the clearance of actinic keratoses and small skin cancers in patients with xeroderma pigmentosum [4]. Importantly, it has been demonstrated that ionophoric zinc can affect the posttranscriptional regulation of gene expression, thereby inducing cytotoxicity in cancer cells [64]. It can also sensitise cancer cells to other anticancer therapeutic modalities [65]. It would be worth considering the therapeutic potential of zinc pyrithione and the related zinc ionophores for skin cancer therapy [66].

4.2 Modulation of signalling pathways and the tumour microenvironment

Zn supplementation in rats decreased the incidence of chemical-induced tongue SCC and elicited a reduced proliferative/inflammatory cancer phenotype [55]. It could also be shown that Zn supplementation that suppressed tongue cancer development also attenuated miR-31 and miR-21 expression [55]. These investigations should be extended to CSCC.

Furthermore, it has been demonstrated that the administration of zinc can re-establish the chemosensitivity of cancer cells by reactivating p53 and increasing the immunogenic potential of cancer cell death [41,67]. This phenomenon has not been studied in CMM cells.

5. Conclusions

Proper functioning of zinc homeostatic proteins and appropriate dietary zinc intake seem to be important in epidermal homeostasis and defence against skin cancer development (Figure 1). Prolonged dietary zinc deficiency causes aberrant miRNA expression in the skin, which is associated with chronic inflammation and

may contribute to carcinogenesis. The immuno--modulatory role of MT together with the ability to affect the activity of transcription factors and enzymes altering cell proliferation and differentiation might contribute to the progression of skin cancers such as CSCC and CMM. It seems worthwhile to further examine the role of zinc in skin because clarifying this issue can affect our thinking about the pathogenesis of skin diseases and contribute to the identification of new therapeutic targets.

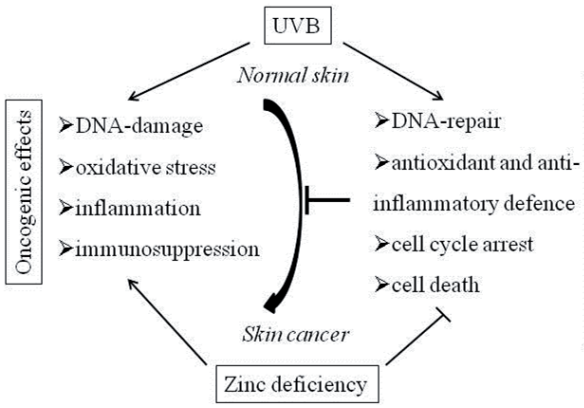

Figure 1: A possible pathogenetic role of zinc in skin carcinogenesis. UVB radiation is thought to be the main pathogenetic factor for skin cancer development [28]. Zinc deficiency can contribute to the formation of a pro-tumorigenic inflammatory microenvironment and impaired host defences against skin carcinogenesis that facilitate carcinoma development [22,34,55]

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Conflicts of Interest

None declared.

References

1. Fartasch, M.; Diepgen, T.L.; Schmitt, J.; Drexler, H. The relationship between occupational sun exposure and non-melanoma skin cancer: Clinical basics, epidemiology, occupational disease evaluation, and prevention. Deutsches Arzteblatt international 2012, 109, 715-720.
2. Whiteman, D.C.; Pavan, W.J.; Bastian, B.C. The melanomas: A synthesis of epidemiological, clinical, histopathological, genetic, and biological aspects, supporting distinct subtypes, causal pathways, and cells of origin. Pigment cell & melanoma research 2011, 24, 879-897.
3. MacKie, R.M.; Hauschild, A.; Eggermont, A.M. Epidemiology of invasive cutaneous melanoma. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2009, 20 Suppl 6, vi1-7.
4. Gupta, M.; Mahajan, V.K.; Mehta, K.S.; Chauhan, P.S. Zinc therapy in dermatology: A review. Dermatology research and practice 2014, 2014, 709152.
5. Molokhia, M.M.; Portnoy, B. Neutron activation analysis of trace elements in skin. Iv. Regional variations in copper, manganese and zinc in normal skin. Br J Dermatol 1970, 82, 254-255.
6. Kawamura, T.; Ogawa, Y.; Nakamura, Y.; Nakamizo, S.; Ohta, Y.; Nakano, H.; Kabashima, K.; Katayama, I.; Koizumi, S.; Kodama, T., et al. Severe dermatitis with loss of epidermal langerhans cells in human and mouse zinc deficiency. J Clin Invest 2012, 122, 722-732.
7. Portnoy, B.; Molokhia, M. Letter: Zinc in acrodermatis enteropathica. Lancet 1974, 2, 663-664.
8. Schwartz, J.R.; Marsh, R.G.; Draelos, Z.D. Zinc and skin health: Overview of physiology and pharmacology. Dermatol Surg 2005, 31, 837-847; discussion 847.
9. Taylor, K.M.; Vichova, P.; Jordan, N.; Hiscox, S.; Hendley, R.; Nicholson, R.I. Zip7-mediated intracellular zinc transport contributes to aberrant growth factor signaling in antihormone-resistant breast cancer cells. Endocrinology 2008, 149, 4912-4920.
10. Taylor, K.M.; Hiscox, S.; Nicholson, R.I.; Hogstrand, C.; Kille, P. Protein kinase ck2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel zip7. Science signaling 2012, 5, ra11.
11. Colvin, R.A.; Holmes, W.R.; Fontaine, C.P.; Maret, W. Cytosolic zinc buffering and muffling: Their role in intracellular zinc homeostasis. Metallomics : integrated biometal science 2010, 2, 306-317.
12. Vallee, B.L.; Falchuk, K.H. The biochemical basis of zinc physiology. Physiol Rev 1993, 73, 79-118.
13. Berg, J.M.; Shi, Y. The galvanization of biology: A growing appreciation for the roles of zinc. Science 1996, 271, 1081-1085.
14. Powell, S.R. The antioxidant properties of zinc. The Journal of nutrition 2000, 130, 1447S-1454S.
15. Maret, W. The function of zinc metallothionein: A link between cellular zinc and redox state. The Journal of nutrition 2000, 130, 1455S-1458S.
16. McGee, H.M.; Woods, G.M.; Bennett, B.; Chung, R.S. The two faces of metallothionein in carcinogenesis: Photoprotection against uvr-induced cancer and promotion of tumour survival. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology 2010, 9, 586-596.
17. Ablett, E.; Whiteman, D.C.; Boyle, G.M.; Green,

A.C.; Parsons, P.G. Induction of metallothionein in human skin by routine exposure to sunlight: Evidence for a systemic response and enhanced induction at certain body sites. The Journal of investigative dermatology 2003, 120, 318-324.
18. Hanada, K.; Sawamura, D.; Hashimoto, I.; Kida, K.; Naganuma, A. Epidermal proliferation of the skin in metallothionein-null mice. The Journal of investigative dermatology 1998, 110, 259-262.
19. Karasawa, M.; Nishimura, N.; Nishimura, H.; Tohyama, C.; Hashiba, H.; Kuroki, T. Localization of metallothionein in hair follicles of normal skin and the basal cell layer of hyperplastic epidermis: Possible association with cell proliferation. The Journal of investigative dermatology 1991, 97, 97-100.
20. Inoue, Y.; Hasegawa, S.; Ban, S.; Yamada, T.; Date, Y.; Mizutani, H.; Nakata, S.; Tanaka, M.; Hirashima, N. Zip2 protein, a zinc transporter, is associated with keratinocyte differentiation. The Journal of biological chemistry 2014, 289, 21451-21462.
21. Li, Y.; Maret, W. Transient fluctuations of intracellular zinc ions in cell proliferation. Experimental cell research 2009, 315, 2463-2470.
22. Emri, E.; Miko, E.; Bai, P.; Boros, G.; Nagy, G.; Rozsa, D.; Juhasz, T.; Hegedus, C.; Horkay, I.; Remenyik, E., et al. Effects of non-toxic zinc exposure on human epidermal keratinocytes. Metallomics : integrated biometal science 2015, 7, 499-507.
23. Bhujade, A.M.; Talmale, S.; Kumar, N.; Gupta, G.; Reddanna, P.; Das, S.K.; Patil, M.B. Evaluation of cissus quadrangularis extracts as an inhibitor of cox, 5-lox, and proinflammatory mediators. J Ethnopharmacol 2012, 141, 989-996.
24. Hseu, Y.C.; Chou, C.W.; Senthil Kumar, K.J.; Fu, K.T.; Wang, H.M.; Hsu, L.S.; Kuo, Y.H.; Wu, C.R.; Chen, S.C.; Yang, H.L. Ellagic acid protects human keratinocyte (hacat) cells against uva-induced oxidative stress and apoptosis through the upregulation of the ho-1 and nrf-2 antioxidant genes. Food Chem Toxicol 2012, 50, 1245-1255.
25. Chapple, S.J.; Siow, R.C.; Mann, G.E. Crosstalk between nrf2 and the proteasome: Therapeutic potential of nrf2 inducers in vascular disease and aging. Int J Biochem Cell Biol 2012, 44, 1315-1320.
26. Surh, Y.J.; Kundu, J.K.; Na, H.K.; Lee, J.S. Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. J Nutr 2005, 135, 2993S-3001S.
27. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology 2007, 39, 44-84.
28. Marrot, L.; Meunier, J.R. Skin DNA photodamage and its biological consequences. Journal of the American Academy of Dermatology 2008, 58, S139-148.
29. de Gruijl, F.R.; van Kranen, H.J.; Mullenders, L.H. Uv-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. Journal of photochemistry and photobiology. B, Biology 2001, 63, 19-27.
30. Pfeifer, G.P.; Besaratinia, A. Uv wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology 2012, 11, 90-97.
31. Hanada, K.; Sawamura, D.; Tamai, K.; Baba, T.; Hashimoto, I.; Muramatsu, T.; Miura, N.; Naganuma, A. Novel function of metallothionein in photoprotection: Metallothionein-null mouse exhibits reduced tolerance against ultraviolet b injury in the skin. The Journal of investigative dermatology 1998, 111, 582-585.
32. Wang, W.H.; Li, L.F.; Zhang, B.X.; Lu, X.Y. Metallothionein-null mice exhibit reduced tolerance to ultraviolet b injury in vivo. Clinical and experimental dermatology 2004, 29, 57-61.
33. Jourdan, E.; Marie Jeanne, R.; Regine, S.; Pascale, G. Zinc-metallothionein genoprotective effect is independent of the glutathione depletion in hacat keratinocytes after solar light irradiation. Journal of cellular biochemistry 2004, 92, 631-640.
34. Jourdan, E.; Emonet-Piccardi, N.; Didier, C.; Beani, J.C.; Favier, A.; Richard, M.J. Effects of cadmium and zinc on solar-simulated light-irradiated cells: Potential role of zinc-metallothionein in zinc-induced genoprotection. Archives of biochemistry and biophysics 2002, 405, 170-177.
35. Parat, M.O.; Richard, M.J.; Pollet, S.; Hadjur, C.; Favier, A.; Beani, J.C. Zinc and DNA fragmentation in keratinocyte apoptosis: Its inhibitory effect in uvb irradiated cells. J Photochem Photobiol B 1997, 37, 101-106.
36. Saito, T.; Tezuka, T.; Konno, R.; Fujii, N. Protective effects of metallothionein i and ii against metal- and ultraviolet radiation-induced damage in cultured lens epithelial cells. Japanese journal of ophthalmology 2010, 54, 486-493.
37. Stork, C.J.; Martorano, L.M.; Li, Y.V. Uvb radiation induces an increase in intracellular zinc in human epidermal keratinocytes. International journal of molecular medicine 2010, 26, 463-469.
38. Wang, L.; Liu, W.; Parker, S.H.; Wu, S. Nitric oxide synthase activation and oxidative stress, but not intracellular zinc dyshomeostasis, regulate ultraviolet b light-induced apoptosis. Life Sci 2010, 86, 448-454.
39. McCord, M.C.; Aizenman, E. The role of intracellular zinc release in aging, oxidative stress, and alzheimer's disease. Front Aging Neurosci 2014, 6, 77.
40. Liu, B.; Chen, Y.; St Clair, D.K. Ros and p53: A versatile partnership. Free radical biology & medicine 2008, 44, 1529-1535.
41. Cirone, M.; Garufi, A.; Di Renzo, L.; Granato, M.; Faggioni, A.; D'Orazi, G. Zinc supplementation is required for the cytotoxic and immunogenic effects of chemotherapy in chemoresistant p53-functionally deficient cells. Oncoimmunology 2013, 2, e26198.
42. Pedersen, M.O.; Larsen, A.; Stoltenberg, M.; Penkowa, M. The role of metallothionein in oncogenesis and cancer prognosis. Prog Histochem Cytochem 2009, 44, 29-64.
43. Thirumoorthy, N.; Shyam Sunder, A.; Manisenthil

Kumar, K.; Senthil Kumar, M.; Ganesh, G.; Chatterjee, M. A review of metallothionein isoforms and their role in pathophysiology. World journal of surgical oncology 2011, 9, 54.
44. Weinlich, G. Metallothionein-overexpression as a prognostic marker in melanoma. Giornale italiano di dermatologia e venereologia : organo ufficiale, Societa italiana di dermatologia e sifilografia 2009, 144, 27-38.
45. Sugita, K.; Yamamoto, O.; Asahi, M. Immunohistochemical analysis of metallothionein expression in malignant melanoma in japanese patients. The American Journal of dermatopathology 2001, 23, 29-35.
46. Emri, E.; Egervari, K.; Varvolgyi, T.; Rozsa, D.; Miko, E.; Dezso, B.; Veres, I.; Mehes, G.; Emri, G.; Remenyik, E. Correlation among metallothionein expression, intratumoural macrophage infiltration and the risk of metastasis in human cutaneous malignant melanoma. Journal of the European Academy of Dermatology and Venereology : JEADV 2013, 27, e320-327.
47. Faller, W.J.; Rafferty, M.; Hegarty, S.; Gremel, G.; Ryan, D.; Fraga, M.F.; Esteller, M.; Dervan, P.A.; Gallagher, W.M. Metallothionein 1e is methylated in malignant melanoma and increases sensitivity to cisplatin-induced apoptosis. Melanoma research 2010, 20, 392-400.
48. Koga, Y.; Pelizzola, M.; Cheng, E.; Krauthammer, M.; Sznol, M.; Ariyan, S.; Narayan, D.; Molinaro, A.M.; Halaban, R.; Weissman, S.M. Genome-wide screen of promoter methylation identifies novel markers in melanoma. Genome Res 2009, 19, 1462-1470.
49. Pula, B.; Tazbierski, T.; Zamirska, A.; Werynska, B.; Bieniek, A.; Szepietowski, J.; Rys, J.; Dziegiel, P.; Podhorska-Okolow, M. Metallothionein 3 expression in normal skin and malignant skin lesions. Pathology oncology research : POR 2015, 21, 187-193.
50. Zamirska, A.; Matusiak, L.; Dziegiel, P.; Szybejko-Machaj, G.; Szepietowski, J.C. Expression of metallothioneins in cutaneous squamous cell carcinoma and actinic keratosis. Pathology oncology research : POR 2012, 18, 849-855.
51. Guo, X.; Xu, Y.; Zhao, Z. In-depth genomic data analyses revealed complex transcriptional and epigenetic dysregulations of braf (v600e) in melanoma. Molecular cancer 2015, 14, 60.
52. Carruthers, C.; Suntzeff, V. Calcium, copper, and zinc in the epidermal carcinogenesis of mouse and man. Cancer research 1946, 6, 296.
53. Lazarczyk, M.; Pons, C.; Mendoza, J.A.; Cassonnet, P.; Jacob, Y.; Favre, M. Regulation of cellular zinc balance as a potential mechanism of ever-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. The Journal of experimental medicine 2008, 205, 35-42.
54. Schetter, A.J.; Heegaard, N.H.; Harris, C.C. Inflammation and cancer: Interweaving microrna, free radical, cytokine and p53 pathways. Carcinogenesis 2010, 31, 37-49.
55. Alder, H.; Taccioli, C.; Chen, H.; Jiang, Y.; Smalley, K.J.; Fadda, P.; Ozer, H.G.; Huebner, K.; Farber, J.L.; Croce, C.M., et al. Dysregulation of mir-31 and mir-21 induced by zinc deficiency promotes esophageal cancer. Carcinogenesis 2012, 33, 1736-1744.
56. Bruegger, C.; Kempf, W.; Spoerri, I.; Arnold, A.W.; Itin, P.H.; Burger, B. Microrna expression differs in cutaneous squamous cell carcinomas and healthy skin of immunocompetent individuals. Experimental dermatology 2013, 22, 426-428.
57. Wang, A.; Landen, N.X.; Meisgen, F.; Lohcharoenkal, W.; Stahle, M.; Sonkoly, E.; Pivarcsi, A. Microrna-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. PloS one 2014, 9, e103206.
58. Choi, D.K.; Li, Z.J.; Chang, I.K.; Yeo, M.K.; Kim, J.M.; Sohn, K.C.; Im, M.; Seo, Y.J.; Lee, J.H.; Kim, C.D., et al. Clinicopathological roles of s100a8 and s100a9 in cutaneous squamous cell carcinoma in vivo and in vitro. Archives of dermatological research 2014, 306, 489-496.
59. Muller-Decker, K. Cyclooxygenase-dependent signaling is causally linked to non-melanoma skin carcinogenesis: Pharmacological, genetic, and clinical evidence. Cancer metastasis reviews 2011, 30, 343-361.
60. Weng, H.; Deng, Y.; Xie, Y.; Liu, H.; Gong, F. Expression and significance of hmgb1, tlr4 and nf-kappab p65 in human epidermal tumors. BMC cancer 2013, 13, 311.
61. Haase, H.; Rink, L. The immune system and the impact of zinc during aging. Immunity & ageing : I & A 2009, 6, 9.
62. Soehnge, H.; Ouhtit, A.; Ananthaswamy, O.N. Mechanisms of induction of skin cancer by uv radiation. Frontiers in bioscience : a journal and virtual library 1997, 2, d538-551.
63. Cherian, M.G.; Jayasurya, A.; Bay, B.H. Metallothioneins in human tumors and potential roles in carcinogenesis. Mutation research 2003, 533, 201-209.
64. Zheng, J.; Zhang, X.X.; Yu, H.; Taggart, J.E.; Ding, W.Q. Zinc at cytotoxic concentrations affects posttranscriptional events of gene expression in cancer cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2012, 29, 181-188.
65. Kim, K.W.; Speirs, C.K.; Jung, D.K.; Lu, B. The zinc ionophore pci-5002 radiosensitizes non-small cell lung cancer cells by enhancing autophagic cell death. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2011, 6, 1542-1552.
66. Lamore, S.D.; Wondrak, G.T. Zinc pyrithione impairs zinc homeostasis and upregulates stress response gene expression in reconstructed human epidermis. Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine 2011, 24, 875-890.
67. Margalit, O.; Simon, A.J.; Yakubov, E.; Puca, R.; Yosepovich, A.; Avivi, C.; Jacob-Hirsch, J.; Gelernter, I.; Harmelin, A.; Barshack, I., et al. Zinc supplementation augments in vivo antitumor effect of chemotherapy by restoring p53 function. International journal of cancer. Journal international du cancer 2012, 131, E562-568.

Metallo-Cancer-Omics

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There is still a lot of unknown related to our perceptiveness to civilization and other illnesses including tumour ones often connected with environmental changes. There is also still an enormous field for cutting-edge research necessary to establish a role the unique tiny particles playing in the whole concert leading to our fitness or illness or, telling in other words, to normal or pathological functioning of our body cells. Studying metallome as the whole picture composed from metals, peptides, proteins and cell parts belongs to the most challenging issues of present biomedicine. Here, we summarize the omics advances in this field with special focus on *in vivo* imaging systems

Keywords: cancer; metallothionein; metals; advanced materials; imaging

1. Introduction

Recent advances in understanding the human genome have been made possible due to multidisciplinary cooperation between life sciences and technology. Genomics has succeeded in producing complete genomic DNA sequences of numerous species, but we are still some way from understanding differences between normal and pathological processes of cells and organisms [1]. Currently, attention is paid towards proteomics providing information about proteins localizations, structures and function, and most importantly, interaction with other proteins [2]. Recent progresses in high-throughput sample separation and mass spectrometry have impacted positively the proteomic characterization of proteins in systems biology [3]. Metalloproteins belong to the most diverse classes of protein, with intrinsic metal atoms providing a catalytic, regulatory and structure role essential to proteins function [4]. Transition metals such as copper, iron and zinc play important roles in life. Zn, the most abundant cellular transition metal, plays a vital role for functions of more than 300 enzymes, in DNA stabilization and in gene expression [5]. As some metals are crucial for body function, dyshomeostasis or deficiency of these elements can result in disease [6-8]. The Metallome is the distribution of inorganic species in cell. Metallomics and metalloproteomics are emerging fields addressing the role, uptake, transport and storage of the trace metals essential for life. Metallomics is defined as the analysis of the entirety of metal and metalloid species within a cell or tissue, whereas metalloproteomics focuses on exploration of the function of metals associated with proteins [9].

There are three main approaches being developed in metallomics and metalloproteomics:

- The first is and widely used is mass spectrometry, particularly electrospray ionisation mass spectrometry (ESI-MS) and inductively coupled plasma mass spectrometry connected with laser ablation (LA-ICP-MS). This connection allows us to see the lateral distribution of elements on the sample surface. These two techniques are ideal partners in comprehensive

structural and functional characterization of metalloproteins. LA-ICP-MS has been extensively developed for elemental mapping in bio-imaging applications. [10, 11].

