Prostate Cancer, miRNAs, Metallothioneins and Resistance to Cytostatic Drugs

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Abstract: MicroRNAs (miRNAs) transtranslationally repressing their target messenger RNAs due to their gene-regulatory functions play an important but not unexpected role in a tumour development. More surprising are the findings that levels of various miRNAs are well correlated with presence of specific tumours and formation of metastases. Moreover, these small regulatory molecules play a role in the resistance of cancer cells to commonly used anti-cancer drugs, such as cisplatin, anthracyclines, and taxanes. In that respect, miRNAs become a very attractive target for potential therapeutic interventions. Improvements in the sensitivity of miRNAs detection techniques led to the discovery of circulating miRNAs which became a very attractive non-invasive biomarker of cancer with a substantial predictive value. In this review, the authors focus on i) oncogenic and anti-tumour acting miRNAs, ii) function of miRNAs in tumour progression, iii) possible role of miRNAs in resistance to anticancer drugs, and iv) diagnostic potential of miRNAs for identification of cancer from circulating miRNAs with special emphasis on prostate cancer. Moreover, relationship between miRNAs and expression of metallothionein is discussed as a possible explanation of resistance against platinum based drugs.

Keywords: Prostate carcinoma, markers, miRNA, circulating miRNAs, metallothionein, anti-cancer drugs, platinum based cytostatics, zinc, resistance, metastases.

INTRODUCTION

Cancer is the leading cause of mortality in Western countries and the second leading cause of death in developing countries [1]. The burden of cancer is rising in economically developing countries: their population is growing and aging and it adopts cancer-associated lifestyle choices including smoking, physical inactivity, and “westernized” diets [2].

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males. The prevalence of prostate cancer increases with age. It does, however, usually respond to treatment and, if localized, may be curable. The rate of tumour growth varies from very slow to moderately rapid, and some patients may have prolonged survival, even after the cancer has metastasized to distant sites. Prostate tissue responds to steroid hormones; the prostate cancer is likewise characterised by transition from androgen independent (AD) stage - where anti-androgen therapy (often castration) can be applied - to androgen independent (AI) stage, where the therapeutic prognosis rapidly declines. Androgen receptor (AR), also known as NR3C4, is cytoplasmically located receptor which after binding its ligand testosterone or dihydrotestosterone translocates to the nucleus and activates specific gene expression there.

A change from healthy cell to cancer one is often characterised by a loss of E-cadherin, a cell adhesion molecule important for the maintenance of tissue integrity. The loss of E-cadherin permits cells to detach from the original niche, form a tumour mass and/or invade blood or lymphatic system. This biochemical and morphological change is known as epithelial-to-mesenchymal transition (EMT). Migrating cells can re-express cadherins in other tissues or organs where they home and thus form secondary lesions. Gene expression of E-cadherins is regulated by promoter methylation of CpG island, which is induced by transcriptional repressors.

Other extracellular matrix interacting or cell adhesion molecules such as integrins [3] are involved in transition from healthy to transformed cell and can become targets of pharmacological intervention [4]. Tumour formation has been analysed from various aspects (genome, transcriptome, metabolome, and other -omes). In this review, two aspects of prostate cancer are discussed - zinc metabolism and function of miRNAs, and the focus is given on the role of these factors in response to chemotherapy and acquired resistance to anticancer drugs.

ZINC METABOLISM AND METALLOTHIONEINS

Among other characteristics, healthy and particularly tumorous prostate tissue is unique in its relation to zinc ions Fig. (1). Healthy prostate is highly specialized in zinc accumulating processes: intracellular zinc level ranges in up to tenfold concentrations compared to most other tissues [5]. In contrast, a significant decrease of intracellular zinc concentration has been observed already from early stages of tumorigenesis [6-9]. Although some conflicting results were reported [10], the decrease of prostate’s tissue zinc may be considered as well evidenced and established [11]. Due to the fact that zinc cannot freely pass through the membranes, the crucial role in the maintenance of intracellular zinc level is provided by zinc-transporting proteins, ZIPS (Zrt-Irt like protein or Zinc Iron permease) and ZnTzs (Zinc transporters). Zinc transport has been discussed in detail in several recent reviews [12, 13]; this review emphasises in particular the decrease of cellular zinc in prostate cancer notably caused by down-regulation of zinc transporter ZIP1 expression [11, 14, 15]. Mechanisms causing the down-regulation of ZIP1 and other transporters in prostate cancer have been addressed recently [16]. Since no mutations have been identified in the zinc transporters’ genes [12, 14], the attention focused on epigenetic processes and association of silenced activator protein AP-2α and ZIPs have been studied in situ model [17]. ZIP1 is very likely down-regulated by Ras Responsive Element Binding Protein-1 (RREB-1) which was shown to be up-regulated in prostate cancer due to Ras-Raf-MEK-ERK cascade [18]. Mihelich et al. also report a possible regulatory role of miRNA on zinc transporters: a miRNA cluster miR-183-96-182 (miR-96 and miR-183 are expressed as a cluster with miR-182) was found overexpressed in prostate tissue [19]. The overexpression of this cluster was associated with suppression of multiple zinc transporters, including ZIP1.

