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-

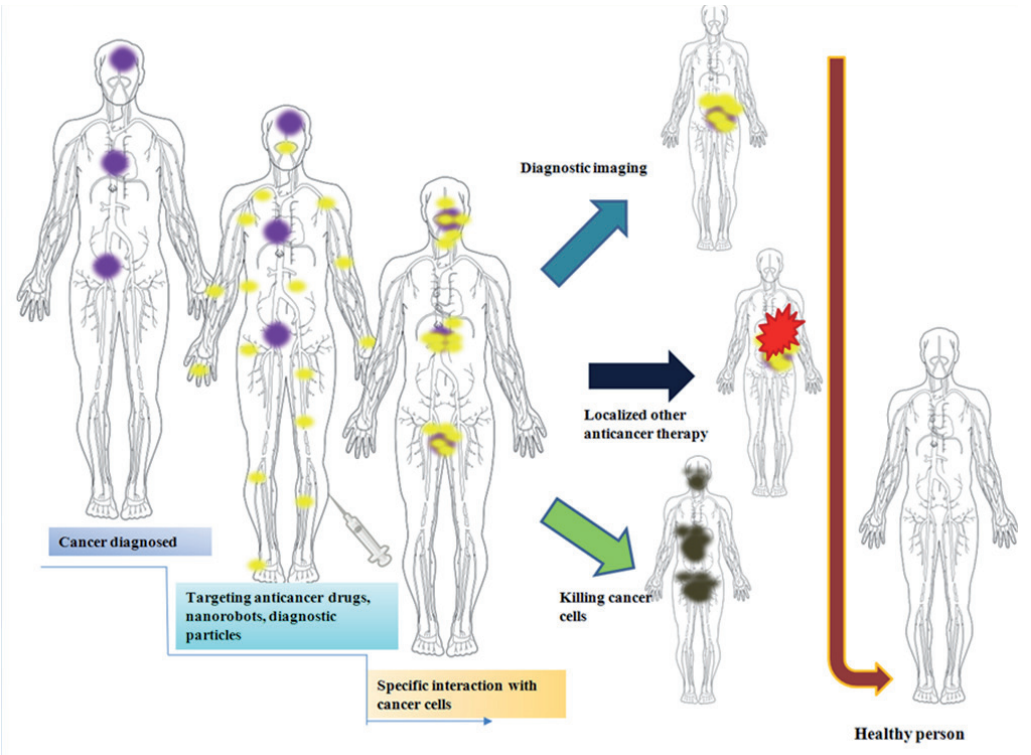

Figure 1: Advanced nanomedicine will be able to provide much earlier diagnosis and/or therapy of cancer. A patient who is suspected to have cancer will likely undergo an application (targeting anticancer drugs, nanorobots, diagnostic particles) into the bloodstream. Then, special particles will specifically 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

11. 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.