- Second approach is high-throughput X-ray absorption spectroscopy (HT-XAS) to provide direct metal analysis of proteins and proteomic metals distribution in tissues and cells [12].
- Third approach is computational bioinformatics analysis of the obtained results. Compared to genomics and proteomics, metallomics and metalloproteomics are relatively new fields that require the design and development of completely new analytical and computing approaches for dada analysis [13]. It has to be acknowledged that genomics and proteomics already have collected large amount of data that can be reused in metallomic and metalloproteomic studies to speed up advancement of these new disciplines. This is certainly a considerable advantage, but these data provide only a part of the complete picture – it has to be completed by additional numerous measurements

2. Metallothioneins

Metallothionein is one of the interesting proteins known as marker of heavy metal poisoning, with potential to be considered as a tumour diseases marker [14, 15]. Metallothioneins (MTs) are low-molecular mass intracellular proteins rich in cysteine, which are able to bind metals in their structure. Previously it was thought that MTs were involved only in storage, homeostasis and detoxification of metal ions, but based on recent findings, they are also involved in inhibition of apoptosis, immunomodulation, cell proliferation, regulation of transcription, and enzymes activation via zinc administration to proteins and via regulation of zinc ions concentration [16, 17]. MT genes are regulated in tissue- and isoform-specific manner by numerous factors, including general responsiveness to zinc and other dietary factors, inflammation and environmental stress. Hence changes in MT gene expression have been reported for many diseases [17]. The chemical reactivity of MTs makes the level of MT induction a factor to contend with in the efficacy of treatment with certain drugs, e.g. cancer chemotherapeutic agents, especially platinum drugs [18] and anthracyclines[19]. An area that also has received considerable attention is the value of MTs as biomarkers for zinc status [20], metal exposure [21] and the prognosis of certain cancers [22]. In addition, there is some evidence that increased heavy metal content and MTs in tumour tissues is connected to increased invasiveness and metastasizing of a tumour [16, 23-25]. Aside from understanding of the role of MTs and both essential and non-essential metals in carcinogenesis and tumour growth, the study of metal distribution within a tumour can answer many important questions about the growth of the tumour and its regulation [26, 27]. Understanding of this phenomenon can subsequently lead to our discovering of new approaches to tumour growth inhibition.

3. Suitable animal models

Suitable animal models for various cancers are indispensable to studying the above-mentioned aspects *in vivo*. Animal models have to be very similar to human cancers to bring real and clinically utilisable results. The MeLiM (Melanoma-bearing Libechov Minipig) strain of miniature pigs with hereditary malignant melanoma has been established in the Institute of Animal Physiology and Genetics (IAPG), the Academy of Sciences of the Czech Republic, v.v.i. in Libechov. Melanoma in this strain shows many histopathological [28-30], immunohistochemical [31], biochemical [32], and molecular biological similarities [33] to human melanoma. Another cancer model is an inoculated syngenic sarcoma in the Lewis rat [28-30, 34, 35]. The R5-28 tumour cell line was established from histologically verified sarcoma that appeared spontaneously in one female of the Lewis rat. These cells, when inoculated subcutaneously, develop in rapidly growing sarcomas. In the both models, animals with either progression or spontaneous regression of tumours appear.

4. Advanced nanomaterials for *in vivo* imaging

Advanced nanomaterials due to their easy penetration to tissues belong among modern methods for studying tumour progression [36]. Generally, 200 nm is considered as the upper limit for the size of nanoparticles, while the minimum diameter should be about 10 nm. Certainly, nanoparticle property requirements also depend on tumour characteristics including cancer type, stage of the disease, location in the body, tumour vascularisation and properties of the interstitial matrix or host species [37]. These requirements are summarized in a review by Adiseshaiah et al. [38]. Magnetic nanoparticles are well-established elements that offer controlled size, ability to be manipulated externally, and enhancement of contrast in magnetic resonance imaging (MRI). Iron-based nanoparticles in particular have been used as therapeutic agents with specific application as contrasting agents for MRI and magnetically targeted drug delivery to the tumour cell (Fig. 1).

Molecular imaging refers to the characterization and measurement of biological processes at the cellular and/or molecular level, its modalities include optical bioluminescence, optical fluorescence, ultrasound, X ray methods including CT, MRI, magnetic resonance spectroscopy (MRS), single-photon-emission computed tomography (SPECT) and positron emission tomography (PET) [39, 40]. In the last decade, molecular imaging, a subfield of func-

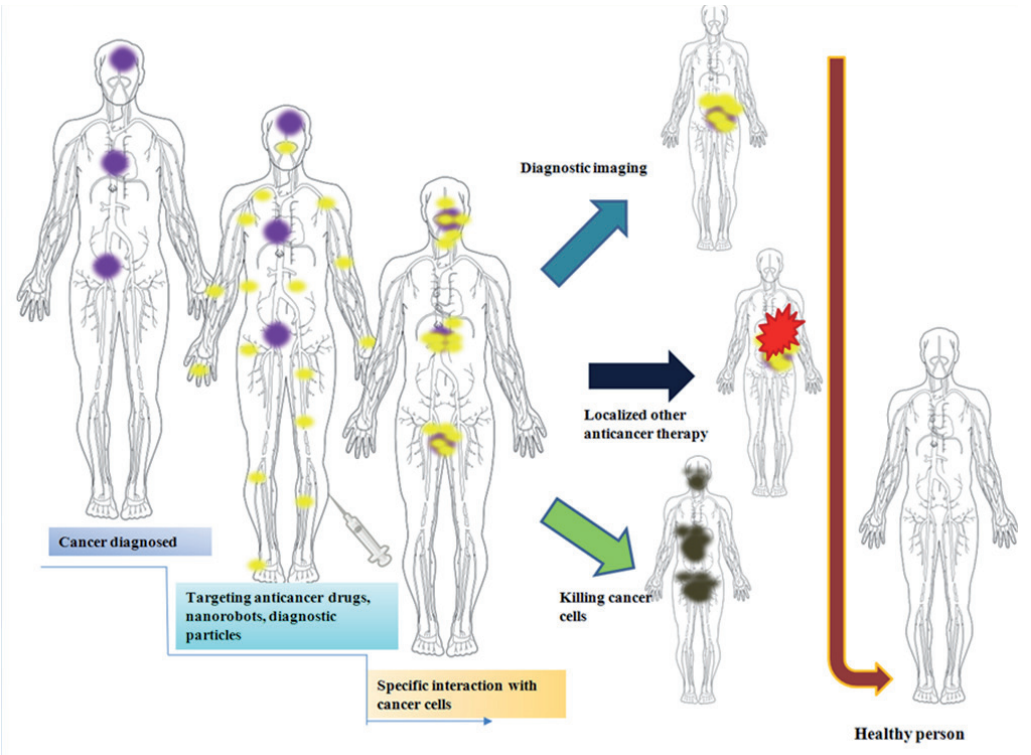

Figure 1: Advanced nanomedicine will be able to provide much earlier diagnosis and/or therapy of cancer. A patient who is suspected to have cancer will likely undergo an application (targeting anticancer drugs, nanorobots, diagnostic particles) into the bloodstream. Then, special particles will specifically interact with cancer cells. The effect obtained is possible to use for diagnostic imaging (sensor test chips containing thousands of nanowires, able to detect proteins and other biomarkers left behind by cancer cells, which could enable the detection and diagnosis of cancer in the early stages from a few drops of a patient's blood), localized other anticancer therapy (chemotherapy, brachytherapy) and or killing all cancer cells in human body. The final stage will be the curing of the patient

tional imaging, has become an essential tool in the arsenal of bio-imaging, understood as the range of all imaging technologies covering the full scale of biological and medical applications from molecule to patient [37, 41-44].

5. Conclusions

Using of omics approaches based on advanced materials is of great importance for the field of suggestions, construction and employment of diagnostic methods and treatment protocols. Those based on metals have numerous advantages including low cost and stability.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Lander, E.S., Initial impact of the sequencing of the human genome. Nature, 2011. 470(7333): p. 187-197.
2. Anderson, N.L. and N.G. Anderson, The human plasma proteome - History, character, and diagnostic prospects. Molecular & Cellular Proteomics, 2002. 1(11): p. 845-867.
3. Aebersold, R. and M. Mann, Mass spectrometry-based proteomics. Nature, 2003. 422(6928): p. 198-207.
4. Shi, W.X., et al., Metalloproteomics: High-throughput structural and functional annotation of proteins in structural genomics. Structure, 2005. 13(10): p. 1473-1486.
5. Maret, W., Zinc and the Zinc Proteome, in Metallomics and the Cell, L. Banci, Editor. 2013. p. 479-501.
6. Cerchiaro, G., T.M. Manieri, and F.R. Bertuchi, Analytical methods for copper, zinc and iron quantification in mammalian cells. Metallomics, 2013. 5(10): p. 1336-1345.
7. Easter, R.N., et al., Vascular metallomics: Copper in the vasculature. Vascular Medicine, 2010. 15(1): p. 61-69.
8. Gladyshev, V.N. and Y. Zhang, Comparative Genomics Analysis of the Metallomes, in Metallomics and the Cell, L. Banci, Editor. 2013. p. 529-580.
9. Shi, W. and M.R. Chance, Metallomics and metalloproteomics. Cell. Mol. Life Sci., 2008. 65(19): p. 3040-3048.
10. Becker, J.S., et al., Imaging of uranium on rat brain sections using laser ablation inductively coupled plasma mass spectrometry: a new tool for the study of critical substructures affined to heavy metals in tissues. Rapid Communications in Mass Spectrometry, 2008. 22(18): p. 2768-2772.
11. Zoriy, M.V., et al., Comparative imaging of P, S, Fe, Cu, Zn and C in thin sections of rat brain tumor as well as control tissues by laser ablation inductively coupled plasma mass spectrometry. Spectrochimica Acta Part B-Atomic Spectroscopy, 2008. 63(3): p. 375-382.
12. Shi, W.X., et al., Characterization of metalloproteins by high-throughput X-ray absorption spectroscopy. Genome Research, 2011. 21(6): p. 898-907.
13. Shi, W.X. and M.R. Chance, Metalloproteomics: forward and reverse approaches in metalloprotein structural and functional characterization. Current Opinion in Chemical Biology, 2011. 15(1): p. 144-148.
14. Eckschlager, T., et al., Metallothioneins and cancer. Curr. Protein Pept. Sci., 2009. 10(4): p. 360-375.
15. Adam, V., et al., Vertebrate metallothioneins as target molecules for analytical techniques. TRAC-Trends Anal. Chem., 2010. 29(5): p. 409-418.
16. Franklin, R.B. and L.C. Costello, Zinc as an anti-tumor agent in prostate cancer and in other cancers. Arch. Biochem. Biophys., 2007. 463(2): p. 211-217.
17. Li, Y. and W. Maret, Human metallothionein metallomics. J. Anal. At. Spectrom., 2007. 23(8): p. 1055-1062.
18. Boulikas, T. and M. Vougiouka, Cisplatin and platinum drugs at the molecular level (review). Oncology Reports, 2003. 10(6): p. 1663-1682.
19. Shuai, Y., et al., Global gene expression profiles of MT knockout and wild-type mice in the condition of doxorubicin-induced cardiomyopathy. Toxicology Letters, 2011. 200(1-2): p. 77-87.
20. Grider, A., L.B. Bailey, and R.J. Cousins, ERYTHROCYTE METALLOTHIONEIN AS AN INDEX OF ZINC STATUS IN HUMANS. Proceedings of the National Academy of Sciences of the United States of America, 1990. 87(4): p. 1259-1262.
21. Satarug, S., et al., Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. Archives of Environmental Health, 2002. 57(1): p. 69-77.
22. Theocharis, S.E., et al., Metallothionein expression in human neoplasia. Histopathology, 2004. 45(2): p. 103-118.
23. Il'yasova, D. and G.G. Schwartz, Cadmium and renal cancer. Toxicol. Appl. Pharmacol., 2005. 207(2): p. 179-186.
24. Bertin, G. and D. Averbeck, Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie, 2006. 88(11): p. 1549-1559.
25. Fabrik, I., et al., Employment of electrochemical techniques for metallothionein determination in tumor cell lines and patients with a tumor disease. Electroanalysis, 2008. 20(14): p. 1521-1532.
26. Vandenoord, J.J. and M. Deley, DISTRIBUTION OF METALLOTHIONEIN IN NORMAL AND PATHOLOGICAL HUMAN SKIN. Archives of Dermatological Research, 1994. 286(1): p. 62-68.
27. Raleigh, J.A., et al., Relationship of hypoxia to

metallothionein expression in murine tumors. International Journal of Radiation Oncology Biology Physics, 1998. 42(4): p. 727-730.
28. Fortyn, K., et al., Exceptional occurrence and extent of malignant melanoma in pig. Veterinarni Medicina, 1998. 43(3): p. 87-91.
29. Horak, V., et al., Hereditary melanoblastoma in miniature pigs and its successful therapy by devitalization technique. Cell. Mol. Biol., 1998. 45(7): p. 1119-1129.
30. Vincent-Naulleau, S., et al., Clinical and histopathological characterization of cutaneous melanomas in the melanoblastoma-bearing Libechov minipig model. Pigment Cell Research, 2004. 17(1): p. 24-35.
31. Geffrotin, C., et al., Opposite regulation of tenascin-C and tenascin-X in MeLiM swine heritable cutaneous malignant melanoma. Biochimica Et Biophysica Acta-General Subjects, 2000. 1524(2-3): p. 196-202.
32. Borovansky, J., et al., Biochemical characterization of a new melanoma model the minipig MeLiM strain. Melanoma Research, 2003. 13(6): p. 543-548.
33. Pohlreich, P., et al., Detection of neoplastic cells in blood of miniature pigs with hereditary melanoma. Veterinarni Medicina, 2001. 46(7-8): p. 199-204.
34. Morakova, A., et al., Immune characterization of the Lewis rats inoculated with K2 sarcoma cell line and newly derived R5-28 malignant cells. Folia Biologica, 2005. 51(6): p. 159-165.
35. Strnadel, J., et al., Multiplex analysis of cytokines involved in tumour growth and spontaneous regression in a rat sarcoma model. Folia Biologica, 2007. 53(6): p. 216-219.
36. Buzea, C., Pacheco, II, and K. Robbie, Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases, 2007. 2(4): p. MR17-MR71.
37. Drbohlavova, J., et al., Nanocarriers for anticancer drugs - New trends in nanomedicine. Curr. Drug Metab., 2013. 14(5): p. 547-564.
38. Adiseshaiah, P.P., J.B. Hall, and S.E. McNeil, Nanomaterial standards for efficacy and toxicity assessment. Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol., 2010. 2(1): p. 99-112.
39. Cai, W.B. and X.Y. Chen, Multimodality molecular imaging of tumor angiogenesis. Journal of Nuclear Medicine, 2008. 49: p. 113S-128S.
40. Chen, K. and X.Y. Chen, Design and Development of Molecular Imaging Probes. Current Topics in Medicinal Chemistry, 2010. 10(12): p. 1227-1236.
41. Ryvolova, M., et al., Modern Micro and Nanoparticle-Based Imaging Techniques. Sensors, 2012. 12(11): p. 14792-14820.
42. Sobrova, P., et al., Quantum dots and prion proteins: Is this a new challenge for neurodegenerative diseases imaging? Prion, 2013. 7(5): p. 349-358.
43. Kratka, Z., et al., Modern imaging techniques. J. Metallomics Nanotech., 2014. 1(1): p. 10-14.
44. Moulick, A., et al., Application of CdTe/ZnSe Quantum Dots in In Vitro Imaging of Chicken Tissue and Embryo. Photochem. Photobiol., 2015. 91(2): p. 417-423.

REVIEW

Nanotransporters for anticancer drugs, modifications, target molecules

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The nanoparticle based drugs, mostly in liposomes, are already approved for clinical use or under clinical investigation. Many attempts are made to improve nanoparticles sizes, shapes and surface modifications that lead to prolongation of drug circulation in blood stream and targeting to cancer cells. Thus small molecules like polyethylene glycol and targeting ligands like folic acid, peptides, antibodies, aptamers and nucleic acids are bound on the surface of nanoparticles with the aim to increase specific cell uptake. Very promising are multifunctional nanoparticles that combine both diagnostic as well as delivery role together.

Keywords: Liposomes; ferritin; carbon nanomaterials; magnetic nanoparticles

1. Introduction

The concept of using a particle measured in the nanoscale as carriers of drugs and vaccines appeared over three decades ago. Advances in nano medicine has evolved and has raised hopes for the implementation methods of striking antitumor therapy selectively in tumor mass, while reducing the risk of a wide range of side effects, which are encumbering modern pharmacology. Nanoparticles are attractive as drug delivery platforms because it is relatively easy to influence their properties and modify their features, so that they can be useful in creation of effective and precise medicine carriers. Meaningful are not only dimensions of the carrier enabling tissue penetration, but also their shape, and developed different functionalities of surface.

Current progress in the field of nanobiotechnology has led to the development of a new area of nanomedicine, associated with the application of nano biomaterials, both for diagnostic and therapeutic aims creating a new category of nano particles called theranostics. The main expectations and challenges in this regard relate to nano-magnetic properties, received bioengineering methods, with potential used in the transport of drugs, particularly anticancer drugs used in therapy determined using molecular targets. Unique physicochemical properties of magnetic nanoparticles promise hope for the development of modern cancer nanomedicine, acting, inter alia, technological breakthrough in the area of targeted drug delivery and gene therapy of cancer using magnetic hyperthermia, tissue engineering, marking the tumor cells and the molecular magnetic resonance imaging.

Nanotechnology in medicine and health care was initiated over forty years ago with deliverance of the first therapeutic and diagnostic agents in a safer and more efficient manner [1]. Convergention of diagnosis and therapy carried out through exploitation of nanopar-

ticles resulted with increasing number of theradiagnostics went out from research stage and being commercialized or having reached clinical stage.

2. Graphene, graphene oxide and carbon nanotubes

Graphene is a two-dimensional layer of carbon atoms with a thickness of single atom, of a hexagonal arrangement of atoms in a shape of honeycomb, and is often visualized as a homogeneous network of a large size. Graphene has many extraordinary properties such as an extremely high mechanical strength and flexibility, good thermal and electrical conductivity, is nearly transparent. Medical applications of graphene are built around its bacteriostatic and bactericidal properties, which are pledged with selected other features open extremely wide field of possibilities. Graphene oxide (GO) can be prepared by oxidation of graphite and in its structure are oxygen atoms in epoxy, hydroxy and carboxy groups. Biomedical applications relate to the whole family of graphene derivatives. Antibacterial properties of graphene and graphene oxide correspond to two phenomena. The first is a purely mechanical effect of destroying cell membranes by the sharp edges of graphene flakes and GO. The second is destructive to many strains of bacterial interaction of oxygen introduced into the cells on the surface of the GO [2].

Medical uses of carbon materials are intensively researched especially carbon nanotubes (CNTs). Carbon nanotubes (CNTs) take the form of a hollow cylinder with a rolled-up graphene built. CNTs are used as drug carriers enable the continuous and constant dosing of pathological cells. It may additionally comprise an antibody or specifically targeting the enzyme activity [3]. An example would be the use of MWNTs containing cisplatin, the use of which resulted in inhibition of tumor cell growth [4]. Similar results were obtained by combining doxorubicin with carbon nanotubes in breast cancer [5], or the attachment of carboplatin in the treatment of bladder cancer [6]. Nanomedicine is an extremely important area in which nanotubes can find a variety of uses, both in therapy and diagnosis. Efforts are also made in attempts to combine carbon nanotubes with active particles to create modern target drug transporters, which are particularly important for the pharmaceutical industry [7,8].

The use of carbon nanotubes as a carrier is possible thanks to the progress of research on the chemical modification [9]. Carbon nanotubes can be subjected to functionalization with different active particles responsible for target recognition (targeted therapy), imaging and treatment. In this way, a multifunctional system for transporting a drug can greatly improve the pharmacological profile [9,10]. Carbon nanotubes are also used as nanocontainers. Nanotubes filled with different chemical substances can be used in tumor therapy, diagnostic, and as contrast agents [10].

The first clinical tests are run on coating with nanotubes metal or metal oxide nanoparticles, and at the same time obtaining a surface ligands (folic acid or the corresponding glycoprotein) providing transport of nanoparticles to the tumor cells. Such particles after intravenous administration to achieve the target are subjected to an external magnetic field, which leads to a controlled heating of the metal particles and, consequently, destruction of the transformed cells. The results indicate that this method is more accurate than chemotherapy, carries also less risk of side effects and generates lower costs.

Carbon nanotubes also fulfill a role of gene carrier. Gene therapy is a promising treatment for cancer and genetic disorders. For the transport of viral genes there are special and non-viral carriers (e. g. liposomes, polymers, micro- and nanoparticles). The first ones carry a risk of side effects such as immune response, inflammation and oncogenesis. In contrast, no viral carriers, but more secure, do not always provide the appropriate level of gene expression. Therefore, researchers are making efforts to seek new, more efficient vehicles [7]. High molecular weight and a cationic nature of functionalized carbon nanotubes (f-CNT) allow electrostatic interaction with plasmid DNA. In order to test the ability of f-CNTs to form complexes with nucleic acids and their translocation were combined in various ratios f-CNT and the plasmid

DNA containing the gene of β-galactosidase marker. Obtained images demonstrated the presence of CNT-DNA complexes. F-CNT nanotubes were present in the form of beams, between which there plasmids in the form of annular clusters or super-coiled structures. The study of gene expression level of β-galactosidase showed penetration of these complexes to the cells. Furthermore, it was found that 5-10 times greater levels of gene expression for f-CNT complexes and DNA than for the same helix [11,12].

Carbon nanotubes have also been used as carriers of antigens. Connection of the external walls of the nanotubes with synthetically produced peptides, as for example. Epitopes of antigens create a system which can induce an immune response in a living body [13].

3. Magnetic nanoparticles

Magnetic nanoparticles (MNP) are made of an inorganic core, e.g. iron oxide, cobalt or nickel coated with substances being compatible with respect to the tissue, to which has been implemented [14]. One of the most important features is the MNP to superparamagnetism used in clinical diagnostic techniques. Introduction of MNP to the tested tissue bears effect of disorder of the local magnetic field in the tissue resulting in decrease of the relaxation time, the phenomenon used in magnetic resonance imaging [15,16]. Using MNP significantly improves possibilities of distinction between tumor and healthy tissue. Among the available contrast agents using nanoparticles there are superparamagnetic iron oxide, used for liver imaging called Combidex used in the diagnosis of metastases with a diameter of 5-10 nm in the lymph nodes [17]. In addition to tumor tissue imaging MNP are used to observe the cardiovascular system, especially in the detection of atherosclerotic plaques, and other diseases of the cardiovascular system. MNP can be further combined with organic dyes and fluorescent like rhodamine or fluorescein isothiocyanate (FITC), allowing to define the extent of tumor resection intraoperative study.