The reduction of the cellular zinc in prostate cancer is not only a minor consequence of wide myriad of genetic aberrations, but rather an important step in the pathogenesis. It has been experimentally demonstrated that the loss of zinc accumulation in situ is es-
sential for prostate cancerogenesis [20]. Thus, the complex understanding of the intracellular zinc, respectively ZIP1 regulation, is of high importance with particular application in targeted therapies.

Recently, the role of zinc has been extensively studied. Possible impacts of zinc in cancer development include influence on gene transcription, energetic metabolism, cell migration and invasivity, and cell cycle [21-24]. Interestingly, conflicting results were published regarding various tumours. Zinc may promote cancer proliferation in some cancers, while in others it exerts an opposite effect. These carcinogenic/protective effects of zinc(II) seem to be very complex and they manifest in cancer-dependent manner [22].

The impact of zinc on energetic metabolism of prostatic cells is well-established. Zinc has inhibitory effects on mitochondrial enzyme aconitase which catalyses the conversion of citrate to isocitrate and thus enables the utilization of citrate in Krebs cycle. Due to aconitase inhibition, prostate cells cannot fully utilise citrate oxidation [25]. As an opposite, because zinc(II) level is decreased in cancer, the inhibitory effect of zinc(II) on aconitase is abolished, citrate can enter the Krebs cycle, and cancer cells can then become more energy-efficient [5, 26]. One may speculate that this “energetic turnover” may provide prostate cancer cells with growth advantage over healthy counterparts.

Zinc is also known to play an important role in apoptosis induction. Increased intracellular concentration of zinc induces release of cytochrome C, which initiates caspase cascade leading to apoptosis [27, 28]. However, the apoptogenic effects of zinc are cell-specific and in other cell types zinc can play a protective role against apoptosis induced by other factors [22, 29].

As a result of disturbances in zinc homeostasis in prostate tumours, several studies reported changes in the serum zinc(II) level in prostate cancer patients. Studies performed by Adamamoye et al. and Goel et al. revealed significantly lower level in patients of all PSA levels [30, 31]. Similar reduction was observed in group of 41 participants using whole blood analysis of zinc(II) level [32]. However, a study on larger set of participants (1,175 US participants) by Park et al. did not observe any difference and no association between serum zinc level and prostate cancer risk in cancer and control groups [33].

The disturbances of zinc homeostasis can be reflected in the activity of transcriptional networks through many regulatory proteins containing zinc in their catalytic centre or in their structure. It is estimated that about 1% of human genome is coding for zinc-finger transcription factors for regulation of gene expression. Zinc fingers are small structural protein motifs accommodating one or two zinc atoms that help to stabilise protein folding.
Micro-RNAs (miRNAs) are another factor often connected with tumour development. In this review, we demonstrate that zinc homeostasis and miRNAs are in prostate carcinoma part of mutually interlinked network where these factors might influence each other Fig. (1).

One of the proteins with zinc-finger domains plausible connecting miRNA metabolism with zinc homeostasis is Lin28 homolog B. This protein is overexpressed in hepatocellular carcinoma and ovarian primitive germ cell tumours [34]. Lin28 was found to inhibit the biogenesis of let-7 miRNAs through a special domain that contains motif zinc-knuckle and specifically binds to the terminal loop of pre-let-7 [35]. Similarly, miR-138 was found to regulate the epithelial-to-mesenchymal transition via direct targeting of zinc finger E-box-binding homeobox 2 in head and neck squamous cell carcinoma and thus promote cancer progression [36].

Conversely, there were also miRNAs identified with the ability to down-regulate the expression of proteins with zinc fingers domains. Using the bioinformatic approach, miR-181a inhibits the expression of large number of zinc fingers proteins by directly targeting their coding sequences [37]. These inhibitory effects might be due to multiple target sites mostly located in the regions coding for the ZNF C2H2 domain within the ZNF gene [37]. As one of these mechanisms, promyelocytic leukaemia zinc finger (PLZF) was identified as a repressor of miR-221 and miR-222 by direct binding to their putative regulatory region [38]. Through this pathway, the progression of melanoma may be controlled.