Other application of MNP is delivery of medicine to specific pathological tissue by utilizing the affinity of the ligands used in surface and magnetism which allows manipulating with pharmaceuticals through the external magnetic field. Biocompatibility, non-toxicity and high level of accumulation in tumors cause that magnetic nanoparticles are also used in intracellular hyperthermia. This therapy involves the use of MNP and alternating magnetic field to produce a significant amount of heat in tumor cells. Depending on the temperature and time of generated heat it causes death of the tumor cell or at least increase their sensitivity to radiotherapy or chemotherapy [18].

4. Liposomes and their modificationsce

Liposomes have been the most successful drug delivery carriers. Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Because of their biocompatibility, biodegradability, low toxicity and possibility to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased rate of both as an investigational system and commercially as a drug delivery system. The drugs inside the liposomes are protected from oxidation and degradation during circulation in bloodstream. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ, or system where the contents will be utilized [19]. Since the introduction of Doxil (a PEGylated liposomal doxorubicin), several products have been approved worldwide [20]. Liposomes are mostly used for the passive targeting having blood lifetime one or two days. It is required that the size of liposomes is less than 200 nm to facilitate fenestration through the leaky blood vessels around the tumour site. In general, the release of drug from liposome has to be slow enough not to let free drug to be removed quickly from the bloodstream.

Most of the liposomes for cancer treatment were approved on the basis of reduced side effects due to their passive targeting capabilities. Other liposomal anticancer products, such as DaunoXome and Myocet were primarily used to reduce toxicity in comparison to free

doxorubicin and not to sustained release of encapsulated drug [21].

Except of conventional liposomes there are modified ones called stealth liposomes [22]. Coating liposomes with PEG reduces the percentage of uptake by macrophages and leads to a prolonged circulation and, therefore, make available abundant time for these liposomes to leak from the circulation. Stealth liposomes are transporters with a membrane composed of phospholipid bilayer used to deliver drugs into a cell. A liposome can be made of naturally phospholipids with mixed lipid chains coated by polymers of PEG and colloidal in nature. Stealth liposomes are a new generation of compounds used for controlled drug release [23]. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil (Janssen Biotech, Inc., Horsham, USA) or Caelyx (Schering-Plough Corporation, Kenilworth, USA) for the treatment of solid tumors [24]. There are plenty of anticancer liposomes under clinical trials. To the group belong PEGylated lipoplatin, S-CKD602 [25] and NL CPT-11 containing cisplatin, CKD-602 (a camptothecin analogue) and irinotecan (CPT-11), respectively. From the trials it follows that lipoplatin is less toxic and of the same activity like cisplatin applicable for various cancers [26] [27] [28].

To improve efficacy of cancer treatment by liposomes it is necessary to modify either liposomes or their surface by peptides, RNA or antibodies which also serve for targeted delivery to specific cancer tissue. For example, dual-ligand liposomal delivery system for targeting the delivery of paclitaxel to lung cancer was prepared. The specific ligand peptide HAIYPRH (T7) and the cationic cell-penetrating peptide TAT were connected with phospholipid via a polyethylene glycol (PEG) spacer to prepare the dual-ligand liposomes (T7/TAT-LP-PTX) [29]. Active targeting molecules displayed better cell selectivity but were shadowed by the poor tumor penetration effect. Cell penetrating peptides could increase the uptake of the carriers but were limited by their non-specificity. Dual-ligand system may possess a synergistic effect and create a more ideal drug delivery effect. Thus, liposome system modified with RGD, TAT and cleavable PEG was designed [30]. Liposomal drug delivery system conjugated with cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk) was developed to improve therapeutic efficacy in a mice model of bone metastasis from prostate cancer [31].

5. Natural nanotransporters – ferritin

Ferritin belongs to proteins with cage like structure, which can be used to bind molecules in its cavity. Maxiferritins are formed from 24 subunits 12 nm in diameter with 8 nm cavities with MW = 480 kDa and miniferritins formed from 12 subunits 8 nm in diameter with 5 nm diameter cavities of MW = 240 kDa [32]. Mostly maxiferritins are used, especially horse spleen ferritin for its commercial availability. Ferritin wide occurrence as well as its ability to reversibly store and release iron ions to the living cells has attracted the interest of researchers worldwide.

It was found that the cavity can be utilized for storage of other ions and molecules and can be utilized for synthesis of nanoparticles with defined size. Apart from interior cavity, the surface of apoferritin can be modified. This offers further possibility of delivering encapsulated drug to a target cell in more effective way and minimalizing thus side effects particularly toxicity to nontarget organs by drugs.

Apoferritin was employed to encapsulate anticancer drugs cisplatin and carboplatin [33] [34]. It is well known, that clinical application of platinum-based anticancer drugs is largely limited by severe general toxicity and drug resistance. Drug delivery systems with tumor-targeting potential are highly desired for improving the efficacy and applicability of these drugs. The delivery of platinum drugs cisplatin, carboplatin and oxaliplatin by encapsulating each of them in the cavity of apoferritin was studied. The encapsulation was achieved through reassembly route at pH 2.0 and 7.4, respectively, in saturated drug solution. Carboplatin and oxaliplatin complexes in apoferritin exhibit a marginal cytotoxicity towards this cell line under similar concentrations [34]. A novel antibody-drug

conjugate was synthesized incorporating ferritin cisplatin nanoparticles [35]. An average of three molecules of monoclonal antibody (mAb) Ep1 to the human melanoma-specific antigen CSPG4 were conjugated to a single ferritin cage encapsulating about 50 cisplatin molecules. The flow cytometry demonstrated specific binding to a CSPG4(+) melanoma cell line, but not to a CSPG4(-) breast carcinoma cell line. As compared to the cisplatin-containing ferritin nanoparticle alone, which inhibited thymidine incorporation more efficiently in breast carcinoma than melanoma cells, the mAb-derivatized nanoparticle had a 25-fold preference for the latter. Anticancer activity was also studied on a methylene blue-encapsulated apoferritin complex. The complex shows cytotoxic effects on MCF-7 human breast adenocarcinoma cells when irradiated at the appropriate wavelength [36].

Ferritin can be genetically modified to present a peptide sequence on the surface [37]. Thus Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys (RGD4C)-modified ferritin can efficiently target tumors through RGD-integrin interaction. It was shown that after being precomplexed with Cu(II), doxorubicin can be loaded onto RGD modified apoferritin with high efficiency. These doxorubicin-loaded ferritin nanocages showed on U87MG subcutaneous tumor models a longer circulation half-life, higher tumor uptake, better tumor growth inhibition, and less cardiotoxicity than free doxorubicin [37]. Ma-Ham et al. [38] studied daunomycin, an anthracycline antibiotic drug, that is used for specific types of cancer treatment such as acute myeloid leukemia and acute lymphocytic leukemia, encapsulated within apoferritin cage.

The apoferritin-doxorubicin complex has been formed by reassembly of the apoferritin sphere in the presence of doxorubicin [39]. The doxorubicin encapsulation was carried out using direct and step-wise change of pH of the solution from 2.5 to 7.4. It was found that up to 28 molecules of doxorubicin can be capsulated per apoferritin protein and no significant drug leakage occurs during the first two days.

Magnetic particle mediated transport in combination with nanomaterial based drug carrier has a great potential for targeted cancer therapy. Doxorubicin encapsulated into the apoferritin was conjugated with magnetic particles and investigated by capillary electrophoresis with laser-induced fluorescence detection (CE--LIF). This combination of magnetic particles and drug encapsulated in apoferritin can be potentially used for magnetic resonance imaging, thermotherapy and chemotherapy [40]. Apoferritin and liposome encapsulated forms of doxorubicin were prepared and their toxicity were compared with doxorubicin alone and Myocet on prostate cell lines [41]. Three different prostatic cell lines PNT1A, 22Rv1, and LNCaP were chosen. The toxicity was compared using the MTT assay, real-time cell impedance-based cell growth method (RTCA), and flow cytometry. The efficiency of doxorubicin entrapment was 56% in apoferritin cages and 42% in the liposome carrier. Apodox IC50 was determined as follows: 603.1, 1344.2, and 931.2 nM for PNT1A, 22Rv1, and LNCaP, respectively.

6. Conclusions

The main objective of research in recent years is to provide a multifunctional nanoparticles and nanomaterials whose properties can be controlled in the body through the local environment and external factors, e. g. external magnetic field. Many pharmaceutical companies have their own research programs aimed at the introduction of new products based on nanoparticles and nanomaterials and improve current pharmaceuticals. Nanosubstances appeared to be commercially and started to be used widely in the diagnosis or treatment of cancer, among others. Intensive nanotechnology research in the future will lead to extend the functions of nanoparticles in nanodiagnostics, in nanopharmacology and in many new medical applications.

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References

1. Strebhardt, K.; Ullrich, A. Paul ehrlich's magic

bullet concept: 100 years of progress. Nat. Rev. Cancer 2008, 8, 473-480.
2. Geim, A.K.; Novoselov, K.S. The rise of graphene. Nat. Mater. 2007, 6, 183-191.
3. Terranova, M.L.; Sessa, V.; Rossi, M. The world of carbon nanotubes: An overview of cvd growth methodologies. Chem. Vapor Depos. 2006, 12, 315-325.
4. Tripisciano, C.; Kraemer, K.; Taylor, A.; Borowiak-Palen, E. Single-wall carbon nanotubes based anticancer drug delivery system. Chem. Phys. Lett. 2009, 478, 200-205.
5. Ali-Boucetta, H.; Al-Jamal, K.T.; Kostarelos, K. Multi-walled carbon nanotube-doxorubicin supramolecular complexes for cancer therapeutics. Crc Press-Taylor & Francis Group: Boca Raton, 2008; p 16-18.
6. Hampel, S.; Kunze, D.; Haase, D.; Kramer, K.; Rauschenbach, M.; Ritscbel, M.; Leonbardt, A.; Thomas, J.; Oswald, S.; Hoffmann, V., et al. Carbon nanotubes filled with a chemotherapeutic agent: A nanocarrier mediates inhibition of tumor cell growth. Nanomedicine 2008, 3, 175-182.
7. Bianco, A.; Kostarelos, K.; Prato, M. Applications of carbon nanotubes in drug delivery. Curr. Opin. Chem. Biol. 2005, 9, 674-679.
8. Prato, M.; Kostarelos, K.; Bianco, A. Functionalized carbon nanotubes in drug design and discovery. Accounts Chem. Res. 2008, 41, 60-68.
9. Cui, D.X.; Tian, F.R.; Ozkan, C.S.; Wang, M.; Gao, H.J. Effect of single wall carbon nanotubes on human hek293 cells. Toxicol. Lett. 2005, 155, 73-85.
10. Pastorin, G.; Wu, W.; Wieckowski, S.; Briand, J.P.; Kostarelos, K.; Prato, M.; Bianco, A. Double functionalisation of carbon nanotubes for multimodal drug delivery. Chem. Commun. 2006, 1182-1184.
11. Pantarotto, D.; Singh, R.; McCarthy, D.; Erhardt, M.; Briand, J.P.; Prato, M.; Kostarelos, K.; Bianco, A. Functionalized carbon nanotubes for plasmid DNA gene delivery. Angew. Chem.-Int. Edit. 2004, 43, 5242-5246.
12. Singh, R.; Pantarotto, D.; McCarthy, D.; Chaloin, O.; Hoebeke, J.; Partidos, C.D.; Briand, J.P.; Prato, M.; Bianco, A.; Kostarelos, K. Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: Toward the construction of nanotube-based gene delivery vectors. J. Am. Chem. Soc. 2005, 127, 4388-4396.
13. Ito, A.; Shinkai, M.; Honda, H.; Kobayashi, T. Medical application of functionalized magnetic nanoparticles. J. Biosci. Bioeng. 2005, 100, 1-11.
14. Mnneil, S.E. Nanotechnology for the biologist. J. Leukoc. Biol. 2005, 78, 585-594.
15. Jain, T.K.; Richey, J.; Strand, M.; Leslie-Pelecky, D.L.; Flask, C.A.; Labhasetwar, V. Magnetic nanoparticles with dual functional properties: Drug delivery and magnetic resonance imaging. Biomaterials 2008, 29, 4012-4021.
16. Sun, C.; Lee, J.S.H.; Zhang, M.Q. Magnetic nanoparticles in mr imaging and drug delivery. Adv. Drug Deliv. Rev. 2008, 60, 1252-1265.
17. Harisinghani, M.G.; Barentsz, J.; Hahn, P.F.; Deserno, W.M.; Tabatabaei, S.; van de Kaa, C.H.; de la Rosette, J.; Weissleder, R. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. N. Engl. J. Med. 2003, 348, 2491-U2495.
18. Maier-Hauff, K.; Rothe, R.; Scholz, R.; Gneveckow, U.; Wust, P.; Thiesen, B.; Feussner, A.; von Deimling, A.; Waldoefner, N.; Felix, R., et al. Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: Results of a feasibility study on patients with glioblastoma multiforme. J. Neuro-Oncol. 2007, 81, 53-60.
19. Hofheinz, R.D.; Gnad-Vogt, S.U.; Beyer, U.; Hochhaus, A. Liposomal encapsulated anti-cancer drugs. Anti-Cancer Drugs 2005, 16, 691-707.
20. Allen, T.M.; Cullis, P.R. Liposomal drug delivery systems: From concept to clinical applications. Adv. Drug Deliv. Rev. 2013, 65, 36-48.
21. Huynh, N.T.; Passirani, C.; Saulnier, P.; Benoit, J.P. Lipid nanocapsules: A new platform for nanomedicine. Int. J. Pharm. 2009, 379, 201-209.
22. Sharma, A.; Sharma, U.S. Liposomes in drug delivery: Progress and limitations. Int. J. Pharm. 1997, 154, 123-140.
23. Gabizon, A.A. Stealth liposomes and tumor targeting: One step further in the quest for the magic bullet. Clin. Cancer Res. 2001, 7, 223-225.
24. Romberg, B.; Hennink, W.E.; Storm, G. Sheddable coatings for long-circulating nanoparticles. Pharm. Res. 2008, 25, 55-71.
25. Zamboni, W.C.; Ramalingam, S.; Friedland, D.M.; Edwards, R.P.; Stoller, R.G.; Strychor, S.; Maruca, L.; Zamboni, B.A.; Belani, C.P.; Ramanathan, R.K. Phase i and pharmacokinetic study of pegylated liposomal ckd-602 in patients with advanced malignancies. Clin. Cancer Res. 2009, 15, 1466-1472.
26. Boulikas, T. Clinical overview on lipoplatin (tm): A successful liposomal formulation of cisplatin. Expert Opin. Investig. Drugs 2009, 18, 1197-1218.
27. Canta, A.; Chiorazzi, A.; Carozzi, V.; Meregalli, C.; Oggioni, N.; Sala, B.; Crippa, L.; Avezza, F.; Forestieri, D.; Rotella, G., et al. In vivo comparative study of the cytotoxicity of a liposomal formulation of cisplatin (lipoplatin (tm)). Cancer Chemother. Pharmacol. 2011, 68, 1001-1008.
28. Casagrande, N.; De Paoli, M.; Celegato, M.; Borghese, C.; Mongiat, M.; Colombatti, A.; Aldinucci, D. Preclinical evaluation of a new liposomal formulation of cisplatin, lipoplatin, to treat cisplatin-resistant cervical cancer. Gynecol. Oncol. 2013, 131, 744-752.
29. Wang, R.H.; Cao, H.M.; Tian, Z.J.; Jin, B.; Wang, Q.; Ma, H.; Wu, J. Efficacy of dual-functional liposomes containing paclitaxel for treatment of lung cancer. Oncol. Rep. 2015, 33, 783-791.
30. Mei, L.; Fu, L.; Shi, K.R.; Zhang, Q.Y.; Liu, Y.Y.; Tang, J.; Gao, H.L.; Zhang, Z.R.; He, Q. Increased tumor targeted delivery using a multistage liposome system functionalized with rgd, tat and cleavable peg. Int. J. Pharm. 2014, 468, 26-38.
31. Wang, F.F.; Chen, L.; Zhang, R.; Chen, Z.P.; Zhu, L. Rgd peptide conjugated liposomal drug delivery system for enhance therapeutic efficacy in treating bone metastasis from prostate cancer. J. Control. Release 2014, 196, 222-233.
32. Theil, E.C.; Liu, X.F.S.; Tosha, T. Gated pores in

the ferritin protein nanocage. Inorg. Chim. Acta 2008, 361, 868-874.
33. Yang, Z.; Wang, X.Y.; Diao, H.J.; Zhang, J.F.; Li, H.Y.; Sun, H.Z.; Guo, Z.J. Encapsulation of platinum anticancer drugs by apoferritin. Chem. Commun. 2007, 3453-3455.
34. Xing, R.M.; Wang, X.Y.; Zhang, C.L.; Zhang, Y.M.; Wang, Q.; Yang, Z.; Guo, Z.J. Characterization and cellular uptake of platinum anticancer drugs encapsulated in apoferritin. J. Inorg. Biochem. 2009, 103, 1039-1044.
35. Falvo, E.; Tremante, E.; Fraioli, R.; Leonetti, C.; Zamparelli, C.; Boffi, A.; Morea, V.; Ceci, P.; Giacomini, P. Antibody-drug conjugates: Targeting melanoma with cisplatin encapsulated in protein-cage nanoparticles based on human ferritin. Nanoscale 2013, 5, 12278-12285.
36. Yan, F.; Zhang, Y.; Yuan, H.K.; Gregas, M.K.; Vo-Dinh, T. Apoferritin protein cages: A novel drug nanocarrier for photodynamic therapy. Chem. Commun. 2008, 4579-4581.
37. Zhen, Z.P.; Tang, W.; Chen, H.M.; Lin, X.; Todd, T.; Wang, G.; Cowger, T.; Chen, X.Y.; Xie, J. Rgd-modified apoferritin nanoparticles for efficient drug delivery to tumors. ACS Nano 2013, 7, 4830-4837.
38. Ma-Ham, A.H.; Wu, H.; Wang, J.; Kang, X.H.; Zhang, Y.Y.; Lin, Y.H. Apoferritin-based nanomedicine platform for drug delivery: Equilibrium binding study of daunomycin with DNA. J. Mater. Chem. 2011, 21, 8700-8708.
39. Kilic, M.A.; Ozlu, E.; Calis, S. A novel protein-based anticancer drug encapsulating nanosphere: Apoferritin-doxorubicin complex. J. Biomed. Nanotechnol. 2012, 8, 508-514.
40. Blazkova, I.; Nguyen, H.V.; Dostalova, S.; Kopel, P.; Stanisavljevic, M.; Vaculovicova, M.; Stiborova, M.; Eckschlager, T.; Kizek, R.; Adam, V. Apoferritin modified magnetic particles as doxorubicin carriers for anticancer drug delivery. Int. J. Mol. Sci. 2013, 14, 13391-13402.
41. Gumulec, J.; Fojtu, M.; Raudenska, M.; Sztalmachova, M.; Skotakova, A.; Vlachova, J.; Skalickova, S.; Nejdl, L.; Kopel, P.; Knopfova, L., et al. Modulation of induced cytotoxicity of doxorubicin by using apoferritin and liposomal cages. Int. J. Mol. Sci. 2014, 15, 22960-22977.

Gene therapy in management of lung cancer

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Lung cancer is the most common cause of death, related to malignant disease. Both major types - small cell and non-small cell lung cancer (SCLC and NSCLC, respectively) possess properties, which significantly affect the survival rates of onco-patients. During the treatment, there occur several issues, including development of chemoresistance, fast metastatic expansion or inoperability. Although, there are only few examples of how gene therapy can be helpful in combating the cancer, its potential is undisputed. Presented study describes and discusses the current status and possibilities of gene therapy, including the viral and non-viral vectors, antisense strategy of gene silencing and the latest technology using CRISPR-Cas9 in treatment of lung cancer.

Keywords: Antisense; CRISPR-Cas9; Chemoresistance; NSCLC; SCLC; Vectors

1. Introduction

Lung cancer is the most common cause of cancer-related deaths worldwide. Despite advances in diagnostics and therapeutics of lung cancer, a 5-year survival rate is still reaching only about 15% [1]. This disease largely affects the socioeconomic statuses of patients and their families, as well as the society. Clinical and molecular evidence has proven that lung cancer is a heterogeneous disease, which demonstrates significant implications in diagnosis [2] and treatment [3]. An increasing number of clinical trials have emphasized targeted and personalized treatments that specifically benefit patients diagnosed by using observed gene expression profiles. The term lung cancer usually refers to tumors that originate from the lining cells of the respiratory tract (epithelial cells) [4]. Based on differences in biological characteristics, lung cancer is classified into two types, namely non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts approximately 85% of lung cancer cases [5]. Platinum-based chemotherapy is prescribed as the standard first-line therapy in patients with advanced NSCLC. However, resistance to platinum-based drugs reduces the survival rate which, as a result, has not improved to anything like the extent seen in other cancers [6]. Advances in the understanding of molecular genetics in NSCLC have led to the identification of key genetic aberrations in NSCLC. These genetic aberrations occur in oncogenes that encode signalling proteins that are crucial for cellular proliferation and survival [7]. Genetic profiling has identified driver mutations in over 60% of lung adenocarcinomas, with 9–14% being new targetable oncogenes such as HER2, BRAF, PIK3CA, and RET [8]. SCLC accounts 10–15% of all lung cancer cases and represents the most aggressive subset of lung cancer. Treatment of SCLC has changed minimally over the last few decades. Patients continue to receive non-targeted, chemotherapy regimens consisting of etoposide plus platinum agents, often combined with irradiation. Although SCLC patients respond well to this first-line treatment, relapse is virtually inevitable and resultant tumours are resistant to further therapy [9]. Based on above

mentioned information, in lung carcinoma management, the personalized medicine is at the forefront, with the goal to cure patients with a predicted modality to be efficacious based on the molecular characteristics of the tumor. Such approach can offer increase of survival rates of oncopatients, significant reduction of tumor chemoresistence and decrease of a number of potential relapses. Gene therapy can be a powerful weapon to combat lung cancer and to elevate the therapeutic successes.