Alongside to zinc fingers, miRNAs may regulate the expression of other zinc-related proteins associated with tumours – matrix metalloproteases (MMPs). The expression of this large family of zinc endopeptidases is under the control of multiple signalling pathways responding to various hormones, cytokines, and growth factors. MMPs are also regulated post-transcriptionally by controlling mRNA stability, protein translation, and recently by miRNAs [39]. For instance, matrix metalloprotease-2 was found to be regulated by miR-21 in a model of myocardial infarction. MiR-21 directly targets PTEN and through this pathway regulates an increase of MMP-2 in infarct area [40].

miRNA

Among all previously described factors involved in the initiation and development of prostate cancer another element interconnecting several cellular processes may be traced. This element is represented by microRNAs (miRNAs), short non-coding regulatory molecules involved in multitude of processes in eukaryotic cells. They play a role in virtually each step of tumour formation and progression [41-43]. miRNAs networks affect apoptotic pathways, cellular growth, responsiveness to growth factors and anticancer drugs, inhibit expression of tumour suppressor genes or permit expression of oncogenes Fig. (2). Classical textbooks refer to carcinogenesis as a harmonic process caused by a loss of function of tumour suppressor genes and simultaneous activation of oncogenic genes. Recent progress in miRNAs function studying did not change this definition substantially; it only extended our understanding of regulation of this intrinsic network by miRNAs which can be likewise characterised as oncogenic miRNAs and anti-tumour miRNAs. Oncogenic miRNAs are those that directly or indirectly suppress gene expression of tumour suppressors or pro-apoptotic genes and vice versa anti-tumorigenic miRNAs are those that reduce expression of oncogenic proteins. miRNAs are involved in nearly all types of cancer studied so far and they target
classical oncological pathways. However, certain miRNAs were specifically associated with defined tumour types suggesting that they are involved in specific processes related to a cancer type or a tissue of origin.

With regard to the number of genes regulated by miRNAs it is not surprising that these small regulatory molecules play a role also in the resistance of cancer cells to various anti-cancer drugs. In that respect, miRNAs become very attractive target for potential therapeutic interventions. Recent research has revealed existence of miRNAs circulating in human blood serum. More surprisingly, it was found that levels of various miRNAs are altered in correlation to various physiological changes and some of these changes are well correlated with tumour existence. This makes circulating miRNAs a very attractive non-invasive cancer biomarker.

ROLE OF miRNAs IN TUMORGENESIS

Loss of Tumour Suppressors

Cellular transformation is usually initiated by aberrant expression or function of genes affecting cell cycle and/or cell proliferation. These changes can be caused by a mutation in the coding sequence of tumour suppressors, by deletion of gene loci or altered regulation of gene expression. Genes for miRNAs, which are often located inside introns of structural genes or are independent mono- or polycistronic units, are subject to the same changes. Chromosomal rearrangements leading to deletion of large DNA segments lead to deletions of genes for miRNAs suppressing expression of oncogenic proteins. Chromosomal translocations or retroviral insertions place miRNA genes to the vicinity of promoter and activate transcription of miRNAs which reduce levels of tumour suppressor genes. Under physiological conditions, abnormal proliferation or cell cycle regulation initiates process of apoptosis. High levels of miRNAs targeting pro-apoptotic genes will allow cells to escape this regulatory feedback and further promote cancer development.

Importance of miRNAs is underscored by the fact that nearly half of the genes coding miRNAs are located at fragile sites or at regions with lost homozygosity [44]. For example, a loss of p-arm of chromosome 1 is a common finding in sporadic colon carcinomas. Among many genes associated with DNA repair, checkpoint functions, tumour suppressors, etc. are also multiple miRNAs [45]. The most critical is miR-34a, directly regulated by tumour suppressor gene p53 [46] and classified now as tumour suppressor itself. Ectopic miR-34a expression induces apoptosis and a cell cycle arrest in G1 phase. Downstream targets of miR-34 are Bcl2, MYCN [47], NOTCH1, Delta1, CDK4 and p53. These are all altered in cancer, and miR-34a cooperatively regulates expression of the E-cadherin transcriptional repressors ZEB1 (also known as SIP1), factors previously implicated in EMT and carcinogenesis of various tumours, including prostate cancer, is associated with epithelial-to-mesenchymal transition (EMT). It is a process of dedifferentiation important for many embryonic processes and wound healing. This process is associated with changes in epithelial cell morphology and gene expression. These cells might gain invasive properties associated with metastasis caused by loss of cell adhesion molecules such as E-cadherin or by increased expression of mesenchymal markers. Among most often studied regulators of mesenchymal phenotype belongs a family of E-box binding proteins (Snail, Slug, ZEB1, and ZEB2). In cells undergoing EMT transition the expression of miR-200 family (miR-200a, -200b, -200c, -141, and -429) was markedly reduced. These miRNAs cooperatively regulate expression of the E-cadherin transcriptional repressors ZEB1 (also known as deltaEF1) and ZEB2 (also known as SIP1), factors previously implicated in EMT and tumour metastasis. Inhibition of the microRNAs was sufficient to induce EMT in a process requiring up-regulation of ZEB1 and/or SIP1. Conversely, ectopic expression of these microRNAs in mesenchymal cells initiated epithelial to mesenchymal transition (EMT) [58]. The regulatory loop comprises also p53, repressing EMT by direct activation of miR-200 and -192 transcription [59]. Another microRNA often attenuated in human carcinomas is miR-199a-3p. Formari et al. [60] has found that miR-199a-3p target of mammalian rapamycin (mTOR) and c-Met (Hepatocyte Growth Factor Receptor). Restoring levels of miR-199a-3p in hepatocellular carcinoma led to G1-phase cell cycle arrest, reduced invasive capability, and enhanced sensitivity to doxorubicin-induced apoptosis.
Increase of Oncogenic miRNAs

There are certain miRNAs, which can suppress carcinogenesis, but others can facilitate the process of malignant transformation and tumour development. These miRNAs are characterised as oncogenic miRNAs, actively acting on 3’UTRs of p53, pro-apoptotic genes or other tumour suppressor genes. In the case of prostate cancer, the androgen receptor (AR) became an interesting therapeutic target.

3’UTR of AR is much longer than originally assumed (~6 kb) and contains many binding sites for miRNAs. Thirteen miRNAs (miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-34c, miR-371-3p, miR-421, miR-449a, miR-449b, miR-634, miR-654-5p, and miR-9) were validated to regulate this UTR [61]. Fifteen miRNAs binding AR mRNAs decreased androgen-induced proliferation of prostate cancer cells. In particular, analysis of prostate cancer miRNAs binding AR mRNAs decreased androgen-induced proliferation of prostate cancer cells. In particular, analysis of prostate cancers confirmed a negative correlation of miR-34a and miR-34c expression with AR levels.

A strategy to down-regulate AR level in combination with antiandrogen therapy may prevent or delay the development of androgen-independent (AI) stage. miRNA mimetics targeting AR 3’UTR are good candidates for such approach. Sikand et al. [62] demonstrated that over-expression of miR-488* downregulates transcriptional activity of AR in androgen-dependent AD as well as in AI cells and blocks the proliferation and induces apoptosis in PCA cells.

Another miRNA upregulated in prostate cancer is miR-375 with approximately 9 fold increased level as compared to non-cancerous tissue [63]. MiR-375 negatively regulates Sec23A protein, a mammalian homolog of yeast Sec23, involved in endoplasmic reticulum / Golgi trafficking. In cancer cells with high level of miR-375, Sec23A is expressed at low level. It was shown that ectopic expression of Sec23A reduces cell growth; therefore, it is reasonable to assume that reduced levels of Sec23A may speed up cell growth. However, the exact mechanism of such action is so far unclear.

Some of miRNAs do not have clearly defined position in carcinogenesis process. For example, miR-125b is an oncogenic miRNA directly inhibiting expression of tumour suppressor p53 or pro-apoptotic genes Puma and Bak1 [64]. Though, in certain cases it was found to be downregulated in multiple samples of various PC [56]. These discrepancies may be biologically relevant or may reflect experimental bias indigenous to the experimental system. The studies are often performed on cell lines derived from primary tumours but the expansion in non-physiological conditions can lead to irrevocable changes in gene expression patterns. Comparison of primary tissue samples with cell lines can lead to inconclusive results.

miRNA AND FORMATION OF METASTASES

Advanced prostate cancer tends to form metastases predominantly localised in bones. Current understanding of the molecular mechanism remains incomplete. So far it is clear that epithelial-to-mesenchymal transition associated with the loss of attachment and migration of cells is mediated by a loss of E-cadherin. There are two main repressors of E-cadherin expression – ZEB-1 and ZEB-2. Both proteins form a repressor complex with histone deacetylase (HDAC) which reduces histone acetylation and thus leads to DNA methylation and transcription inactivation of target genes.

Saini et al. has identified miR-203 as one of important regulators of the metastatic process in prostate cancer [65]. The miRNA is specifically attenuated in bone metastases and ectopic expression of miR-203 reduces development of metastases in bone metastatic model of prostate cancer. This is achieved via inhibition of several critical steps of the metastatic cascade including epithelial-to-mesenchymal transition, invasion and motility. MiR-203 regulates several pro-metastatic genes including ZEB2, Bmi, Survivin, and Runx2. Tryndiak et al. [66] found that increase of miR-200b and -200c upregulates expression of E-cadherin through direct targeting of ZEB-1 and -2.