2. Gene therapy

Gene therapy can be defined as the transfer of genetic material into a cell for therapeutic purposes [10]. Gene therapy was conceptualized in 1972, by authors who urged caution before commencing human gene therapy studies. The first gene therapy experiment approved by the US Food and Drug Administration occurred in 1990, when Ashanti DeSilva was treated for adenosine deaminase deficiency with severe combined immunodeficiency. By January 2014, some 2000 clinical trials had been conducted or approved and no one was FDA approved for clinical utilization in management of lung cancer. Despite these facts, gene therapy still offers huge potential, which nevertheless encounters numerous obstacles. The aim of present study is to summarize the approaches employable for gene therapy of lung cancer and to highlight their possible advantages and disadvantages. Given the large size and the negative charge of these macromolecules, their delivery is typically mediated by carriers or vectors as is discussed below.

2.1 Gene delivery

Gene therapy relies on the principle of introducing exogenous DNA into malignant cells causing them to die. Since lung cancer can be a highly disseminated malignancy, the gene therapeutic agent must be administered systemically, obligating a high level of targeting of tumor tissue and the use of delivery vehicles designed for systemic circulation of the therapeutic DNA [11]. The possible target cells include not only the tumor cells and the immune cells but also surrounding normal tissue. Gene therapy of tumor cells could result in correction of their abnormal growth and re-establishment of apoptosis, or in increased drug or radio-sensitivity of the tumor cells. Gene modification of tumor cells could also enhance their immunogenicity [12]. Various physical and biological methods are available to deliver genes into target cells. Which delivery method one chooses depends generally on the local, regional, or systemic route of administration chosen or needed to reach the tumor [13]. Physical methods, such as calcium phosphate precipitation, electroporation, direct microinjection, and gene gun, may be suitable for introducing naked DNA into established cell lines *in vitro*, but are generally of low efficiency and are often impractical for *in vivo* applications. Nevertheless, gene delivery to lung tumors by aerosolization of adenoviral vectors incorporated into calcium phosphate precipitates resulted in much greater expression in tumors than in normal lung tissue [14]. Biological vectors - genetically modified, replication-defective viruses are effective by exploiting their natural tropism for mammalian cells and biological life cycles to achieve gene transfer and gene expression. Retroviral vectors can infect a variety of cell types and have the advantage of being able to integrate into the target cell genome. However, because retroviral-mediated transduction might result in permanent integration of the foreign gene into the target cell, the promoter used to drive the transcription of the foreign gene must be carefully selected. Moreover, one of the biggest challenges facing viral vectors in gene delivery is the host immune response. Cell-mediated responses to viral vectors have been documented, but this response may be dependent on the route of administration [15] and vector serotype [16]. For instance, a potent immune response to adeno-associated virus-ovalbumin was observed when vector was administered intraperitoneally, intravenously, or subcutaneously but not when administered intramuscularly [17]. A replication-deficient type 5 adenovirus (Adp53) in which the viral E1 gene was replaced with a wt p53 expression cassette driven by cytomegalovirus promoter has been evaluated in two Phase I clinical trials in NSCLC patients [18,19]. From these trials, it

was found that disease stabilization lasted up to 14 months, and >50% tumor regression was seen in two patients. The observed response rate in this heavily pretreated group of patients with progressive disease was encouraging.

Non-viral gene delivery strategies are generally regarded as safer and less immunogenic alternatives to viral vectors. Nonviral methods of gene delivery have recently expanded and several effective nanomaterials exist including lipid-based [20], polymeric [21], and inorganic nanoparticles [22], some of which have reached clinical trials. Modern non-virals vector are characterized by a high level of transfection efficiency, low production costs, they are easy to prepare and enable a flexible size of DNA to be transported. As was shown by Ji and colleagues, restoration of wt-FUS function in 3p21.3--deficient NSCLC cells significantly inhibits tumor cell growth by induction of apoptosis and alteration of cell cycle kinetics [23]. A Phase I clinical trial is underway to evaluate delivery of the FUS1 gene using repeated intravenous injection of liposomal particles composed of DOTAP and cholesterol.

2.2 Antisense oligonucleotides (ASOs)

The field of oligonucleotides (ODNs) has been developed in a sophisticated manner and novel pharmaceutics appear to emerge based on ODNs. Evidently, several approaches using ODNs could be done at the gene therapy level using the ODN genes. This will reduce cost, toxicity and ensure the presence of steady state levels of a therapeutic ODN in the cytoplasm or nucleus [24]. Because nucleases that cleave the phosphodiester linkage in DNA are expressed in almost every cell, unmodified DNA molecules are generally degraded before they reach their targets. Therefore, antisense drug candidate molecules are generally modified during the drug discovery phase of their development [25]. Amongst the most succesfull nucleic acid backbone modifications belong phosphorothioates, morpholinos, locked nucleic acids (LNAs), ribozymes or peptide nucleic acids (PNAs) [26-28]. In lung cancer management, the rational drug design has resulted in agents directed against a number of important cellular targets, including the mRNA of bcl-2, cyclin D1, protein kinase (PK) C-alpha, PKA-I, H-ras, c-raf, R1 and R2 subunit of ribonucleotide reductase, and transforming growth factor beta2 [29,30]. Saini and Klein demonstrated that NSCLC and mesothelioma cells can be significantly weakened by using CD1 ASOs within the meaning of cell proliferation and CD1 de novo synthesis [30]. Other suitable targets are cyclooxygenases. For instance cyclooxygenase 2 (COX-2), overexpressed in several tumor entities, can be efficiently blocked, as was shown by Windhovel and coworkers [31]. Interestingly, they tested twelve phosphorothioates and a range of activities was reached on protein, RNA and growth level. This points at importance of selection of a duplexing site on mRNA sequence, which subsequently affects the translation into protein.

2.3 CRISPR-Cas9

CRISPR-Cas9 is a versatile genome editing technology for studying the functions of genetic elements. The bacterial type II clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) systems have recently shown great potential for RNA--guided genome editing, including multiplexing genome engineering, homologous recombination, gene targeting and regulation of transcription [32]. Using both, viral (lentivirus) and non-viral (nanoparticles) mediated delivery of guide RNA, Platt et al. simultaneously modeled the dynamics of KRAS, p53, and LKB1, the top three significantly mutated genes in lung adenocarcinoma [33]. CRISPR-Cas9 technology arises many discussions in the scientific community, particularly due to its exceptional properties, thus it can be expected that CRISPR-Cas9 can significantly affect future of modern medicine, not only within the meaning of malignant diseases.

3. Conclusion

Although limitations still exist to the widespread application of gene therapy, the strategy has been shown to be applicable in several clinical situations. Currently, the most fundamental issue is development of efficient and non-immunogenic vectors for delivery of

nucleic acids, which are easily cleaved by endonucleases. Another option is further development of nucleic acids backbone modifications, which provide higher stability and are nucleases-proof. Gene therapy offers a number of future possibilities from simple blocking of expression through mRNA duplexing to highly effective replacement and deletions of entire dysfunctional genes. Hence, gene therapy can be considered as powerful future-weapon to combat lung cancer.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Siegel, R.; Ma, J.M.; Zou, Z.H.; Jemal, A., Cancer Statistics, 2014. Ca-a Cancer Journal for Clinicians 2014, 64, 9-29.
2. Cha, N.; Lv, M.; Zhao, Y.J.; Yang, D.; Wang, E.H.; Wu, G.P., Diagnostic utility of VEGF mRNA and SP1 mRNA expression in bronchial cells of patients with lung cancer. Respirology 2014, 19, 544-548.
3. Xu, R.; Shang, C.; Zhao, J.G.; Han, Y.; Liu, J.; Chen, K.B.; Shi, W.J., Knockdown of response gene to complement 32 (RGC32) induces apoptosis and inhibits cell growth, migration, and invasion in human lung cancer cells. Molecular and Cellular Biochemistry 2014, 394, 109-118.
4. D'Adda, T.; Pelosi, G.; Lagrasta, C.; Azzoni, C.; Bottarelli, L.; Pizzi, S.; Troisi, I.; Rindi, G.; Bordi, C., Genetic alterations in combined neuroendocrine neoplasms of the lung. Modern Pathology 2008, 21, 414-422.
5. Reck, M.; Heigener, D.F.; Mok, T.; Soria, J.C.; Rabe, K.F., Management of non-small-cell lung cancer: recent developments. Lancet 2013, 382, 709-719.
6. Haghgoo, S.M.; Allameh, A.; Mortaz, E.; Garssen, J.; Folkerts, G.; Barnes, P.J.; Adcock, I.M., Pharmacogenomics and targeted therapy of cancer: Focusing on non-small cell lung cancer. European Journal of Pharmacology 2015, 754, 88-97.
7. Kumarakulasinghe, N.B.; van Zanwijk, N.; Soo, R.A., Molecular targeted therapy in the treatment of advanced stage non-small cell lung cancer (NSCLC). Respirology 2015, 20, 370-378.
8. Oxnard, G.R.; Binder, A.; Janne, P.A., New Targetable Oncogenes in Non-Small-Cell Lung Cancer. Journal of Clinical Oncology 2013, 31, 1097-1104.
9. Byers, L.A.; Rudin, C.M., Small cell lung cancer: Where do we go from here? Cancer 2015, 121, 664-672.
10. Zarogoulidis, P.; Chatzaki, E.; Hohenforst-Schmidt, W.; Goldberg, E.P.; Galaktidou, G.; Kontakiotis, T.; Karamanos, N.; Zarogoulidis, K., Management of malignant pleural effusion by suicide gene therapy in advanced stage lung cancer: a case series and literature review. Cancer Gene Therapy 2012, 19, 593-600.
11. Christensen, C.L.; Zandi, R.; Gjetting, T.; Cramer, F.; Poulsen, H.S., Specifically targeted gene therapy for small-cell lung cancer. Expert Review of Anticancer Therapy 2009, 9, 437-452.
12. Toloza, E.M.; Morse, M.A.; Lyerly, H.K., Gene therapy for lung cancer. Journal of Cellular Biochemistry 2006, 99, 1-23.
13. Woll, P.J.; Hart, I.R., Gene-therapy for lung-cancer. Annals of Oncology 1995, 6, 73-77.
14. Lee, H.Y.; Sub, Y.A.; Lee, J.I.; Hassan, K.A.; Mao, L.; Force, T.; Gilbert, B.E.; Jacks, T.; Kurie, J.A., Inhibition of oncogenic K-ras signaling by aerosolized gene delivery in a mouse model of human lung cancer. Clinical Cancer Research 2002, 8, 2970-2975.
15. Brockstedt, D.G.; Podsakoff, G.M.; Fong, L.; Kurtzman, G.; Mueller-Ruchholtz, W.; Engleman, E.G., Induction of immunity to antigens expressed by recombinant adeno-associated virus depends on the route of administration. Clinical Immunology 1999, 92, 67-75.
16. Wang, L.L.; Figueredo, J.; Calcedo, R.; Lin, J.P.; Wilson, J.M., Cross-presentation of adeno-associated virus serotype 2 capsids activates cytotoxic T cells but does not render hepatocytes effective cytolytic targets. Human Gene Therapy 2007, 18, 185-194.
17. Zhang, C.; Cortez, N.G.; Berns, K.I., Characterization of a bipartite recombinant adeno-associated viral vector for site integration. Human Gene Therapy 2007, 18, 787-797.
18. Roth, J.A.; Swisher, S.G.; Merritt, J.A.; Lawrence, D.D.; Kemp, B.L.; Carrasco, C.H.; El-Naggar, A.K.; Fossella, F.V.; Glisson, B.S.; Hong, W.K., et al., Gene therapy for non-small cell lung cancer: A preliminary report of a phase I trial of adenoviral p53 gene replacement. Seminars in Oncology 1998, 25, 33-37.
19. Swisher, S.G.; Roth, J.A.; Nemunaitis, J.; Lawrence, D.D.; Kemp, B.L.; Carrasco, C.H.; Connors, D.G.; El-Naggar, A.K.; Fossella, F.; Glisson, B.S., et al., Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. Journal of the National Cancer Institute 1999, 91, 763-771.
20. Akinc, A.; Zumbuehl, A.; Goldberg, M.; Leshchiner, E.S.; Busini, V.; Hossain, N.; Bacallado, S.A.; Nguyen, D.N.; Fuller, J.; Alvarez, R., et al., A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nature Biotechnology 2008, 26, 561-569.
21. van der Aa, L.J.; Vader, P.; Storm, G.; Schiffelers, R.M.; Engbersen, J.F.J., Optimization of poly(amido amine)s as vectors for siRNA delivery. Journal of Controlled Release 2011, 150, 177-186.
22. Kakizawa, Y.; Furukawa, S.; Ishii, A.; Kataoka, K., Organic-inorganic hybrid-nanocarrier of siRNA constructing through the self-assembly of calcium

phosphate and PEG-based block aniomer. Journal of Controlled Release 2006, 111, 368-370.
23. Ji, L.; Roth, J.A., Tumor suppressor FUS1 signaling pathway. Journal of Thoracic Oncology 2008, 3, 327-330.
24. Berg, R.W.; Ferguson, P.J.; Vincent, M.D.; Koropatnick, D.J., A „combination oligonucleotide" antisense strategy to downregulate thymidylate synthase and decrease tumor cell growth and drug resistance. Cancer Gene Therapy 2003, 10, 278-286.
25. Bennett, C.F.; Swayze, E.E., RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform. Annual Review of Pharmacology and Toxicology 2010, 50, 259-293.
26. Heasman, J., Morpholino oligos: Making sense of antisense? Developmental Biology 2002, 243, 209-214.
27. Johnston, W.K.; Unrau, P.J.; Lawrence, M.S.; Glasner, M.E.; Bartel, D.P., RNA-catalyzed RNA polymerization: Accurate and general RNA-templated primer extension. Science 2001, 292, 1319-1325.
28. Owczarzy, R.; You, Y.; Groth, C.L.; Tataurov, A.V., Stability and Mismatch Discrimination of Locked Nucleic Acid-DNA Duplexes. Biochemistry 2011, 50, 9352-9367.
29. Davies, A.M.; Gandara, D.R.; Lara, P.N., Jr.; Mack, P.C.; Lau, D.H.M.; Gumerlock, P.H., Antisense oligonucleotides in the treatment of non-small-cell lung cancer. Clinical lung cancer 2003, 4 Suppl 2, S68-73.
30. Saini, S.S.; Klein, M.A., Targeting Cyclin D1 in Non-small Cell Lung Cancer and Mesothelioma Cells by Antisense Oligonucleotides. Anticancer Research 2011, 31, 3683-3690.
31. Windhovel, U.; Haasper, C.; Duchrow, M.; Bruch, H.P.; Broll, R., Effect of cyclooxygenase-2 antisense oligodeoxyribonucleotides in a-549 lung cancer cells. Anticancer Research 2004, 24, 3789-3794.
32. Cong, L.; Ran, F.A.; Cox, D.; Lin, S.L.; Barretto, R.; Habib, N.; Hsu, P.D.; Wu, X.B.; Jiang, W.Y.; Marraffini, L.A., et al., Multiplex Genome Engineering Using CRISPR/Cas Systems. Science 2013, 339, 819-823.
33. Platt, R.J.; Chen, S.D.; Zhou, Y.; Yim, M.J.; Swiech, L.; Kempton, H.R.; Dahlman, J.E.; Parnas, O.; Eisenhaure, T.M.; Jovanovic, M., et al., CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Cell 2014, 159, 440-455.

REVIEW

Separation methods in cancer research, real samples

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Cancer belongs to the most terrifying diseases. Therefore, diagnostic and therapeutic tools are desperately searched. To detect the disease at it early stage, reliable biomarkers have to be employed, however to identify these molecules and find their connection to the type and stage of the disease is a challenging task. To meet this goal, extremely powerful methods have to be used. Among such methods, mass spectrometry occupies the leading position. However, even such powerful technique needs to be coupled with some kind of separation, which simplifies the complex biological sample prior to the analysis.

Keywords: cancer; separation science; capillary electrophoresis; chromatography; mass spectrometry

1. Introduction

Cancer is one of the biggest threats of current population because it is the most common cause of death [1]. In total, about 200 cancer forms have been recognized, however lung cancer, prostate cancer, breast cancer, and colon cancer cause more than 50% of all deaths [1]. The high mortality is caused not only by effectivity of the treatment, which may be in numerous cases quite low, but also by the diagnostics of the disease in the late stage [2]. Therefore, it is obvious that an early diagnosis of cancer is playing a key role in the successful treatment and biomarkers are sought in genome, proteome and metabolome. The main disadvantage connected to modern gene arrays, which are nowadays one of the most commonly used tools for genomic analysis is the fact that they require mRNA as starting material. Therefore, even though one can survey the expression of all genes in cells or tissues, the sample preparation is a drawback.

In proteomic research there are in general two strategies used. One is the 'bottom-up' approach and the other is the 'top-down' method. The bottom-up technique is based on a tryptic digestion of the protein mixture followed by separation of the fragments and analysis by MS, which can be done either on-line by electrospray ionization of off-line by matrix-assisted laser desorption and ionization. The disadvantage of this type of analysis is that limited information about the intact protein is provided. On the other hand, during the "top-down" method, the intact proteins and protein complexes are separated first and then analyzed by MS. Therefore, this approach can be used to obtain molecular information about the intact protein and may be advantageous for the detection of proteins' post-translational modifications [3].

The main problem is the high complexity and wide dynamic range of peptides in body fluids (i.e. blood serum, saliva, sputum, cerebrospinal fluid, etc.). Too many peptides are present spread over a range of concentrations exceeding 1012 in the case of serum. To overcome this obstacle, it is crucial to simplify the complexity and remove the major components of the matrix, which are usually masking the signal

of components of interest by some kind of separation method, which offers high resolution and can cope with a wide dynamic range of peptide concentrations [4].

In both, top-down and bottom-up methods, a powerful separation technique is required to obtain the information of interest. Despite the development of improved analytical tools for analysis of clinical and biochemical samples, gel electrophoresis is still the gold standard used up-to-date. Its two-dimensional variant – 2D gel electrophoresis – is taking advantage of combination with isoelectric focusing. The 2D sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) approach to protein profiling are accessible and economical method that enables the detection of hundreds of proteins on a single gel plate. Resolution has been enhanced by the introduction of immobilized pH gradients, which enable the analyst to tailor the pH gradient for maximum resolution using ultrazoom gels with a narrow pH gradient range. With modern 2D-PAGE, it is not unusual to resolve two proteins that differ in pI by 0.001 U [5]. Another limitation of 2D-PAGE include the labor-intensive and time-consuming nature of the technique, poor reproducibility, limited dynamic range of detection (undetectable for the mass range <20,000 m/z), and under--representation of certain classes of proteins, so that truly comprehensive analysis is impossible. Furthermore, it cannot provide accurate Mr information and it still remains difficult to interface 2-DE directly to MS analysis [4,6].

2. Chromatography

Another separation, which has proven to be applicable for cancer analysis is gas chromatography (GC). GC-MS is reported to have many advantages in the area of clinical samples. It is applicable for variety of samples which include not only serum [7] and/or plasma [8] but also breath gas from the lung cancer patients [9,10]. GC-MS is characterized by minimal sample requirements, rapid analysis and reduced use of expensive labeled substrates. [1]. Besides GC--MS also liquid chromatography is a powerful method providing the valuable information in cancer research. Liquid chromatographic techniques are faster, quantitative, easier to automate, and couple more readily to mass spectrometry than two-dimensional gel electrophoresis [11]. The main area of recent advances in LC-MS technologies is the improvement linked to capillary LC instrumentation that provides improved peak capacities and dynamic range of detection needed to analyze biological samples [12]. The enhancements have been achieved mainly by use of very high pressure, very small porous particles, columns with smaller inner diameter, nanoelectrospray interfaces, and relatively long columns and long gradients for separations. By using smaller inner diameter columns, the sensitivity of the system continues to increase inversely as the mobile phase flow rates drop to as low as 20 nl/min, demonstrating the advantages of ESI-MS analyses at very low liquid flow rates [12]. Separation columns for capillary LC are usually prepared by packing of conventional beads into a fused silica capillary with internal diameter of 10-300 µm. However, the large void volume between the packed particles and the slow diffusional mass transfer of solutes are the major factors limiting the separation efficiency of porous packing materials, especially for proteins and peptides having low diffusivities [11]. Ultra-performance liquid chromatography (UPLC) operates with sub-2 µm chromatographic particles and a fluid system capable of operating at pressures in the 6000–15000 psi range, providing an increased chromatographic selectivity compared with conventional HPLC, which uses larger particles. Increased sensitivity of UPLC compared to conventional HPLC is caused by reduced peak width and therefore by increased S/N ratio [13]. To improve the stationary-phase properties with enhanced mass-transfer abilities even more, the monolithic stationary phases were introduced in which the separation medium consists of a continuous rod of a rigid, porous polymer that has no interstitial volume but only internal porosity consisting of micro and macropores. All of the mobile phase is forced to flow through the channels of the porous separation medium, resulting in enhanced mass transport and improved chromatographic efficiency [11].

3. Capillary electrophoresis (CE)

Compared to chromatography, CE separation is separating the sample based on a completely different principle – ion mobility in the electric field. Therefore, it is providing different sort of information, which may be complementary to the one provided by chromatographic analysis. Moreover, there is a whole group of the electrophoretic methods including capillary zone electrophoresis, capillary gel electrophoresis, capillary micellar chromatography, capillary electrochromatography, capillary isoelectric focusing and/or capillary isotachophoresis. This variability enables the researcher to select the method providing the required information of interest. Moreover, the potential of miniaturization broadens the application potential even further [2].