Additionally, a cooperative action of miR-203 on downregulation of SIRT1 histone deacetylase and disruption of ZEB1 complex by miR-200b up-regulates proapoptotic genes in the p53 pathway. This process results in increased sensitivity of cancer cells to chemotherapy.

miRNAs – THERAPEUTIC TARGET

It is apparent from previous statements that miRNAs represent an interesting therapeutic target or they may become therapeutic agents on their own. Downregulation of oncogenic miRNAs could counteract their tumorigenic properties or improve the sensitivity of lesions to chemotherapy. This was clearly proven by experiments carried out by Blower et al. [64] who tested the response to several anti-cancer drugs in multiple cancer cell lines transfected with precursors and inhibitors of miRNAs previously implicated in cancer biology (let-7i, miR-16, and mir-21) [67]. Changing the cellular levels of miRNAs affected the potencies of number of the anticancer agents by up to 4-fold. The effects differed for various miRNAs and they were also in opposite directions depending on the compound class. These results suggest that pharmacological interactions of miRNAs and individual drugs are very complex. In following text, we attempt to dissect direct links between individual miRNAs and their identified or putative targets and the impact on drug sensitivity.

Introduction of anti-miR-125b into androgen-independent cdc2 cells together with GCP (isosflavone enriched fermentation product having anti-prostate cancer activity [68]) resulted in significant inhibition of the cancer cells as compared to using GCP itself. Similarly, combination of anti-miR-125b with 5 µM cisplatin increased the number of PC cells that entered apoptosis in the presence of androgens [64]. Interestingly, that is not the only example. In lung cancer, reduced expression of miRNA-1 was identified which is normally expressed in bronchial epithelial cells and is downregulated as a consequence of low expression of tumour suppressor C/EBPα, frequently suppressed in cancer cells. Ectopic expression of miRNA-1 in tumour cell lines reverses their tumorigenic properties, such as growth, replication, motility, migration, and clonogenic survival. This is probably a consequence of miR-1 action on oncogenic targets (MET = RTK, Pim-1= Ser/Thr kinase, FoxP1, and HDAC4). Introduction of miR-1 into the cells also induced apoptosis in response to doxorubicin, probably mediated by activation of caspases 3 and 7, and depletion of anti-apoptotic gene Mcl-1 [63]. The effects caused by re-introduction of miR-1 into cancer cells are relatively broad and might suggest that introduction of miR-1 into another types of tumour could have similar antitumour effects. Though, one should be cautious since miR-1 is closely associated with muscle development (mesodermal tissue) and this way it might facilitate EMT.

Interesting relationship between the function of miRNA and response to anti-cancer drugs was identified for let-7. Tumour cells acquire resistance to drugs through various mechanisms including metabolic drug inactivation, drug accumulation in cellular compartments (where the drug is ineffective), and translocating drugs from intra- to extra-cellular spaces. One of such transporters is MDR1 (multi-drug resistance-1), a member of ABC transport family (ATP binding cassette transporters) coding for membrane transporter P-glycoprotein. Substrates for MDR-1 include anthracyclines and taxanes, microtubule targeting drugs. MDR-1 expression is postranscriptionally regulated by IMP-1 (insulin-like growth factor mRNA binding protein). Members of this protein family bind miRNA of various genes and stabilise it from the miRNA degradation. IMP-1 has classic oncofoetal expression pattern and re-
expression occurs in many cancers; it also belongs among proteins which are strongly suppressed by let-7. Downregulation of let-7 leads to activation of protein synthesis of IMP-1 from suppressed miRNA pool which in turn stabilises mRNA for MDR1 and concomitantly increases levels of MDR1 protein in cancer cells. Boyerinas et al. has experimentally shown that let-7g selectively affects the sensitivity of a drug-resistant ovarian cancer cells to taxanes by targeting IMP-1, which in turn causes destabilisation of MDR1 at the mRNA and protein level, and this increases sensitivity of multidrug-resistant cancer cells to taxanes [69]. Other miRNAs directly influencing expression of P-glycoprotein (P-gp) are miR-27a and miR-451 [70]. These miRNAs are upregulated in multidrug resistant cell lines and miRNA mimetics increased level of P-gp in non-resistant cell lines. Treatment of resistant cells with antagonoms reduced level of P-gp. These results suggest that targeting miR-27a and miR-451 can be a therapeutic strategy for modulating MDR in cancer cells.

Another study was looking at miRNA expression changes after transition of primary tumour cells into multidrug resistant population. Head and neck squamous cell carcinoma cell lines UM-SCC-1 and SQ20B were treated with docetaxel at increasing concentrations to develop resistant cell lines. Parental and resistant cells were treated with cisplatin, 5-flourouracil, paclitaxel, methotrexate, and doxorubicin to confirm cross-resistance. The miRNA pattern of resistant cells was then compared with their parental cells. Docetaxel treatment successfully induced primary resistance and multidrug cross-resistance. Resistant cells showed significant downregulation of miR-100, miR-130a, and miR-197, and upregulation in miR-101, miR-181b, miR-181d, and miR-195 expression as compared to their parent cells. Real-time polymerase chain reaction (PCR) analysis confirmed statistically significant downregulation in miR-100 and miR-130a and upregulation in miR-181d expressions [71]. However, the question whether any of identified miRNAs can be used as therapeutic target remains to be resolved.

Doxorubicin is an anticancer drug commonly used for treatment of a number of diverse malignant tumours such as acute leukaemia, non-Hodgkin’s and Hodgkin’s lymphoma and several solid tumours, including neuroblastoma. Despite extensive clinical use, the mechanisms of action of anthracyclines in cancer cells remain a matter of controversy. The following mechanisms were considered: 1) intercalation into DNA, leading to inhibited synthesis of macromolecules; 2) generation of free radicals, leading to DNA damage or lipid peroxidation; 3) DNA binding and alkylation; 4) DNA cross-linking; 5) interference with DNA unwinding or DNA strand separation and helicase activity; 6) direct membrane effects; and 7) inhibition of topoisomerase II [72]. Likewise, with other drugs tumour cells can develop resistance to doxorubicin; manipulation of intracellular miRNAs has a potential to overcome this acquired resistance. Abovementioned work has shown that increase of miR-199a-3p in hepatocellular carcinoma can, among other effects, lead to improved sensitivity to doxorubicin [60].

CISPLATIN RESISTANCE, METALLOTHIONEINS AND miRNAs

Platinum based drugs, namely cisplatin, carboplatin and oxaliplatin became nearly indispensable in our arsenal of anti-cancer drugs nevertheless the fact that cancer cells develop resistance to these compounds Fig. (3). Kumar et al. have compared miRNA signature of cisplatin-resistant versus cisplatin-sensitive ovarian cancer cell lines [73]. From 1500 analysed miRNAs, 11 up- or downregulated miRNAs have been identified. Moreover, the authors have used Ingenuity Pathway Analysis (IPA) which has identified potential pathways and networks involved in cisplatin resistance. Alternatively, a key element of the drug – which is platinum atom – can be a focus of interest. A possible way how to acquire resistance to heavy metal containing drugs is an expression of proteins with very high affinity for metal forming active centre of the compound. The most commonly present proteins important for intracellular management of cations such as Zn, Cd, As or Pt belong to the family of metallothioneins (MTs). These are very small proteins rich in cysteine, under physiological conditions binding predominantly zinc; however, the affinity of MTs for platinum exceeds the affinity for zinc by several orders of magnitude [74]. It was shown that increased expression of MTs has marked impact on sensitivity of cancer cells to the treatment with cisplatin, however, the mechanism is still unclear but intensively investigated [75-81]. Can miRNAs participate here? There are no data available but our laboratory has used bioinformatic approach and with the aid of TargetScan software (release 6.1) (http://www.targetscan.org/) has identified multiple binding sites for several different miRNAs in 3’UTRs of miRNAs for MT-1 and MT-2 [82]. Yet another miRNA binding sites were extracted from the microRNA.org database [83] as it is shown in Fig. (4).

The precise relationships between levels of metallothioneins at different stages of PCa progression are yet a bit unclear. Tissue zinc(II) levels are increased in benign hypertrophy of prostate; however, in prostatic cancer these ions’ levels are reduced again. This cannot preclude the possibility of gain a growth advantage and expansion in drug resistant fashion in cells with elevated levels of MTs after the use of cisplatin drugs.

A computer analysis has shown that MT-1 and -2 have redundant binding sites for many miRNAs, including miR-23a and -23b which were found to be downregulated in prostate cancer [84]. Downregulation of miR-23 might elevate levels of MTs which in turn reduce effectivity of cisplatin. Another miRNA predicted to interact with MTs mRNA is miR-224 which was found to be upregulated in perineuronal invading (PNI) PCa cells [85] and potentially reduces MTs levels Fig. (1). Surprisingly, downregulation of multiple MTs genes in PNI cancer cells was shown. It is also important to note that not all tumours upregulate miRNA-224 and downregulate MTs. It is tempting to speculate that these two markers can have predictive value in respect to the potential of development of resistance to cisplatin.