Whereas HPLC is more widely used in clinical analysis, CE offers several advantages over HPLC by these including stable constant flow, no gradient, which results in changes in the ideal ionization parameters; fairly robust and inexpensive capillaries; compatible with essentially all buffers and analytes; fast separation; and high resolution [4]. These advantages are especially beneficial when analyzing a large number of heterogeneous samples that contain interfering compounds, such as lipids, precipitates, etc.

From the technical point of view, main advantages are the robustness and the possibility to renew the capillary inner surface by hydroxide. On the other hand, the small volume and therefore the small loading capacity is the limitation. Whereas ml quantities can be loaded onto an LC column, a CE can be filled with a maximum of several hundreds of nanolitres. Although pH stacking and other types of sample preconcentration methods can be used very effectively, a maximum of 30–50% of the total capillary volume can be filled with sample to maintain the separation power, which corresponds to 0.5–2 µl when using 50 or 75 µm internal diameter capillaries with length of 80 –100 cm. The major limitation is due to the dynamic range of the mass spectrometer (4 orders of magnitude at best), and that more abundant peptides will obscure minor signals [14].

4. Data analysis

Commonly, data are presented in two ways: (A) comparison of peaks from normal and cancerous samples. The peaks are usually not identified and the evaluation is just on the fingerprinting level which is sufficient for medical diagnostics, (B) Selected peaks are at least partly identified by the use of tandem mass spectrometry. The important challenges for proteomic studies are to use the variety of protein databases and algorithms applied for compound identification. One difficulty for peptide identification is to verify the accuracy of the match. The scoring algorithm is usually used to rank the candidates and assign only the highest scoring of all. Another difficulty is that some post-translational modifications of amino acids residues influence the masses the peptides, for example, phosphoserine usually exhibits a neutral loss of 98 Da (H_3PO_4) because of the elimination of phosphoric acid during MS/MS of phosphopeptides, whereas acetylated lysine exhibits a diagnostic ion at m/z 126.0913 during MS/MS of peptides [15].

5. Conclusions

From the literature review can be clearly concluded that even though MS is extremely powerful, without application of the separation methods, the analysis of bodily fluids is insurmountable problem. Especially, the biomarker research requires high separation power, which is mainly provided by CE-MS and its variants. Even though CE-MS is not so commonly used in clinical practice, the undoubtable advantages provided by this hyphenated technique predispose it for a wide application.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kaluzna-Czaplinska, J.; Jozwik, J. Current applications of chromatographic methods for diagnosis and identification of potential biomarkers in cancer. Trac-Trends Anal. Chem. 2014, 56, 1-12.
2. Yang, Z.Y.; Sweedler, J.V. Application of capillary electrophoresis for the early diagnosis of cancer. Anal. Bioanal. Chem. 2014, 406, 4013-4031.
3. Gao, M.X.; Qi, D.W.; Zhang, P.; Deng, C.H.; Zhang, X.M. Development of multidimensional liquid chromatography and application in proteomic analysis. Expert Rev. Proteomics 2010, 7, 665-678.
4. Kolch, W.; Neususs, C.; Peizing, M.; Mischak, H. Capillary electrophoresis - mass spectrometry as a powerful tool in clinical diagnosis and biomarker discovery. Mass Spectrom. Rev. 2005, 24, 959-977.
5. Issaq, H.J.; Veenstra, T.D. Two-dimensional polyacrylamide gel electrophoresis (2d-page): Advances and perspectives. Biotechniques 2008, 44, 697-700.
6. Yoo, C.; Pal, M.; Miller, F.R.; Barder, T.J.; Huber, C.; Lubman, D.M. Toward high sequence coverage of proteins in human breast cancer cells using on-line monolith-based hplc-esi-tof ms compared to ce ms. Electrophoresis 2006, 27, 2126-2138.
7. Chen, Y.R.; Ma, Z.H.; Li, A.Y.; Li, H.W.; Wang, B.; Zhong, J.; Min, L.S.; Dai, L.C. Metabolomic profiling of human serum in lung cancer patients using liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry and gas chromatography/mass spectrometry. J. Cancer Res. Clin. Oncol. 2015, 141, 705-718.
8. Musharraf, S.G.; Mazhar, S.; Choudhary, M.I.; Rizi, N.; Atta ur, R. Plasma metabolite profiling and chemometric analyses of lung cancer along with three controls through gas chromatography-mass spectrometry. Sci Rep 2015, 5.
9. Ma, W.; Gao, P.; Fan, J.; Hashi, Y.; Chen, Z.L. Determination of breath gas composition of lung cancer patients using gas chromatography/mass spectrometry with monolithic material sorptive extraction. Biomed. Chromatogr. 2015, 29, 961-965.
10. Ma, H.Y.; Li, X.; Chen, J.M.; Wang, H.J.; Cheng, T.T.; Chen, K.; Xu, S.F. Analysis of human breath samples of lung cancer patients and healthy controls with solid-phase microextraction (spme) and flow-modulated comprehensive two-dimensional gas chromatography (gc x gc). Anal. Methods 2014, 6, 6841-6849.
11. Premstaller, A.; Oberacher, H.; Walcher, W.; Timperio, A.M.; Zolla, L.; Chervet, J.P.; Cavusoglu, N.; van Dorsselaer, A.; Huber, C.G. High-performance liquid chromatography-electrospray ionization mass spectrometry using monolithic capillary columns for proteomic studies. Anal. Chem. 2001, 73, 2390-2396.
12. Qian, W.J.; Jacobs, J.M.; Liu, T.; Camp, D.G.; Smith, R.D. Advances and challenges in liquid chromatography-mass spectrometry-based proteomics profiling for clinical applications. Mol. Cell. Proteomics 2006, 5, 1727-1744.
13. Zhao, Y.Y.; Cheng, X.L.; Vaziri, N.D.; Liu, S.M.; Lin, R.C. Uplc-based metabonomic applications for discovering biomarkers of diseases in clinical chemistry. Clin. Biochem. 2014, 47, 16-26.
14. Dakna, M.; He, Z.Y.; Yu, W.C.; Mischak, H.; Kolch, W. Technical, bioinformatical and statistical aspects of liquid chromatography-mass spectrometry (lc-ms) and capillary electrophoresis-mass spectrometry (ce-ms) based clinical proteomics: A critical assessment. J. Chromatogr. B 2009, 877, 1250-1258.
15. Zhang, X.W.; Wei, D.; Yap, Y.; Li, L.; Guo, S.Y.; Chen, F. Mass spectrometry-based „omics“ technologies in cancer diagnostics. Mass Spectrom. Rev. 2007, 26, 403-431.

REVIEW

Fluorescence imaging for specific analysis of cancer cells

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Cancer is a serious disease that causes 25% of deaths in the developed countries. Significant impact on the cancer patients survival has early detection of this disease, therefore great attention is paid to its imaging. Fluorescence imaging represents powerful imaging method for the cell detection. For the successful detection of tumour cells, the specific targeting of fluorescence probes to the tumour tissue has a key role. Interesting materials enabling the imaging of tumour cells are fluorescence nanoparticles. For the accurate imaging, the NPs should be conjugated with targeting ligands and/or constructed as off-on probes.

Keywords: cancer cell; fluorescence; imaging; quantum dots

1. Introduction

Cancer is life-threatening disease, which causes nearly 7 million deaths every year worldwide and represents around 1 trillion dollars economic loss [1,2]. Cancer presents 25% of death caused in the developed countries [3]. But due to an early diagnosis and effective treatment, the mortality caused by this disease decreases and survival time increases [4]. The risk of dying from cancer decreased by 20% between 1991 and 2010 [3]. The cancer is diagnosed in the every third woman and every second man in the United States [5]. Cancer mortality in Czech Republic is about 20% [6,7]. Death rates continue to decline for all 4 major cancer sites (lung, colorectal, breast, and prostate), with lung cancer accounting for almost 40% of the total decline in men and breast cancer accounting for 34% of the total decline in women [4]. The most common causes of death are cancers of the lung, followed by colorectal, breast and stomach [7]. Early detection of cancer can significantly impact survival of cancer patients, so the regular screening is highly recommended [8-10].

2. Fluorescence imaging of cells

Optical methods are relatively cheap non-ionizing techniques based on the specific optical properties and are due to their variability, flexibility, specificity and sensitivity an important tool for non-invasive and objective diagnosis with still improving resolution [11-14]. Fluorescence imaging is the optical method based on the usage of fluorophores, the compounds, that can emit light after absorption of the appropriate wavelength light [15]. Fluorescence detection of cancer cells has the potential to be used in early cancer diagnosis [16]. The fluorescence of the dyes for imaging should be in the „tissue optical window" spectral ranges between 650 and 900 nm [17] or in the infrared spectrum [18]. The long wavelength (far-red to NIR) fluorescent probes are advantageous for *in vivo* bioimaging because of minimum photo-damage to biological samples, deep tissue penetration, and minimum interference from background autofluorescence by biomolecules in the living systems. Therefore great attention is paid in the development of new long wavelength fluorescent probes [19].

3. Fluorescent probes

As smart molecular probes, organic compounds such as fluorescein or rhodamin are widely studied [20,21] and as an universal, genetically encoded fluorescent label, green fluorescent protein, is applied [22]. Promising imaging tools represent inorganic compounds such as nanoparticles (NPs) [23,24]. The NPs accumulate in tumour cells by themselves, or they have to be targeted to the cancer cell surface molecules [25]. Interesting NPs with very good fluorescence properties are QDs [26-28]. They have broad absorption spectrum and a narrow emission spectrum and are photostable [29,30]. Fluorescence properties of QDs can be successfully exploit for imaging of tumour cells as well as in situ investigations of tumour tissue [31,32]. Because of the content of heavy metals, the toxicity of QDs is discussed [33]. CdTe QDs conjugated with antibodies were successfully used for the cancer cells detection [34,35].

Technical developments in fluorescence imaging have enabled recent translation into investigational human studies [36]. NIR fluorescence imaging is in some applications already used in the clinic. Indocyanine green (ICG) is NIR fluorophore used primarily in angiography [37]. ICG enable noninvasive imaging of the lymphatic vasculature and discrimination of malignant from benign breast lesions [36]. ICG is useful for the hepatocellular carcinoma detection [38]. ICG encapsulated within poly-(allylamine) hydrochloride chains cross-linked ionically with sodium phosphate (ICG-NCs) and functionalized with anti-HER2 can be used as theranostic agent for optical imaging and also for the photodestruction of ovarian cancers invitro [39].

Tryptophan is investigated as the key native marker in cells to determine the level of metastasis competence in breast cell lines. The ratio of fluorescence intensity is associated with aggressiveness of the cancer cells [40]. The higher content of tryptophan was detected in the advanced metastatic cancer cell lines against the moderate metastatic and non aggressive cell lines [41].

Cancer cells can reduce of innocuous silver salts and spontaneously generate silver nanoclusters (NCs), which has great fluorescence intensity. The formation of silver NCs also results in drastic reduction of tumour size and/or complete remission of the tumour [42]. Glutathione (GSH) can significantly and selectively enhance the fluorescence intensity of gold NCs. Gold NCs without GSH can selectively image the cancer cells. The liver cancer cells have much higher content of GSH, than other cell types. Therefore gold NCs enabled differentiation of cancer cells from normal ones [43].

For the targeting imaging, gold NCs conjugated with folic acid (FA) were utilized [44]. Folate receptor (FR) is overexpressed by a number of epithelial-derived tumors and minimally expressed in normal tissues. As FA is a high-affinity ligand to FR, and not produced endogenously, the FA-conjugated probes are specific for cancer cells imaging [45]. Folate-functionalized NPs are efficient imaging probes [46]. The overexpression of FR in 90–95% of epithelial ovarian cancers enabled the investigation of intraoperative tumor-specific fluorescence imaging in ovarian cancers surgery using an FR–targeted fluorescent probe [47]. Fluorescence imaging of cancer cells utilized in guidance surgery improves the tumour successful removal [48].

Low cytotoxic fluorescence probe comprises hydroxy-6-methyl-naphthalene-2-carbaldehyde was used for the detection of tyrosine kinase in cancer cells [49]. Lanthanum hexaboride LaB_6 NPs coated with a carbon-doped silica (C-SiO_2) shell to introduce a fluorescent property and improve stability and biocompatibility enabled fluorescent imaging and NIR-triggered photothermal therapy of cancer cells [50]. 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy--D-glucose (2-NBDG) was used as a tracer for detection of hypermetabolic circulating tumor cells (CTC) [51].

4. Turn off-on probes

For the sensitive and selective imaging without false positive results the „off-on“ fluorescence probes have been constructed. Polyethyleneimine-coated CdS/ZnS QDs (PEI-CdS/ZnS QDs) with the electrostaticly absorbed FA were turned off. In the presence of FR, the FA was desorbed and the fluorescence signal of QDs

was detected [45]. The fluorescence-quenching platform based on the biomineralized HAp (hydroxyapatite) has been also internalized for cancer cell detection [52]. Protein LAPTM4B is characteristic for a large number of cancer cells. Small peptide IHGHHIISVG (AP2H) is a targeting ligand for the LAPTM4B and therefore could be used for targeting the fluorescence probe to the cancer cells. Huang et al. constructed the turn-on probe consisted of the peptide and tetraphenylethylene (TPE), an aggregation-induced emission (AIE) fluorophore [21]. Another turn-on probe was designed via hydrogen-bond interaction between FA and carbon dots (CDs) with the passivating agent - poly(acrylate sodium) (PAAS). This probe could be used in the fluorescence-assisted surgical resection and real-time monitoring of the cells [16]. Acid phosphatase (ACP) is an important biomarker and indicator of prostate cancer. An „off-on" probe for ACP detection consisted of a near-infrared mercaptopropionic acid (MPA)--capped CuInS2 QDs [53].

5. Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) with centromeric probes is used to detect chromosomal instability (CIN), which is observed in many cancers. The chromosome doubling could influence the tumours heterogeneity [54]. A multi-gene fluorescence *in situ* hybridization (M-FISH) was used to investigate gene copy number aberrations (CNAs) and it was found, that the gene copy number aberrations (CNAs) of cell cycle-regulated genes can be significant for prognosis in young breast cancer patients [55]. FISH is a useful technique for ALK gene rearrangement analysis and specification of the type of gene irregularities. ALK gene examination could be applied in histological and also in cytological samples. FISH was utilized in the FISH of samples from NSCLC patients [56].

6. Conclusions

Cancer is one of the most serious diseases worldwide. The most important for its succesfull treatment is early detection of tumour in the body. Methods for the fast and easy tumour detection are widely studied. Interesting application represents fluorescence imaging. There are many possibilities of the fluorescence imaging of tumour cells. The most important for the use of fluorescence imaging in human medicine will be the necessity of the construction of the highly sensitive fluorescence detectors to detect the fluorophores deeply in the body.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Fan, Z.; Senapati, D.; Singh, A.K.; Ray, P.C. Theranostic magnetic core-plasmonic shell star shape nanoparticle for the isolation of targeted rare tumor cells from whole blood, fluorescence imaging, and photothermal destruction of cancer. Mol. Pharm. 2013, 10, 857-866.
2. Sultana, S.; Khan, M.R.; Kumar, M.; Kumar, S.; Ali, M. Nanoparticles-mediated drug delivery approaches for cancer targeting: A review. J. Drug Target. 2013, 21, 107-125.
3. Siegel, R.; Ma, J.M.; Zou, Z.H.; Jemal, A. Cancer statistics, 2014. CA-Cancer J. Clin. 2014, 64, 9-29.
4. Siegel, R.; Naishadham, D.; Jemal, A. Cancer statistics, 2012. CA-Cancer J. Clin. 2012, 62, 10-29.
5. Siegel, R.; DeSantis, C.; Virgo, K.; Stein, K.; Mariotto, A.; Smith, T.; Cooper, D.; Gansler, T.; Lerro, C.; Fedewa, S., et al. Cancer treatment and survivorship statistics, 2012. CA-Cancer J. Clin. 2012, 62, 220-241.
6. Zaloudik, J. Onkologicky vyzkum v ceske republice v souvislostech. Klinicka onkologie 2007, 405-407.
7. Ferlay, J.; Steliarova-Foucher, E.; Lortet-Tieulent, J.; Rosso, S.; Coebergh, J.W.W.; Comber, H.; Forman, D.; Bray, F. Cancer incidence and mortality patterns in europe: Estimates for 40 countries in 2012. European Journal of Cancer 2013, 49, 1374-1403.
8. Carter, H.B.; Albertsen, P.C.; Barry, M.J.; Etzioni, R.; Freedland, S.J.; Greene, K.L.; Holmberg, L.; Kantoff, P.; Konety, B.R.; Murad, M.H., et al. Early detection of prostate cancer: Aua guideline. Journal of Urology 2013, 190, 419-426.
9. Esserman, L.J.; Thompson, I.M.; Reid, B. Overdiagnosis and overtreatment in cancer an opportunity for improvement. JAMA-J. Am. Med. Assoc. 2013, 310, 797-798.
10. Horilova, J.; Cunderlikova, B.; Marcek Chorvatova, A. Time- and spectrally resolved characteristics of flavin fluorescence in u87mg cancer cells in culture. Journal of biomedical optics 2015, 20, 51017.
11. Blazkova, I.; Vaculovicova, M.; Eckschlager, T.; Stiborova, M.; Trnkova, L.; Adam, V.; Kizek, R.

Study of fluorescence of doxorubicin in muscle tissue using highly sensitive fluorescence sensing. Chem. Sensors 2014, 4, 1-6.
12. Balas, C. Review of biomedical optical imaging-a powerful, non-invasive, non-ionizing technology for improving in vivo diagnosis. Meas. Sci. Technol. 2009, 20, 1-12.
13. Stemmer, N.; Mehnert, J.; Steinbrink, J.; Wunder, A. Noninvasive fluorescence imaging in animal models of stroke. Curr. Med. Chem. 2012, 19, 4786-4793.
14. Wouters, F.S.; Verveer, P.J.; Bastiaens, P.I.H. Imaging biochemistry inside cells. Trends Cell Biol. 2001, 11, 203-211.
15. Frangioni, J.V. In vivo near-infrared fluorescence imaging. Curr. Opin. Chem. Biol. 2003, 7, 626-634.
16. Liu, Q.L.; Xu, S.H.; Niu, C.X.; Li, M.F.; He, D.C.; Lu, Z.L.; Ma, L.; Na, N.; Huang, F.; Jiang, H., et al. Distinguish cancer cells based on targeting turn-on fluorescence imaging by folate functionalized green emitting carbon dots. Biosens. Bioelectron. 2015, 64, 119-125.
17. Pu, Y.; Tang, R.; Xue, J.P.; Wang, W.B.; Xu, B.G.; Achilefu, S. Synthesis of dye conjugates to visualize the cancer cells using fluorescence microscopy. Appl. Optics 2014, 53, 2345-2351.
18. Rosenblum, L.T.; Kosaka, N.; Mitsunaga, M.; Choyke, P.L.; Kobayashi, H. Optimizing quantitative in vivo fluorescence imaging with near-infrared quantum dots. Contrast Media & Molecular Imaging 2011, 6, 148-152.
19. Yuan, L.; Lin, W.; Zheng, K.; He, L.; Huang, W. Far-red to near infrared analyte-responsive fluorescent probes based on organic fluorophore platforms for fluorescence imaging. Chemical Society Reviews 2013, 42, 622-661.
20. Chatterjee, K.; Zhang, J.Q.; Honbo, N.; Karliner, J.S. Doxorubicin cardiomyopathy. Cardiology 2010, 115, 155-162.
21. Huang, Y.Y.; Hu, F.; Zhao, R.; Zhang, G.X.; Yang, H.; Zhang, D.Q. Tetraphenylethylene conjugated with a specific peptide as a fluorescence turn-on bioprobe for the highly specific detection and tracing of tumor markers in live cancer cells. Chem.-Eur. J. 2014, 20, 158-164.
22. Chudakov, D.M.; Matz, M.V.; Lukyanov, S.; Lukyanov, K.A. Fluorescent proteins and their applications in imaging living cells and tissues. Physiological Reviews 2010, 90, 1103-1163.
23. Andreadou, I.; Sigala, F.; Iliodromitis, E.K.; Papaefthimiou, M.; Sigalas, C.; Aligiannis, N.; Savvari, P.; Gorgoulis, V.; Papalabros, E.; Kremastinos, D.T. Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. Journal of Molecular and Cellular Cardiology 2007, 42, 549-558.
24. Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol. Rev. 2004, 56, 185-229.
25. Key, J.; Leary, J.F. Nanoparticles for multimodal in vivo imaging in nanomedicine. International Journal of Nanomedicine 2014, 9, 711-726.
26. Zhu, Y.; Hong, H.; Xu, Z.P.; Li, Z.; Cai, W. Quantum dot-based nanoprobes for in vivo targeted imaging. Curr. Mol. Med. 2013, 13, 1549-1567.
27. Wang, Y.C.; Hu, R.; Lin, G.M.; Roy, I.; Yong, K.T. Functionalized quantum dots for biosensing and bioimaging and concerns on toxicity. ACS Appl. Mater. Interfaces 2013, 5, 2786-2799.
28. Moulick, A.; Blazkova, I.; Milosavljevic, V.; Fohlerova, Z.; Hubalek, J.; Kopel, P.; Vaculovicova, M.; Adam, V.; Kizek, R. Application of cdte/znse quantum dots in in vitro imaging of chicken tissue and embryo. Photochem. Photobiol. 2015, 91, 417-423.
29. Hong, L.; Wang, Z.; Yuan, L.; Tan, J.H.; Wang, L.X.; Qu, G.B.; Zhang, D.Q.; Lin, R.H.; Liu, S.J. Subcellular distribution of cdse quantum dots (qds) in breast cancer cells. J. Nanosci. Nanotechnol. 2012, 12, 365-367.
30. Gallo, J.; Garcia, I.; Genicio, N.; Penades, S. Cdte-based qds: Preparation, cytotoxicity, and tumor cell death by targeting transferrin receptor. Part. Part. Syst. Charact. 2014, 31, 126-133.
31. Hu, W.Q.; Fang, M.; Zhao, H.L.; Yan, S.G.; Yuan, J.P.; Peng, C.W.; Yang, G.F.; Li, Y.; Li, J.D. Tumor invasion unit in gastric cancer revealed by qds-based in situ molecular imaging and multispectral analysis. Biomaterials 2014, 35, 4125-4132.
32. Moulick, A.; Blazkova, I.; Milosavljevic, V.; Fohlerova, Z.; Hubalek, J.; Kopel, P.; Vaculovicova, M.; Adam, V.; Kizek, R. Application of cdte/znse quantum dots in in vitro imaging of chicken tissue and embryo. Photochemistry and Photobiology 2014.
33. Lin, G.M.; Ding, Z.C.; Hu, R.; Wang, X.M.; Chen, Q.; Zhu, X.M.; Liu, K.; Liang, J.H.; Lu, F.Q.; Lei, D.L., et al. Cytotoxicity and immune response of cdse/zns quantum dots towards a murine macrophage cell line. RSC Adv. 2014, 4, 5792-5797.
34. Wang, Z.Y.; Zong, S.F.; Chen, H.; Wang, C.L.; Xu, S.H.; Cui, Y.P. Sers-fluorescence joint spectral encoded magnetic nanoprobes for multiplex cancer cell separation. Adv. Healthc. Mater. 2014, 3, 1889-1897.
35. Chang, B.X.; Yang, X.J.; Wang, F.; Wang, Y.S.; Yang, R.; Zhang, N.; Wang, B.Q. Water soluble fluorescence quantum dot probe labeling liver cancer cells. J. Mater. Sci.-Mater. Med. 2013, 24, 2505-2508.
36. Sevick-Muraca, E.M. Translation of near-infrared fluorescence imaging technologies: Emerging clinical applications. Annual Review of Medicine, Vol 63 2012, 63, 217-231.
37. Mielke, D.; Malinova, V.; Rohde, V. Comparison of intraoperative microscopic and endoscopic icg angiography in aneurysm surgery. Neurosurgery 2014, 10, 418-425.
38. Morita, Y.; Sakaguchi, T.; Unno, N.; Shibasaki, Y.; Suzuki, A.; Fukumoto, K.; Inaba, K.; Baba, S.; Takehara, Y.; Suzuki, S., et al. Detection of hepatocellular carcinomas with near-infrared fluorescence imaging using indocyanine green: Its usefulness and limitation. International Journal of Clinical Oncology 2013, 18, 232-241.
39. Bahmani, B.; Guerrero, Y.; Vullev, V.; Singh, S.P.; Kundra, V.; Anvari, B. Icg-loaded polymeric nanocapsules functionalized with