Another way to control levels of MTs in cells and the way how platinum based drugs are sequestered is through regulators of MTs expression. A key regulator of intracellular free zinc level is metal regulatory transcription factor 1 (MRTF-1, also called MRE-binding factor) [86]. This 753 amino acids transcription factor directly responds to the elevated zinc(II) level and induces the transcription of MT and main zinc transporter responsible for its export, ZnT-1 [87-89]. This autoregulatory loop maintains narrow optimal limits of intracellular zinc(II); when the level of MT and ZnT-1 is elevated, more free zinc(II) and cisplatin may be buffered (i.e. bound to MT)
and more zinc may leave cells (through larger amount of membrane transporters). MTF1 has rather (over 5 kb) long 3'-UTR and per-
haps not surprisingly miRDB software has predicted over 45 miR-
NAs interacting with this mRNA. It is reasonable to speculate that
elevated expression of MTs in cisplatin resistant cancer can be at
least partially related to mis-regulation of miRNAs targeting MTs
or MTF1. It can be also assumed that introduction of miRNAs bind-
ning MTs mRNA may have a beneficial effect on sensitivity of cells
to the treatment with platinum compounds.

**EPIGENETIC CONTROL OF miRNAs EXPRESSION**

Epigenetic regulation is a common mechanism to control gene
expression. Gene silencing is often accompanied by methylation of
CpG dinucleotide; it takes place in so-called CpG islands inside or
in the vicinity of gene promoters and leads to changes in chromatin
structure. Modification of chromatin includes histone modification,
permitting or prohibiting gene transcription. The transcriptional
control is executed by several enzymes, including DNA methyl-
transferases (DNMTs), histone acetyltransferases (HATs), histone
deacetylases (HDACs), histone methyltransferases (HMTs), and
histone demethylases (HDMTs) [90, 91]. Function and activity of
individual enzymes is regulated by chromatin remodelling com-
plexes such as polycomb group (PCG) proteins. Epigenetic regula-
tion is commonly subject to alteration in process of malignant trans-
fomation. Chromatin remodelling enzymes can be themselves
targets of miRNAs. One such example is regulation of enhancer of
zeste homolog 2 (EZH2), a histone methyltransferase and part of
the PcG complex. It was demonstrated that in PCa cells EZH2 acts
as an oncogene and is directly regulated by miR-101 and miR-26a
[92]. Interestingly, there are androgen receptor binding sites in
miR-101 promoter and AR binding activates miR-101 expression.
This indicates that miR-101 can be therapeutic target to reduce PCa
cells growth and invasiveness. This idea is further supported by the
finding that genomic loss of miR-101 negatively correlates with an
increased expression of EZH2 in PCa [93]. Another miRNA di-
rectly connected with the regulation of epigenetic state of cell is
miR-449a; it controls expression of histone deacetylase (HDAC1)
in PCa cells [94]. Histone deacetylases are frequently overex-
pressed in a broad range of cancer and they promote cell prolifera-
tion and survival. Noon et al. have found that miR-449a is down-
regulated in PCa and its re-introduction into cancer cells causes cell
cycle arrest, apoptosis, and senescent-like phenotype.

Alternatively miRNAs themselves can be subject of epigenetic
regulation. Formosa et al. has applied demethylating agent 5-aza-
2'-deoxycytidine (azaC) to normal prostate epithelial and carcino-
ma cells and subsequently examined changes in expression of
miRNAs [95]. Multiple miRNAs had modified their expression in
the presence of azaC. Moreover, analysis of CpG islands present in
miRNA promoters revealed changes in DNA methylation. The
authors showed that in prostate cancer miR-132 is silenced by DNA
methylation. Another study by Hult et al. has identified miR-205,
miR-21, and miR-196b to be epigenetically repressed, and miR-615
epigenetically activated in prostate cancer [96].

**CIRCULATING miRNAs**

Development of new highly sensitive methods of miRNA de-
tection led to identification of miRNAs circulating in blood. Circu-
lating miRNAs are present in bloodstream in a very stable extracel-
lar form resistant to RNA digestion by ubiquitously present RNA-
ases. The mechanism of mRNA release from cells to extracellular
space is unknown as well as it is unclear whether it is an active
secretion mechanism or a by-product of non-apoptotic or apoptotic
cell death. It is also not clear whether the circulating miRNAs have
any physiological function, such as translating information to
neighbouring cells or even distant organs. Current assumption is
that miRNAs are encapsulated in membrane vesicles such as
exosomes; however, recent research suggests that this assumption is
false or at least incomplete. Arroyo et al. [97] has demonstrated that
miRNAs circulating in human plasma form a RNA-ase resistant
complex with Argonouate2 protein, the key factor of miRNA medi-
sated silencing and part of the RISC complex.