anti-her2 for targeted fluorescence imaging and photodestruction of ovarian cancer cells. Reporters, Markers, Dyes, Nanoparticles, and Molecular Probes for Biomedical Applications V 2013, 8596.
40. Zhang, L.; Pu, Y.; Xue, J.P.; Pratavieira, S.; Xu, B.G.; Achilefu, S.; Alfano, R.R. Tryptophan as the fingerprint for distinguishing aggressiveness among breast cancer cell lines using native fluorescence spectroscopy. Journal of biomedical optics 2014, 19.
41. Pu, Y.; Xue, J.P.; Xu, B.G.; Wang, W.B.; Gu, Y.Q.; Tang, R.; Achilefu, S.; Ackerstaff, E.; Koutcher, J.A.; Alfano, R.R. Investigation of native fluorescence spectral difference among prostate cancer cell lines with different risk levels. Optical Biopsy Xi 2013, 8577.
42. Gao, S.P.; Chen, D.H.; Li, Q.W.; Ye, J.; Jiang, H.; Amatore, C.; Wang, X.M. Near-infrared fluorescence imaging of cancer cells and tumors through specific biosynthesis of silver nanoclusters. Sci Rep 2014, 4.
43. Zhang, X.D.; Wu, F.G.; Liu, P.D.; Gu, N.; Chen, Z. Enhanced fluorescence of gold nanoclusters composed of haucl4 and histidine by glutathione: Glutathione detection and selective cancer cell imaging. Small 2014, 10, 5170-5177.
44. Ding, C.Q.; Tian, Y. Gold nanocluster-based fluorescence biosensor for targeted imaging in cancer cells and ratiometric determination of intracellular ph. Biosens. Bioelectron. 2015, 65, 183-190.
45. Zhang, Y.; Liu, J.M.; Yan, X.P. Self-assembly of folate onto polyethyleneimine-coated cds/zns quantum dots for targeted turn-on fluorescence imaging of folate receptor overexpressed cancer cells. Anal. Chem. 2013, 85, 228-234.
46. Maity, A.R.; Saha, A.; Roy, A.; Jana, N.R. Folic acid functionalized nanoprobes for fluorescence-, dark-field-, and dual-imaging-based selective detection of cancer cells and tissue. ChemPlusChem 2013, 78, 259-267.
47. van Dam, G.M.; Themelis, G.; Crane, L.M.A.; Harlaar, N.J.; Pleijhuis, R.G.; Kelder, W.; Sarantopoulos, A.; de Jong, J.S.; Arts, H.J.G.; van der Zee, A.G.J., et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: First in-human results. Nature Medicine 2011, 17, 1315-U1202.
48. Metildi, C.A.; Felsen, C.N.; Savariar, E.N.; Nguyen, Q.T.; Kaushal, S.; Hoffman, R.M.; Tsien, R.Y.; Bouvet, M. Ratiometric activatable cell-penetrating peptides label pancreatic cancer, enabling fluorescence-guided surgery, which reduces metastases and recurrence in orthotopic mouse models. Ann. Surg. Oncol. 2015, 22, 2082-2087.
49. Ahn, K.H.; Kim, D.; Kim, S.; Kim, K.H. Fluorescence probe useful for detecting tyrosine kinase for imaging cancer cells or tissues, comprises 3-hydroxy-6-methyl-naphthalene-2-carbaldehyde. WO2014181960-A2; KR2014133730-A, WO2014181960-A2 13 Nov 2014 C12Q-001/48 201478.
50. Lai, B.H.; Chen, D.H. Lab6 nanoparticles with carbon-doped silica coating for fluorescence imaging and near-ir photothermal therapy of cancer cells. Acta Biomater. 2013, 9, 7556-7563.
51. Cai, H.W.; Peng, F.Y. 2-nbdg fluorescence imaging of hypermetabolic circulating tumor cells in mouse xenograft model of breast cancer. J. Fluoresc. 2013, 23, 213-220.
52. Zhang, Y.; Liu, W.; Banks, C.E.; Liu, F.; Li, M.; Xia, F.; Yang, X.L. A fluorescence-quenching platform based on biomineralized hydroxyapatite from natural seashell and applied to cancer cell detection. Sci Rep 2014, 4.
53. Lin, Z.H.; Liu, Z.P.; Zhang, H.; Su, X.G. Near-infrared fluorescence probe for the determination of acid phosphatase and imaging of prostate cancer cells. Analyst 2015, 140, 1629-1636.
54. Ito, H.; Oga, A.; Ikemoto, K.; Furuya, T.; Maeda, N.; Yamamoto, S.; Kawauchi, S.; Itoh, H.; Oka, M.; Sasaki, K. Analysis of centromere signal patterns in breast cancer cells with chromosomal instability using image cytometry combined with centromere fluorescence in situ hybridization. Cytom. Part A 2014, 85A, 809-816.
55. Li, C.Y.; Bai, J.C.; Hao, X.M.; Zhang, S.; Hu, Y.H.; Zhang, X.B.; Yuan, W.P.; Hu, L.P.; Cheng, T.; Zetterberg, A., et al. Multi-gene fluorescence in situ hybridization to detect cell cycle gene copy number aberrations in young breast cancer patients. Cell Cycle 2014, 13, 1299-1305.
56. Wojas-Krawczyk, K.; Krawczyk, P.A.; Ramlau, R.A.; Szumilo, J.; Kozielski, J.; Kalinka-Warzochas, E.; Bryl, M.; Knopik-Nabrowicz, A.; Spychalski, L.; Szczesna, A., et al. The analysis of alk gene rearrangement by fluorescence in situ hybridization in non-small cell lung cancer patients. Wspolczesna Onkol. 2013, 17, 484-492.

Metallothionein and cancer

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Metallothioneins (MT) are small (molecular weight in range 500 – 1400 Da) intracellular proteins rich in cysteine content. In humans four main isoforms of MTs have been discovered so far – MT-1, MT-2, MT-3 and MT-4. All of MTs functions – heavy metals binding, antioxidative, regulation and immunomodulation are involved in MTs roles in cancer. MTs role is double-edged in carcinogenesis; in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer-transformed cell the high MT content increases the tumour malignancy and chemotherapeutics resistance. Expression of MT can be used as marker of tumour diseases. Increased level of MT in serum or full blood was found in many cancer diseases and can be used as early stage biomarker. To decrease tumour resistance, or on the other hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy.

Keywords: metallothionein; cancer; biomarker; chemoresistance; epigenetics

1. Metallothionein

Metallothioneins (MT) are small (molecular weight in range 500 – 1400 Da) intracellular proteins rich in cysteine content. In 1957 MT was discovered in horse liver as cadmium binding and detoxification protein [1]. With progressing research it has been discovered, that metallothioneins are found in all kingdoms – bacteria, animals, fungi and even plants and that they are involved in numerous cell processes according their isoform, oxidation state and metals content [2].

In humans four main isoforms of MTs have been discovered so far – MT-1, and MT-2, which are ubiquitous, MT-3 and MT-4, which are specific for neuronal tissue, but their expression also in skin was found. Human MT genes are localized on chromosome 16q13 and are encoded by a multigene cluster of closely linked genes. Genes for MT consist of 11 MT-1 genes and one gene for every other MT isoform (the MT-2A gene, MT-3 gene and MT-4 gene) [3]. A gene called MT-like 5 (MTL-5) that encodes a testis-specific MT-like protein called tesmin was described in the 11q13 [4]. A number of other MT or MT-like genes and pseudogenes with significant homology to functional MT genes exist in human genome, but their functionality is unknown.

2. Roles of metallothionein in the cells

The first discovered and still not fully understood roles of MT-1 and MT-2 are transporting of essential heavy metals, detoxification of toxic ones and protection against oxidation stress. MT by interaction with other proteins fulfils its function, resulting in different effects in the organism [5]. Interaction of MT with other proteins occurs either directly or via heavy metals administration and redox reactions.

Interaction of MT with ferritin, which causes a redox reaction leads to reduction of Fe^{3+} stored in ferritin with consequent releasing of harmful Fe^{2+} [6]. Interaction of MT with GSH/GSSG modulates Zn transfer between MT and zinc-binding proteins [7]. Apo-MT is able to deactivate Zn-transcription factors and Zn--dependent enzymes, while Zn-MT can activate them. Interaction of MT with zinc-dependent enzymes such as carbonic anhydrase, Cu/Zn SOD, δ-aminolevulinic acid dehydratase via zinc binding is known [5,8]. After interaction of MT with endocytic LDL receptor megalin the uptake of CdMT occurs, resulting in disruption of proximal tubules [9]. MT is involved in many cellular processes, which are regulated by protein-protein interaction. The most known are transcription, apoptosis, immunomodulation and cancerogenesis, interacting with NF κB, p53, specificity protein 1 (Sp1), transcription factor IIA (TFIIA), estrogen receptor (ER), Gal4, and tramrack (TTK), matrix metalloproteinases and other [5,10].

MT-3 has been discovered as neuronal growth inhibition factor. It is involved in growth of neuronal tissue, but its expression has also been found in skin, and epithelial tissues. It also binds heavy metals, but its main function is regulation of neuronal development [11].

MT-4, discovered few years ago is the least known MTs isoform. It is found in stratified squamous epithelia, including the oesophagus, upper stomach, tail, footpads and neonatal skin [12]. It helps to regulate stomach acid pH, taste and texture discrimination of the tongue and help protect against sunburn and other skin traumas [13].

3. Connection of metallothionein with cancer diseases

All of MTs functions – heavy metals binding, antioxidative, regulation and immunomodulation are involved in MTs roles in cancer. MTs role is double-edged in carcinogenesis, in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer-transformed cell the high MT content increases the tumour malignancy [14]. Proliferative, anti--apoptic function of MTs, (de)activation of transcription factors, ROS scavenging are beneficial for cancer cell to survive, proliferate and defend against organisms' immune system.

All isoforms, except the least known MT-4 have been found to be involved in cancer. However, according to last findings MT-4 –expression at mRNA level was found in lung cancer (NSLC) [15] and squamous cell carcinoma [16].

The most about MT up/down regulation is known about MT-1 and MT-2 [8,17-19]. Disclosed MT expression as a useful diagnostic factor for tumour progression and drug resistance was reported in a variety of malignancies e.g. leukaemia, melanoma, breast, ovarian, renal, lung, pancreatic, gall bladder, oesophageal, and basal cell carcinomas [20-27]. One may suggest that MTs may lead to a protection of tumour cells against apoptosis and support the metastatic behaviour of tumours and/or cancer cell proliferation. On the other hand in some other studies devoted to colorectal and bladder cancer and others, no significant correlation between MT expression and prognosis was observed [28,29]. In recent years, common MT polymorphisms were identified and associated with, particularly, western lifestyle diseases such as cancer, complications of atherosclerosis, and type 2 diabetes mellitus along with related complications [30]. MT is often downregulated at epigenetic level in numerous cancers [31-36].

MT-3 expression was found to be corrected with lung cancer [20], skin cancer [37], breast cancer [38], prostate cancer [39], oesophageal squamous cell carcinoma [40], bladder cancer [41] and renal carcinoma [42]. Moreover, MT-3 is a putative tumour suppressor gene, that is frequently inactivated in paediatric acute myeloid leukemia [33].

4. Metallothionein and resistance to chemotherapeutics

In MT-related chemoresistance heavy metals chelation and antioxidative functions are involved. It can contribute not only to cancer cell survival, but also to decreasing of side effect of chemotherapy, especially anthracyclines [8]. To decrease tumour resistance, or on the other

hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy, including using antisense mRNA, siRNA, microRNA, and DNA methylation, can be considered [8,38,43].

5. Metallothionein as cancer biomarker

From numerous studies it is known, that expression of MT both on nucleic acids and protein level can be used as marker of tumour diseases [15,26,44,45]. Also methylation of MT promoters has been shown to be usable as a biomarker [34]. Increased level of MT in serum or full blood has been found in many cancer diseases and can be used as early stage biomarker [26,45,46]. Also changes in Zn isotopes in tumour tissues can be usable for detection of cancer. The authors hypothesize, that higher content of light isotopes in tumour tissue than in control tissues (both non tumour and healthy volunteers) is caused by preferential chelation of light isotopes by MT via SH groups [47].

6. Conclusion

MTs role is double-edged in carcinogenesis; in healthy cells with high MT content it has protective effects, while in healthy cells with low MT content, carcinogenesis via environmental factors occurs, on the other hand, at cancer--transformed cell the high MT content increases the tumour malignancy and chemotherapeutics resistance. Expression of MT both on nucleic acids and protein level can be used as marker of tumour diseases. Increased level of MT in serum or full blood has been found in many cancer diseases and can be used as early stage biomarker. To decrease tumour resistance, or on the other hand, to improve non-target cells tolerance to chemotherapeutic multiple approaches are tested, including specific increasing or decreasing of MT expression based on specific compounds or targeted gene therapy, can be considered.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Margoshes, M.; Vallee, B.L. A cadmium protein from equine kidney cortex. Journal of the American Chemical Society 1957, 79, 4813-4814.
2. Coyle, P.; Philcox, J.C.; Carey, L.C.; Rofe, A.M. Metallothionein: The multipurpose protein. Cell. Mol. Life Sci. 2002, 59, 627-647.
3. Vasak, M.; Meloni, G. Chemistry and biology of mammalian metallothioneins. J. Biol. Inorg. Chem. 2011, 16, 1067-1078.
4. Olesen, C.; Moller, M.; Byskov, A.G. Tesmin transcription is regulated differently during male and female meiosis. Molecular Reproduction and Development 2004, 67, 116-126.
5. Zalewska, M.; Trefon, J.; Milnerowicz, H. The role of metallothionein interactions with other proteins. Proteomics 2014, 14, 1343-1356.
6. Orihuela, R.; Fernandez, B.; Palacios, O.; Valero, E.; Atrian, S.; Watt, R.K.; Dominguez-Vera, J.M.; Capdevila, M. Ferritin and metallothionein: Dangerous liaisons. Chem. Commun. 2011, 47, 12155-12157.
7. Maret, W. Fluorescent probes for the structure and function of metallothionein. J. Chromatogr. B 2009, 877, 3378-3383.
8. Krizkova, S.; Ryvolova, M.; Hrabeta, J.; Adam, V.; Stiborova, M.; Eckschlager, T.; Kizek, R. Metallothioneins and zinc in cancer diagnosis and therapy. Drug Metabolism Reviews 2012, 44, 287-301.
9. Klassen, R.B.; Crenshaw, K.; Kozyraki, R.; Verroust, P.J.; Tio, L.; Atrian, S.; Allen, P.L.; Hammond, T.G. Megalin mediates renal uptake of heavy metal metallothionein complexes. American Journal of Physiology-Renal Physiology 2004, 287, F393-F403.
10. Krizkova, S.; Fabrik, I.; Adam, V.; Hrabeta, P.; Eckschlager, T.; Kizek, R. Metallothionein - a promising tool for cancer diagnostics. Bratislava Medical Journal-Bratislavske Lekarske Listy 2009, 110, 93-97.
11. Uchida, Y.; Takio, K.; Titani, K.; Ihara, Y.; Tomonaga, M. The growth inhibitory factor that is deficient in the alzheimers-disease brain is a 68-amino acid metallothionein-like protein. Neuron 1991, 7, 337-347.
12. Quaife, C.J.; Findley, S.D.; Erickson, J.C.; Froelick, G.J.; Kelly, E.J.; Zambrowicz, B.P.; Palmiter, R.D. Induction of a new metallothionein isoform (mt-iv) occurs during differentiation of stratified squamous epithelia. Biochemistry 1994, 33, 7250-7259.
13. Thirumoorthy, N.; Sunder, A.S.; Kumar, K.T.M.;

Kumar, M.S.; Ganesh, G.N.K.; Chatterjee, M. A review of metallothionein isoforms and their role in pathophysiology. World J. Surg. Oncol. 2011, 9.
14. McGee, H.M.; Woods, G.M.; Bennett, B.; Chung, R.S. The two faces of metallothionein in carcinogenesis: Photoprotection against uvr-induced cancer and promotion of tumour survival. Photochem. Photobiol. Sci. 2010, 9, 586-596.
15. Werynska, B.; Pula, B.; Muszczynska-Bernhard, B.; Gomulkiewicz, A.; Piotrowska, A.; Prus, R.; Podhorska-Okolow, M.; Jankowska, R.; Dziegiel, P. Metallothionein if and 2a overexpression predicts poor outcome of non-small cell lung cancer patients. Exp. Mol. Pathol. 2013, 94, 301-308.
16. Nakano, M.; Sogawa, C.A.; Sogawa, N.; Mishima, K.; Yamachika, E.; Mizukawa, N.; Fukunaga, J.; Kawamoto, T.; Sawaki, K.; Sugahara, T., et al. Expression pattern of cisplatin-induced metallothionein isoforms in squamous cell carcinoma. Anticancer Res. 2003, 23, 299-303.
17. Cherian, M.G.; Jayasurya, A.; Bay, B.H. Metallothioneins in human tumors and potential roles in carcinogenesis. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 2003, 533, 201-209.
18. Theocharis, S.E.; Margeli, A.P.; Klijanienko, J.T.; Kouraklis, G.P. Metallothionein expression in human neoplasia. Histopathology 2004, 45, 103-118.
19. Eckschlager, T.; Adam, V.; Hrabeta, J.; Figova, K.; Kizek, R. Metallothioneins and cancer. Current Protein & Peptide Science 2009, 10, 360-375.
20. Werynska, B.; Pula, B.; Kobierzycki, C.; Dziegiel, P.; Podhorska-Okolow, M. Metallothioneins in the lung cancer. Folia Histochemica Et Cytobiologica 2015, 53, 1-10.
21. Goldmann, T.; Ribbert, D.; Suter, L.; Brode, M.; Otto, F. Tumor characteristics involved in the metastatic behaviour as an improvement in primary cutaneous melanoma prognostics. Journal of Experimental & Clinical Cancer Research 1998, 17, 483-489.
22. Jin, R.X.; Huang, J.X.; Tan, P.H.; Bay, B.H. Clinicopathological significance of metallothioneins in breast cancer. Pathology & Oncology Research 2004, 10, 74-79.
23. Huang, G.W.; Yang, L.Y. Metallothionein expression in hepatocellular carcinoma. World Journal of Gastroenterology 2002, 8, 650-653.
24. Neville, C.S.; Smith, C.; Van Huizen, I.; Yamasaki, Y.; Moussa, M.; Xuan, J.; Leal, J.; Chin, J.L.; Izawa, J.I. Metallothionein expression as a prognostic factor for renal cell carcinoma. Journal of Urology 2005, 173, 100-100.
25. Grove, P.S.; Chang, W.W.L.; Gangarosa, L.M. Metallothionein expression in the pancreas and pancreatic cancer. Gastroenterology 1999, 116, A1128-A1128.
26. Krizkova, S.; Masarik, M.; Majzlik, P.; Kukacka, J.; Kruseova, J.; Adam, V.; Prusa, R.; Eckschlager, T.; Stiborova, M.; Kizek, R. Serum metallothionein in newly diagnosed patients with childhood solid tumours. Acta Biochimica Polonica 2010, 57, 561-566.
27. Surowiak, P.; Materna, V.; Kaplenko, I.; Spaczynski, M.; Dietel, M.; Lage, H.; Zabel, M. Augmented expression of metallothionein and glutathione s-transferase pi as unfavourable prognostic factors in cisplatin-treated ovarian cancer patients. Virchows Archiv 2005, 447, 626-633.
28. Jasani, B.; Schmid, K.W. Significance of metallothionein overexpression in human. Histopathology 1997, 31, 211-214.
29. Ofner, D.; Maier, H.; Riedmann, B.; Bammer, T.; Rumer, A.; Winde, G.; Bocker, W.; Jasani, B.; Schmid, K.W. Immunohistochemical metallothionein expression in colorectal adenocarcinoma - correlation with tumor stage and patient survival. Virchows Archiv 1994, 425, 491-497.
30. Raudenska, M.; Gumulec, J.; Podlaha, O.; Sztalmachova, M.; Babula, P.; Eckschlager, T.; Adam, V.; Kizekb, R.; Masarik, M. Metallothionein polymorphisms in pathological processes. Metallomics 2014, 6, 55-68.
31. Alkamal, I.; Ikromov, O.; Tolle, A.; Fuller, T.F.; Magheli, A.; Miller, K.; Krause, H.; Kempkensteffen, C. An epigenetic screen unmasks metallothioneins as putative contributors to renal cell carcinogenesis. Urologia Internationalis 2015, 94, 99-110.
32. Faller, W.J.; Rafferty, M.; Hegarty, S.; Gremel, G.; Ryan, D.; Fraga, M.F.; Esteller, M.; Dervan, P.A.; Gallagher, W.M. Metallothionein 1e is methylated in malignant melanoma and increases sensitivity to cisplatin-induced apoptosis. Melanoma Research 2010, 20, 392-400.
33. Tao, Y.F.; Xu, L.X.; Lu, J.; Cao, L.; Li, Z.H.; Hu, S.Y.; Wang, N.N.; Du, X.J.; Sun, L.C.; Zhao, W.L., et al. Metallothionein iii (mt3) is a putative tumor suppressor gene that is frequently inactivated in pediatric acute myeloid leukemia by promoter hypermethylation. Journal of Translational Medicine 2014, 12.
34. Jadhav, R.R.; Ye, Z.Q.; Huang, R.L.; Liu, J.; Hsu, P.Y.; Huang, Y.W.; Rangel, L.B.; Lai, H.C.; Roa, J.C.; Kirma, N.B., et al. Genome-wide DNA methylation analysis reveals estrogen-mediated epigenetic repression of metallothionein-1 gene cluster in breast cancer. Clinical Epigenetics 2015, 7.
35. Peng, D.F.; Hu, T.L.; Jiang, A.X.; Washington, M.K.; Moskaluk, C.A.; Schneider-Stock, R.; El-Rifai, W. Location-specific epigenetic regulation of the metallothionein 3 gene in esophageal adenocarcinomas. Plos One 2011, 6.
36. Tse, K.Y.; Liu, V.W.S.; Chan, D.W.; Chiu, P.M.; Tam, K.F.; Chan, K.K.L.; Liao, X.Y.; Cheung, A.N.Y.; Ngan, H.Y.S. Epigenetic alteration of the metallothionein 1e gene in human endometrial carcinomas. Tumor Biology 2009, 30, 93-99.
37. Slusser, A.; Zheng, Y.; Zhou, X.D.; Somji, S.; Sens, D.A.; Sens, M.A.; Garrett, S.H. Metallothionein isoform 3 expression in human skin, related cancers and human skin derived cell cultures. Toxicology Letters 2015, 232, 141-148.
38. Lai, Y.Y.; Yip, G.W.C.; Bay, B.H. Targeting metallothionein for prognosis and treatment of breast cancer. Recent Patents Anti-Canc. Drug Discov. 2011, 6, 178-185.
39. Wei, H.; Desouki, M.M.; Lin, S.; Xiao, D.; Franklin, R.B.; Feng, P. Differential expression

of metallothioneins (mts) 1, 2, and 3 in response to zinc treatment in human prostate normal and malignant cells and tissues. Molecular Cancer 2008, 7.
40. Smith, E.; Drew, P.A.; Tian, Z.Q.; De Young, N.J.; Liu, J.F.; Mayne, G.C.; Ruszkiewicz, A.R.; Watson, D.I.; Jamieson, G.G. Metallothionien 3 expression is frequently down-regulated in oesophageal squamous cell carcinoma by DNA methylation. Molecular Cancer 2005, 4.
41. Sens, M.A.; Somji, S.; Lamm, D.L.; Garrett, S.H.; Slovinsky, F.; Todd, J.H.; Sens, D.A. Metallothionein isoform 3 as a potential biomarker for human bladder cancer. Environmental Health Perspectives 2000, 108, 413-418.
42. Hoey, J.G.; Garrett, S.H.; Sens, M.A.; Todd, J.H.; Sens, D.A. Expression of mt-3 mrna in human kidney, proximal tubule cell cultures, and renal cell carcinoma. Toxicology Letters 1997, 92, 149-160.
43. Heger, Z.; Merlos, M.A.R.; Krizkova, S.; Ruttkay-Nedecky, B.; Zalewska, M.; Planells, E.M.P.; Pelfrene, A.; Pourrut, B.; Stiborova, M.; Eckschlager, T., et al. Metallothionein as a scavenger of free radicals – new cardioprotective therapeutic agent or initiator of tumor chemoresistance? Curr. Drug Targets 2015, in press.
44. Gumulec, J.; Raudenska, M.; Adam, V.; Kizek, R.; Masarik, M. Metallothionein - immunohistochemical cancer biomarker: A meta-analysis. Plos One 2014, 9.
45. Tariba, B.; Zivkovic, T.; Krasnici, N.; Marijic, V.F.; Erk, M.; Gamulin, M.; Grgic, M.; Pizent, A. Serum metallothionein in patients with testicular cancer. Cancer Chemotherapy and Pharmacology 2015, 75, 813-820.
46. Krejcova, L.; Fabrik, I.; Hynek, D.; Krizkova, S.; Gumulec, J.; Ryvolova, M.; Adam, V.; Babula, P.; Trnkova, L.; Stiborova, M., et al. Metallothionein electrochemically determined using brdicka reaction as a promising blood marker of head and neck malignant tumours. International Journal of Electrochemical Science 2012, 7, 1767-1784.
47. Larner, F.; Woodley, L.N.; Shousha, S.; Moyes, A.; Humphreys-Williams, E.; Strekopytov, S.; Halliday, A.N.; Rehkamper, M.; Coombes, R.C. Zinc isotopic compositions of breast cancer tissue. Metallomics 2015, 7, 107-112.

REVIEW

Zinc and metallothionen in prostate cancer: A review

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Zinc is an essential and the second most abundant trace element in humans. Zinc is a structural component of different proteins involved in the transcriptional machinery, such as transcription factors and ribosomes and the presence of zinc is also necessary for DNA synthesis. Zinc not only improves cell mediated immune functions but also functions as an antioxidant and anti-inflammatory agent. Oxidative stress and chronic inflammation have been implicated in development of many cancers. Various types of tumor cell lines have been used to investigate the cellular effects of zinc ions and its connection with metallothioneins (MTs). Dietary zinc deficiency is associated with oxidative stress in the reproductive organs with consequent decline in MTs levels indicative of association of MTs expression and apoptosis and perturbation of homeostatic zinc level. Treatment of prostate cancer with zinc causes an increase in MTs expression, which is significantly associated with resistance to cisplatin chemotherapy and radiotherapy in prostate cancer. We review the role and recent advances of zinc and MTs in prostate cancer in the last years.

Keywords: Zinc; metallothionein; cancer; prostate cancer

1. Zinc in medicine

Zinc is the second most common trace metal in the human body. It is involved in numerous aspects of biology cellular, zinc is known to be an essential component of DNA-binding proteins with zinc fingers, as well as copper/zinc superoxide dismutase and several proteins involved in DNA repair. Thus, the zinc plays an important role in immune function, protein synthesis, DNA synthesis and cell division [1, 2]. The role of zinc in cancer has received increasing attention in last years. Dietary deficiencies in the intake of zinc can contribute to oxidative modifications to DNA that increase risk for cancer development [3, 4]. Various types of cancer have been used to investigate the cellular effects of zinc ions and its connection with MTs [5]. MTs are low weight proteins involved in several key cellular processes such as metal ions homeostasis, detoxification and scavenging of free radicals [5, 6]. In humans, MTs are encoded by 17 genes, from which thirteen code for MT-1, two for MT-2 and one gene each codes for MT-3 and MT-4 [7, 8]. MTs were shown to protect cells against oxidative stress damage and participate in differentiation, proliferation and apoptosis of normal and cancer cells [9]. Their altered mRNA expression has been correlated with metal toxicity and a variety of cancers. The different MTs genes have been found in normal human prostatic tissue. Different studies showed the relationship between the gene expression of MTs and the cellular zinc homeostasis in relation to the diseases of the prostate and the potential of MTs as a candidate biomarker for prostate cancer and the utilization of zinc in prostate cancer prevention and treatment [10, 11]. For this reason, we show in this review an overview the importance of the regulation of zinc and/or relationship with the MTs in prostate cancer in recent years.

2. Zinc in medicine

Zinc is an essential and the second most abundant trace element in humans. It is critical for the growth, development and differentiation of cells, as well as for RNA transcription, DNA synthesis, cell division and cell activation [12]. The major manifestations of zinc deficiency include growth retardation, hypogonadism in males, cell-mediated immune dysfunctions, rough skin, hyperammonemia and cognitive impairment. Zinc is a structural component of different proteins involved in the transcriptional machinery, such as transcription factors and ribosomes and the presence of zinc is also necessary for DNA synthesis. Furthermore, zinc is a second messenger of mitogenic signaling [13]. Zinc also plays an important role in synaptic function. At cellular level, zinc is a modulator of synaptic activity and neuronal plasticity in both development and adulthood [14]. Zinc has many beneficial roles in normal growth and development, cellular homeostasis, cell survival, and numerous biochemical functions, including protein synthesis, gene expression, and nucleic acid metabolism [15, 16]. Zinc deficiency affects cells involved in both innate and adaptive immunity at the survival, proliferation and maturation levels. These cells include monocytes, polymorphonuclear-, natural killer-, T-, and B-cells. T cell functions and the balance between the different T helper cell subsets are particularly susceptible to changes in Zinc status [17]. Zinc affects the monocytes/macrophages in several ways. Zinc is required for the development of monocytes/macrophages and regulates their functions such as phagocytosis and proinflammatory cytokine production. LPS stimulation of zinc sufficient monocytes results in down-regulation of inflammatory cytokines such as TNF, IL-1, IL-6 and IL-8 [18, 19, 20, 21, 22]. Zinc deficiencies occur due to malabsorption syndromes and other gastrointestinal disorders, chronic liver and renal diseases, sickle cell disease, malignancy, cystic fibrosis, pancreatic insufficiency, rheumatoid arthritis and other chronic conditions [23]. Several other diseases including infectious diseases, cancer, chronic diseases such as bronchial asthma and Alzheimer disease, skin lesions, growth retardation, impaired wound healing, anemia, mental retardation which were observed even in mild zinc deficiency and/or alterations in zinc status [24, 25]. Zinc has a key role in apoptosis regulation. Zinc chelation in cell culture medium causes apoptosis and subsequent addition of Zinc protects cells against the undergoing apoptosis even if it was added to the cell culture only a short time after an apoptotic agent [26].

Beneficial therapeutic response of zinc supplementation has been observed in the diarrhoea of children, chronic hepatitis C, shigellosis, leprosy, tuberculosis, pneumonia, acute lower respiratory tract infection, common cold, and leishmaniasis [27, 28]. Zinc supplementation was effective in decreasing incidences of infections in the elderly, in patients with sickle cell disease (SCD) and decreasing incidences of respiratory tract infections in children. Zinc supplementation was effective in decreasing oxidative stress and generation of inflammatory cytokines such as TNF-alpha and IL-1 beta in elderly individuals and patients with SCD [20, 29, 30].

3. Zinc in cancer prevention

Zinc not only improves cell mediated immune functions but also functions as an antioxidant and anti-inflammatory agent. Oxidative stress and chronic inflammation have been implicated in development of many cancers [31]. The role of zinc in cancer has received increasing attention. In patients with head and neck cancer, Prasad et al showed that nearly 65% of these patients were zinc deficient based on their cellular zinc concentrations. Natural killer (NK) cell activity and IL-2 generation were also affected adversely. Th2 cytokines were not affected. In these patients, zinc status was a better indicator of tumor burden and stage of disease in comparison to the overall nutritional status. NF-κ B is constitutively activated in many cancer cells, and this results in activation of antiapoptotic genes, VEGF, cyclin DI, EGFR, MMP-9 and inflammatory cytokines. Zinc inhibits NF-κ B via induction of A-20 [32]. Increased amounts of zinc transporter LIV-1 (SLC39A6) are present in estrogen receptor–positive breast cancer

and in tumors that spread to lymph nodes. The LIV-1 subfamily of ZIP zinc transporters consists of nine human sequences. It is a highly conserved group of eight transmembrane domain proteins, which are situated on the plasma membrane and which are responsible for zinc transport into cells. LIV-1 has been used as a reliable marker of luminal A type clinical breast cancer [33]. In different publications there has been showed the concept that zinc is involved in the pathogenesis of prostate cancer (PCa); and that zinc could be efficacious in the prevention and treatment of prostate cancer [34, 35].

4. Zinc and metallothionein in cancer

MT has been found to be involved in apoptosis, immunomodulation, transcription regulation, cell proliferation, and activation of enzymes via administration of zinc to the proteins [36]. In Figure 1, we show schematic representation of the MTs functions. Many of these interactions are driven by zinc(II) ions. Disturbing of zinc homeostasis can lead to formation of reactive oxygen species, which can result in oxidative stress causing alterations in immunity, aging, and civilization diseases, but also in cancer development [5]. Various types of tumor cell lines have been used to investigate the cellular effects of zinc ions and its connection with MTs. The human genome contains at least 11 functional MTs genes that may be divided into four subgroups (MT1-4). Given their stress--inducible nature and their capacity to chelate toxic metals and electrophiles, many studies have proposed MTs expression to confer resistance to many toxic drugs [37, 38]. Several lines of evidence indicate that MTs may play a role in various carcinogenic processes, as high levels of MT expression have been reported in association with progressive disease and poor prognosis in several tumors [39, 40]. Han et al showed by microarray and validation analyses that MT1H, is down-regulated in many human malignancies. Low expression of MT1H was associated with poor clinical outcomes in both prostate and liver cancer. The promoter region of MT1H was hypermethylated in cancer and that demethylation of the MT1H promoter reversed the suppression of MT1H expression [41]. A number of studies have demonstrated altered MT2A expression in various human tumors, including prostate cancer. Forma et al conducted an association study to examine whether MT2A gene polymorphisms are associated with a risk of prostate cancer. They suggested

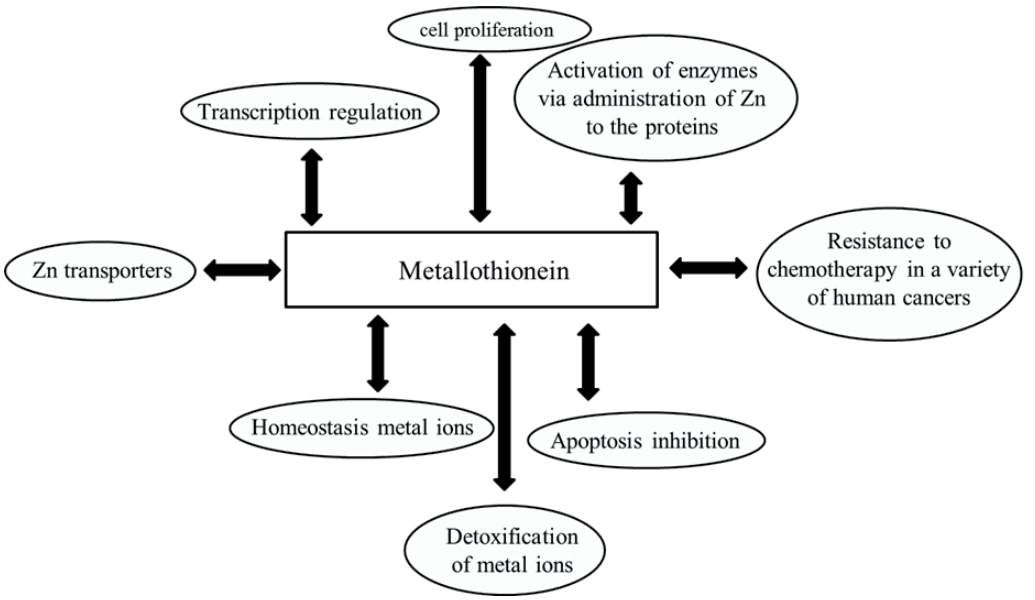

Figure 1: Schematic representation of the MTs functions

that the gen polymorphism rs28366003 SNP in MT2A is associated with the risk of prostate cancer in a Polish population [42].

4.1. Metallothionein and zinc in prostate cancer

Dietary zinc deficiency is associated with oxidative stress in the reproductive organs with consequent decline in MTs levels [43] indicative of association of MTs expression and apoptosis and perturbation of homeostatic zinc level [44]. Several reports have demonstrated MTs overexpression to be a useful prognostic factor for tumor progression and implicated in causing resistance to chemotherapy in a variety of human cancers [45, 46]. MTs are evaluated as trace metal-responsive genes. The different MTs genes have been found in normal human prostatic tissue. MTs protein in the normal human prostate is supported by transcription of mRNA from the MT-1A, MT-1E, MT-1X, and MT-2A genes. Expression of MT-1X mRNA is down-regulated in advanced prostate cancer [47]. Treatment of prostate cancer with zinc causes an increase in MT expression, which is significantly associated with resistance to cisplatin chemotherapy and radiotherapy in prostate cancer and the effect of MTs induction by zinc on resistance to radiotherapy and cisplatin treatment in prostate cancer cells, which may provide unique opportunities to manipulate the cellular events in a prostate cell [48]. Gumulec et al showed also provided evidence of the association between MT expression and prostate tumor progression [49]. Hlavna et al showed that significantly increased microRNA levels, are a large class of single-stranded RNA molecules involved in post-transcriptional gene silencing, of MT2A isoform in tumor cell lines. Contrary to mRNA, significantly reduced level of MTs protein in tumor lines was observed. None of the miRNA analyzed here correlated with MT mRNA level after zinc treatment in the prostate cell lines. It can be assumed according to results that miRNAs act differently in each cell line [50]. The disturbance of zinc homeostasis featured with a significant decrease of cellular zinc level was well documented to associate with the development and progression of human prostate malignancy. Hua Wei et al showed for the first time provided new evidence on zinc regulation of MTs gene expression and elucidated the relationship between the gene expression and the cellular zinc homeostasis in relation to the pathogenesis status of the prostate tissues [10]. The expression of MTs genes was induced by zinc and cadmium in the RWPE-1 and BPH-1 human prostate epithelial cell lines the human prostate gland has low basal expression of the MT-1 and MT-2 proteins. In prostate cancer, MTs protein expression is variable and correlates directly with the increasing Gleason score of the tumor. Albrecht et al showed that the RWPE-1 cells may be a valuable system to define the interplay that occurs between zinc concentration, cadmium exposure, citrate and MT in the normal and malignant prostate epithelial cell [51].

5. Conclusion

Zinc ions contribute to a number of biological processes. Disturbing of zinc homeostasis can lead to formation of reactive oxygen species, which can result in oxidative stress causing alterations in immunity, aging, and civilization diseases, but also in cancer development. It is clear from literature that zinc is of extraordinary and diverse importance in cancer biology. MTs may play a role in various carcinogenic processes, as high levels of MTs expression have been reported in association with prognosis in several tumors. This review reports on the roles of zinc in differential regulation of MTs gene expression in human prostate normal and malignant cell lines. We believe that this review delivered important insight to a new field of research on zinc and its roles in the prevention and intervention of prostate cancer. It is however still necessary to clarify the ambiguity of the association between MTs staining and prostate tumors.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. McCarthy, T.J.; Zeelie, J.J.; Krause, D.J. THE ANTIMICROBIAL ACTION OF ZINC ION ANTIOXIDANT COMBINATIONS. Journal of Clinical Pharmacy and Therapeutics 1992, 17, 51-54.
2. Solomons, N.W. Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. Nutrition Reviews 1998, 56, 27-28.
3. Ho, E. Zinc deficiency, DNA damage and cancer risk. Journal of Nutritional Biochemistry 2004, 15, 572-578.
4. Alam, S.; Kelleher, S.L. Cellular Mechanisms of Zinc Dysregulation: A Perspective on Zinc Homeostasis as an Etiological Factor in the Development and Progression of Breast Cancer. Nutrients 2012, 4, 875-903.
5. Krizkova, S.; Ryvolova, M.; Hrabeta, J.; Adam, V.; Stiborova, M.; Eckschlager, T.; Kizek, R. Metallothioneins and zinc in cancer diagnosis and therapy. Drug Metab. Rev. 2012, 44, 287-301.
6. Ruttkay-Nedecky, B.; Nejdl, L.; Gumulec, J.; Zitka, O.; Masarik, M.; Eckschlager, T.; Stiborova, M.; Adam, V.; Kizek, R. The Role of Metallothionein in Oxidative Stress. Int. J. Mol. Sci. 2013, 14, 6044-6066.
7. Mehus, A.A.; Muhonen, W.W.; Garrett, S.H.; Somji, S.; Sens, D.A.; Shabb, J.B. Quantitation of Human Metallothionein Isoforms: A Family of Small, Highly Conserved, Cysteine-rich Proteins*. Molecular & Cellular Proteomics 2014, 13, 1020-1033.
8. Mididoddi, S.; McGuirt, J.P.; Sens, M.A.; Todd, J.H.; Sens, D.A. Isoform-specific expression of metallothionein mRNA in the developing and adult human kidney. Toxicology Letters 1996, 85, 17-27.
9. Werynska, B.; Pula, B.; Kobierzycki, C.; Dziegiel, P.; Podhorska-Okolow, M. Metallothioneins in the lung cancer. Folia Histochem. Cytobiol. 2015, 53, 1-10.
10. Wei, H.; Desouki, M.M.; Lin, S.; Xiao, D.; Franklin, R.B.; Feng, P. Differential expression of metallothioneins (MTs) 1, 2, and 3 in response to zinc treatment in human prostate normal and malignant cells and tissues. Mol. Cancer 2008, 7.
11. Lin, S.-f.; Wei, H.; Maeder, D.; Franklin, R.B.; Feng, P. Profiling of zinc-altered gene expression in human prostate normal vs. cancer cells: a time course study. Journal of Nutritional Biochemistry 2009, 20, 1000-1012.
12. Horecka, A.; Pasternak, K. ZINC IN MEDICINE AND TREATMENT. Journal of Elementology 2014, 19, 607-616.
13. Grummt, F.; Weinmanndorsch, C.; Schneiderschaulies, J.; Lux, A. Zinc as a 2nd messenger of mitogenic induction - effects on diadenosine tetraphosphate (ap4a) and DNA-synthesis. Experimental Cell Research 1986, 163, 191-200.
14. Prakash, A.; Bharti, K.; Majeed, A.A. Zinc: indications in brain disorders. Fundam. Clin. Pharmacol. 2015, 29, 131-149.
15. Truong-Tran, A.Q.; Carter, J.; Ruffin, R.; Zalewski, P.D. New insights into the role of zinc in the respiratory epithelium. Immunol. Cell Biol. 2001, 79, 170-177.
16. Haase, H.; Beyersmann, D. Intracellular zinc distribution and transport in C6 rat glioma cells. Biochemical and Biophysical Research Communications 2002, 296, 923-928.
17. Bonaventura, P.; Benedetti, G.; Albarede, F.; Miossec, P. Zinc and its role in immunity and inflammation. Autoimmun. Rev. 2015, 14, 277-285.
18. Bao, B.; Prasad, A.S.; Beck, F.W.J.; Fitzgerald, J.T.; Snell, D.; Bao, G.W.; Singh, T.; Cardozo, L.J. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. American Journal of Clinical Nutrition 2010, 91, 1634-1641.
19. Hirano, T.; Murakami, M.; Fukada, T.; Nishida, K.; Yamasaki, S.; Suzuki, T. Roles of zinc and zinc signaling in immunity: Zinc as an intracellular signaling molecule. In Advances in Immunology, Vol 97; Alt, F. W.; Austen, K. F.; Honjo, T.; Melchers, F.; Uhr, J. W.; Unanue, E. R., Eds.; 2008; Vol. 97, pp. 149-176.
20. Prasad, A.S. Zinc: role in immunity, oxidative stress and chronic inflammation. Current Opinion in Clinical Nutrition and Metabolic Care 2009, 12, 646-652.
21. Rosenkranz, E.; Prasad, A.; Rink, L. Immunobiology and Hematology of Zinc. In Zinc in Human Health; Rink, L., Ed. 2011; Vol. 76, pp. 195-233.
22. Wong, C.P.; Ho, E. Zinc and its role in age-related inflammation and immune dysfunction. Mol. Nutr. Food Res. 2012, 56, 77-87.
23. John, E.; Laskow, T.C.; Buchser, W.J.; Pitt, B.R.; Basse, P.H.; Butterfield, L.H.; Kalinski, P.; Lotze, M.T. Zinc in innate and adaptive tumor immunity. J. Transl. Med. 2010, 8.
24. Evans, G.W. ZINC AND ITS DEFICIENCY DISEASES. Clinical Physiology and Biochemistry 1986, 4, 94-98.
25. Haase, H.; Rink, L. The immune system and the impact of zinc during aging. Immunity & ageing : I & A 2009, 6, 9.
26. Apostolova, M.D.; Ivanova, I.A.; Cherian, M.G. Signal transduction pathways, and nuclear translocation of zinc and metallothionein during differentiation of myoblasts. Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire 2000, 78, 27-37.
27. Karamyyar, M.; Gheibi, S.; Noroozi, M.; Kord Valeshabad, A. Therapeutic effects of oral zinc supplementation on acute watery diarrhea with moderate dehydration: a double-blind randomized clinical trial. Iranian journal of medical sciences 2013, 38, 93-99.
28. Overbeck, S.; Rink, L.; Haase, H. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. Archivum Immunologiae Et Therapiae Experimentalis 2008, 56, 15-30.

29. Prasad, A.S.; Beck, F.W.J.; Kaplan, J.; Chandrasekar, P.H.; Ortega, J.; Fitzgerald, J.T.; Swerdlow, P. Effect of zinc supplementation on incidence of infections and hospital admissions in sickle cell disease (SCD) (vol 61, pg 194, 1999). American Journal of Hematology 1999, 62, 127-127.
30. Bao, B.; Prasad, A.S.; Beck, F.W.J.; Snell, D.; Suneja, A.; Sarkar, F.H.; Doshi, N.; Fitzgerald, J.T.; Swerdlow, P. Zinc supplementation decreases oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients. Translational Research 2008, 152, 67-80.
31. Ames, B.N. Micronutrient deficiencies - A major cause of DNA damage. In Cancer Prevention: Novel Nutrient and Pharmaceutical Developments; Bradlow, H. L.; Fishman, J.; Osborne, M. P., Eds.; New York Acad Sciences: New York, 1999; Vol. 889, pp. 87-106.
32. Prasad, A.S.; Beck, F.W.J.; Snell, D.C.; Kucuk, O. Zinc in Cancer Prevention. Nutrition and Cancer-an International Journal 2009, 61, 879-887.
33. Taylor, K.M.; Morgan, H.E.; Smart, K.; Zahari, N.M.; Pumford, S.; Ellis, I.O.; Robertson, J.F.R.; Nicholson, R.I. The emerging role of the LIV-1 subfamily of zinc transporters in breast cancer. Mol. Med. 2007, 13, 396-406.
34. Costello, L.C.; Franklin, R.B. The intermediary metabolism of the prostate: A key to understanding the pathogenesis and progression of prostate malignancy. Oncology 2000, 59, 269-282.
35. Costello, L.C.; Feng, P.; Milon, B.; Tan, M.; Franklin, R.B. Role of zinc in the pathogenesis and treatment of prostate cancer: critical issues to resolve. Prostate Cancer and Prostatic Diseases 2004, 7, 111-117.
36. Krizkova, S.; Ryvolova, M.; Gumulec, J.; Masarik, M.; Adam, V.; Majzlik, P.; Hubalek, J.; Provaznik, I.; Kizek, R. Electrophoretic fingerprint metallothionein analysis as a potential prostate cancer biomarker. Electrophoresis 2011, 32, 1952-1961.
37. Chun, J.H.; Kim, H.K.; Kim, E.; Kim, I.H.; Kim, J.H.; Chang, H.J.; Choi, I.J.; Lim, H.S.; Kim, I.J.; Kang, H.C.; Park, J.H.; Bae, J.M.; Park, J.G. Increased expression of metallothionein is associated with irinotecan resistance in gastric cancer. Cancer Res. 2004, 64, 4703-4706.
38. Yap, X.; Tan, H.-Y.; Huang, J.; Lai, Y.; Yip, G.W.-C.; Tan, P.-H.; Bay, B.-H. Over-expression of metallothionein predicts chemoresistance in breast cancer. Journal of Pathology 2009, 217, 563-570.
39. Ioachim, E.E.; Goussia, A.C.; Agnantis, N.J.; Machera, M.; Tsianos, E.V.; Kappas, A.M. Prognostic evaluation of metallothionein expression in human colorectal neoplasms. J. Clin. Pathol. 1999, 52, 876-879.
40. Saga, Y.; Hashimoto, H.; Yachiku, S.; Tokumitsu, M.; Kaneko, S. Immunohistochemical expression of metallothionein in human bladder cancer: Correlation with histopathological parameters and patient survival. Journal of Urology 2002, 168, 2227-2231.
41. Han, Y.C.; Zheng, Z.L.; Zuo, Z.H.; Yu, Y.P.; Chen, R.; Tseng, G.C.; Nelson, J.B.; Luo, J.H. Metallothionein 1h tumour suppressor activity in prostate cancer is mediated by euchromatin methyltransferase 1. Journal of Pathology 2013, 230, 184-193.
42. Forma, E.; Krzeslak, A.; Wilkosz, J.; Jozwiak, P.; Szymczyk, A.; Rozanski, W.; Brys, M. Metallothionein 2A genetic polymorphisms and risk of prostate cancer in a Polish population. Cancer Genet. 2012, 205, 432-435.
43. Agrawal, R.; Bedwal, R.S. Effect of dietary zinc deficiency on metallothionein concentration of epididymal luminal fluids of weanling Wistar albino rats. Indian Journal of Experimental Biology 2003, 41, 118-122.
44. Joshi, S.; Nair, N.; Bedwal, R.S. Dietary Zinc Deficiency Effects Dorso-lateral and Ventral Prostate of Wistar Rats: Histological, Biochemical and Trace Element Study. Biol. Trace Elem. Res. 2014, 161, 91-100.
45. Theocharis, S.E.; Margeli, A.P.; Koutselinis, A. Metallothionein: A multifunctional protein from toxicity to cancer. International Journal of Biological Markers 2003, 18, 162-169.
46. Theocharis, S.E.; Margeli, A.P.; Klijanienko, J.T.; Kouraklis, G.P. Metallothionein expression in human neoplasia. Histopathology 2004, 45, 103-118.
47. Garrett, S.H.; Sens, M.A.; Shukla, D.; Flores, L.; Somji, S.; Todd, J.H.; Sens, D.A. Metallothionein isoform 1 and 2 gene expression in the human prostate: Downregulation of MT-1X in advanced prostate cancer. Prostate 2000, 43, 125-135.
48. Smith, D.J.; Jaggi, M.; Zhang, W.; Galich, A.; Du, C.; Sterrett, S.P.; Smith, L.M.; Balaji, K.C. Metallothioneins and resistance to cisplatin and radiation in prostate cancer. Urology 2006, 67, 1341-1347.
49. Gumulec, J.; Masarik, M.; Krizkova, S.; Hlavna, M.; Babula, P.; Hrabec, R.; Rovny, A.; Masarikova, M.; Sochor, J.; Adam, V.; Eckschlager, T.; Kizek, R. Evaluation of alpha-methylacyl-CoA racemase, metallothionein and prostate specific antigen as prostate cancer prognostic markers. Neoplasma 2012, 59, 191-200.
50. Hlavna, M.; Raudenska, M.; Hudcova, K.; Gumulec, J.; Sztalmachova, M.; Tanhaeuserova, V.; Babula, P.; Adam, V.; Eckschlager, T.; Kizek, R.; Masarik, M. MicroRNAs and zinc metabolism-related gene expression in prostate cancer cell lines treated with zinc(II) ions. International Journal of Oncology 2012, 41, 2237-2244.
51. Albrecht, A.L.; Singh, R.K.; Somji, S.; Sens, M.A.; Sens, D.A.; Garrett, S.H. Basal and metal-induced expression of metallothionein isoform 1 and 2 genes in the RWPE-1 human prostate epithelial cell line. Journal of Applied Toxicology 2008, 28, 283-293.

LABORATORY REPORT

World Cancer Day in the Laboratory of Metallomics and Nanotechnologies at Mendel University in Brno

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Laboratory of metallomics and nanotechnologies attended the important event of traditional World cancer day (WCD) which was held on 4. February 2015. On this occasion were prepared a series of lectures aimed for introduction of nanotechnology research as an improvement for cancer diagnosis in early stages, treatment and progressive ways of healing the oncologic patients by gene therapy or rapid cancer diagnosis.

Keywords: World cancer day ; cancer; Laboratory of metallomics

1. Introduction

Program of WCD 2015 was dedicated to ten researchers from the Laboratory of metallomics and nanotechnologies and their lectures (Fig. 1). The whole series of lectures were introduced by information of the League against cancer Prague activities and regular actions also organized under its auspices on the Laboratory of metalomics and nanotechnology ground. For interest was introduced the cooperation of both subjects which has been continued on the project level since 2006.

The connection of some viruses and tumor diseases has been not fully understood yet, so the various relations between malignant progression and presence of SV40 (simian vacuolating virus 40) viral DNA, distributed between 1955 and 1963 in poliomyelitis vaccine as a contamination, were discussed. The ability of virus interference with the function of tumor suppressor p53 was described in laboratory animal tests; however the connections in human have not been clearly described yet.

The main part of lectures was given to nanomaterials and their application in diagnosis and treatment of oncology diseases. Technologies for earlier identification of malignant changes of carcinoma are rapidly developing field of research and for that reason is annually discussed on lecture summarized the actual knowledge. In particular, combination of analytical protocols and nanomaterials for in vivo imaging or magnetic separation of target molecules – biomarkers which increases or decreases in dependence on the condition of the organism, shows the wide range of application scale. The most discussed possibilities of employing the nanotechnologies are drug delivery together with the decrease of non-target toxicity. Some of nanotransporters have been employed in clinical practice (Myocet, Abraxan) with excellent therapeutic results, which promise a great potential of this field of research. For increase the specifity against tumor tissue could be nanotransporters modified by a "controller" enabled the specific recognition of cancer cells based on their typical properties (e.g. the presence of receptors). Smart nanoconstructs are able to drug release in one particular place and eliminate the toxicity and side effects of cytostatics used in the treatment. In the case of gene therapy based on replacing the defective genes or eliminating its ability to encode a target protein, are employed similar nanotechnologies. Although, the idea of gene therapy is due to the ethical issues complicating acceleration to the clinical applications, the Gendicin (gene which encoded wt-p53 in recombinant adenoviral vec-

tor) have been already used successfully for the treatment of malignant cancer squamous cell carcinoma of head and neck in China. Another obstacle that complicates practical application of gene therapy is the need to use viral vectors for gene integration into genome. Although, viruses are recombinantly attenuated, the undesirable massive immune response threatens in some cases. Separate category of nanomaterials, the carbon nanotubes and their modification by drugs or targeting ligands, show a huge application potential. In this case, the nanotoxicologic studies confirm the biocompatability and bioavailability of carbon materials. In vivo and in vitro experiments demonstrate the carbon based carriers improve the passive intake of drugs by cancer cells, while the modification by targeting ligand rapidly increase the elimination of development of different tumor types. The unique properties of nanomaterials could be used not only for drug delivery, but for earlier diagnostic by targeted tumor imaging, both of fluorescence measurement or magnetic resonance where the nanomaterials are employed as contrast agents. In the field of fluorescence are highly discussed semiconductor crystals – quantum dots (QDs) due its higher quantum yield and the possibility of their modification. The main disadvantage of QDs is potential toxicity depending on the forming material, however it has been experimentally demonstrated the toxicity of QDs is negligible in comparison with the presence of the free metal ions. Moreover, the carbon quantum dots especially developed with less toxicity due to toxic metal elimination.

Further lectures discussed especially early diagnosis of cancer with the use of biomarkers. Although, the body fluids are the complex matrixes and most of known biomarkers are present in concentrations of the order of less than other matrix compounds, it is necessary to perform a separation step before analysis. The modern analytical methods for biomarkers analysis allow huge possibilities of high resolution and sensitive detection. One of the interesting biomarker is metal-binding protein; metallothionein (MT), its alterations have been identified in a wide range of malignancies. Several studies have been proven the connection of MT into a number of physiological processes such as metal homeostasis and protecting the body against oxidative stress. The main role of MT is the storage and zinc transport thereby contributing to the genes involved to apoptosis, cell proliferation and cellular division. It is therefore not surprising; the level of MT could be used as a supporting tool in diagnostics, prognostics of state of health during and after oncologic treatment.

The word cancer day 2015, held on the ground of Laboratory of metallomics and nanotechnologies, provided scientific and educational program coupled with fruitful discussions with all interested researchers. The conference fulfilled the mission to share the information about cancer treatment and early diagnostics using modern analytical methods and interesting biomolecules.

Figure 1: Series of lectures given by Laboratory of metallomics and nanotechnologies researchers within WCD 2015

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Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform reguirements“ for biomedical papers

Nanotechnology in diagnosis, treatment and prophylaxis of infectious diseases

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Book titled Nanotechnology in diagnosis, treatment and prophylaxis of infectious diseases was released in 2015 by Mahendra Rai from India and Kateryna Kon from Ukraine.

Figure 1: Authors of the book: Mahendra Rai (Sant Gadge Baba Amravati University, Department of Biotechnology) and Kateryna Kon (Kharkiw National Medical University, Department of Microbiology, Virology and Immunology). Adapted from the [1]

The book was created especially considering the still spreading the risk of resistance of infectious pathogens to antimicrobial agents. Resistance to antimicrobial agents has been reaching high levels among all types of microorganisms. Bacteria constantly demonstrate growing rates of resistance to classical and newly introduced antibiotics, fungi increase rates of resistance to antimycotics, viruses, increase rates of resistance to antiviral agents, and even insect vectors carrying microorganisms have been acquiring the ability to develop resistance to the most common insecticidal agents. Because of this, the efforts of scientists all over the world are being directed to the search for new and effective methods to cope with drug resistance. One promising approach is the application of nanotechnology in the battle against microorganisms.

Potential readers may thus become researchers in applied microbiology, biotechnology, pharmacology, nanotechnology, and infection control, students of medical and biological faculties, and clinicians dealing with infectious diseases.

The book consists of 18 chapters by authors from around the world. Our participation in the creation of the book consisted in writing a contribution of the topic *Complexes of Metal-Based Nanoparticles with Chitosan Suppressing the Risk of Staphylococcus aureus and Escherichia coli Infections* [2].

The aim of our study was the use of nanotechnology (silver, selenium, copper or zinc nanoparticles) in complexes with polymer substance chitosan to reduce the risk and spread of dangerous bacterial pathogens. Nanoparticles of metals interacting with cellular components and biomacromolecules including DNA and RNA alter cellular processes.

From the point of view of the antimicrobial activity, the metal nanoparticles at nanomolar concentrations exhibit the excellent results on the inhibition of bacterial strains. In last few years, magnetic nanoparticles have become a very useful tool in variety of research areas such as biotechnology, biomedicine, and magnetic resonance imaging, in waste water treatment or information technology [4].

The superparamagnetic nanoparticles have a fast response to applied magnetic field. They will randomize their directions and become neutral again almost immediately after the field is turned off because the thermal energy will flip the dipoles in random directions [5,6].

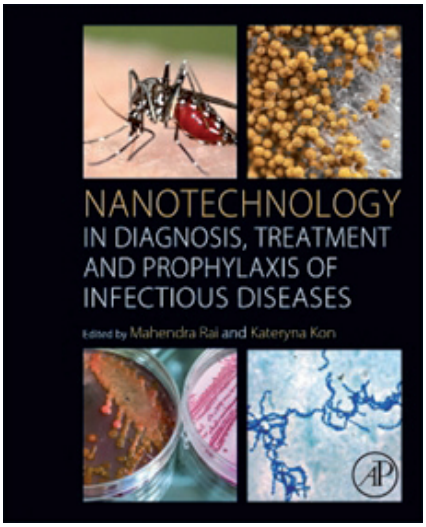

Figure 2: Cover of the book Nanotechnology in diagnosis, treatment and prophylaxis of infectious diseases. Addapted from the [3]

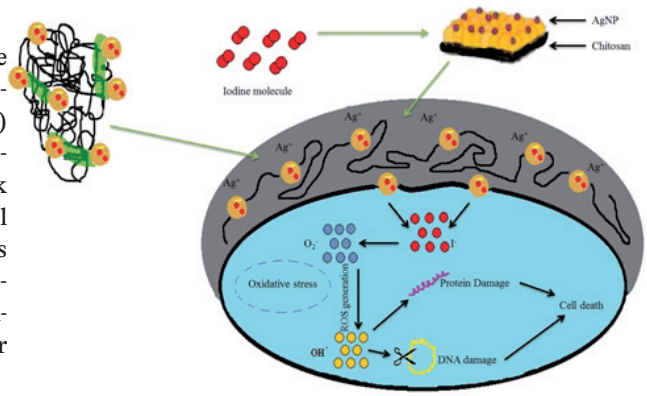

Figure 3: Schematic representation of the proposed mechanism of antibacterial activity of the iodinated chitosan-Ag NPs composite. Addapted from Bajernee et al. [7]

The need of the antimicrobial agents with the similar effect as antibiotics is increasing rapidly. Metal nanoparticles are characterized by significant antimicrobial properties, which lead to elimination of bacterial infections. At present, testing of antimicrobiality of different compounds is very interesting for the research groups worldwide. Possible toxicity of metal nanoparticles to eukaryotes can be eliminated by formation of composites with biopolymers like chitosan. Nanotechnological use of metals in the fight against infectious pathogens due to their antimicrobial properties and low toxicity to the host cell is a promising future.

This scientific book is certainly suitable material for familiarization with modern trends in the treatment of bacterial infections. The covered subjects open horizons in still not very explored areas; however, which offer effective and progressive possibilities in the fight against infectious pathogens. The book is thus uniquely beneficial not only for scientific groups but also for employees of medical fields.

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Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

References

1. www.researchgate.net, 2015.
2. M. Rai and K. Kon Nanotechnology in Diagnosis, Treatment and Prophylaxis of Infectious Diseases, Elsevier, 2015.
3. www.store.elsevier.com, 2015.
4. P. Tartaj, M.D. Morales, S. Veintemillas-Verdaguer, T. Gonzalez-Carreno and C.J. Serna, „The preparation of magnetic nanoparticles for applications in biomedicine", Journal of Physics D-Applied Physics, 2003, pp. R182-R197.
5. A.H. Lu, E.L. Salabas and F. Schuth, „Magnetic nanoparticles: Synthesis, protection, functionalization, and application", Angewandte Chemie-International Edition, 2007, pp. 1222-1244.
6. U. Jeong, X.W. Teng, Y. Wang, H. Yang and Y.N. Xia, „Superparamagnetic colloids: Controlled synthesis and niche applications", Advanced Materials, 2007, pp. 33-60.
7. M. Banerjee, S. Mallick, A. Paul, A. Chattopadhyay and S.S. Ghosh, „Heightened reactive oxygen species generation in the antimicrobial activity of a three component iodinated chitosan-silver nanoparticle composite", Langmuir, 2010, pp. 5901-5908.


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