Regardless of present lack of knowledge of their physiological
function, several studies have shown that circulating miRNAs re-
veal the health status of the body and thus pathological or physio-
logical changes are reflected in a signature of circulating miRNAs.
For example, several miRNAs were found to be upregulated in
pregnant women. Altered levels of several other miRNAs were
identified in human serum; they can be good and early indicators of
tumours as well as prognostic markers for prospective therapy.
Tumour specific dysregulation of miRNAs was described first in
2008 by Lawrie et al. who identified altered expression profile of
miRNAs in serum of patients suffering from diffuse large B-cell
lymphoma [98].

The major advantage of miRNAs as prognostic marker is their
easy isolation from blood serum. Moreover, high sensitivity of ana-
lytical methods allows identification of asymptomatic individuals
with early stage of lung cancer [99]. Furthermore, a specific
miRNA signature can also identify the type of tumour. Neverthe-
less, it is important to notice that circulating miRNAs are correlated
with histopathological parameters and not directly associated with
patient’s outcome in certain malignancies. Brase et al. [100] studied
circulating miRNAs in serum of patients with different types of
prostate cancer. He found that in sera of patients with aggressive
metastatic cancer miR-141 and miR-375 were more abundant and
that their concentration well correlated with prostate tumour pro-
gression. Both miRNAs were also highly upregulated in cancer
tissue. On the other hand, Mahn et al. [101] correlated miRNAs -
26a, -16, and -195 with various types and stages of prostate cancer.
Gonzales et al. [102] identified miR-141 as clinically valuable di-
agnostic biomarker. However, it is hard to speculate at present
which of miRNAs will be clinically useful as tumour marker.

In case of lung cancer, miR-486, miR-30d, miR-1, and miR-499
were identified as significant biomarkers of non-small cell lung
 carcinoma and were associated with overall survival [103]. Surpris-
ingly, this study has shown that several miRNAs previously identi-
fied in cancer tissue were not identified in serum by Solexa se-
quencing. Therefore, the predictive role of serum miRNAs might be important. However, miRNAs identified in serum do not have to be necessarily the same as miRNAs expressed in tumour tissue. It should be taken in consideration that circulating miRNAs probably reflect more general health status and might not be explicitly linked to a specific gene expression pattern in individual tumour cells. In the abovementioned study, clear and significant associations between miRNAs signatures from serum and lung cancer prognosis, potentially useful in clinical practice, were found, but the found mechanism needs additional exploration [103].

Many laboratories generate vast quantity of data on mRNA or miRNA expression in various types of cancer and/or healthy tissues. Extraction of biologically relevant information belongs among the most demanding tasks of contemporary bioinformatics. MicroRNAs are important regulators of signalling pathways. Therefore it is important to take into consideration whether expression of studied miRNAs correspond to identified signalling pathways. A web resource allowing analysis of functional significance of miRNAs in various tissues is now available under the name miTALOS [104]. As a novel feature, this software considers the tissue specific expression signatures of miRNAs and target transcripts to improve the analysis of miRNA regulation in biological pathways. Though, one must be very careful in comparing methods of microRNAs detection regarding their sensitivity and clinical information value. There is a big variation among techniques using quantitative real time PCR based on different systems and comparing data of global expression profiles based on chip hybridisation techniques or with massive parallel sequencing techniques. Only standardised techniques and large patient cohorts will reveal which results are statistically significant, however biologically irrelevant, which are simply false based on applied technique of miRNA isolation or analysis, and those which are truly relevant and will become standards in future diagnostics of tumours.

CONCLUSION

Recent scientific discoveries clearly demonstrate that miRNAs are probably as important in cancer biology as protein coding genes. Moreover, miRNAs were shown to be differently expressed in tumours that had acquired resistance to chemotherapy. On the other side, different levels of intracellular miRNAs can change responsiveness of cells to various cytostatic compounds. Further research is required to identify correlations among specific miRNA expression levels and drug classes. Such research is necessary for future progress toward personalised therapy based on gene expression profile of tumour in a given patient and for rational design of treatment regime.

Many questions remain unanswered. The very complex nature of regulatory networks (including miRNAs, protein expression, modifications and interactions) obscures truly relevant interplays. On the other hand, oversimplification can preclude an understanding of important synergies among players. The scientists have to eternally balance on the edge between these two extremes and perhaps then, answers relevant for future treatment and therapy might be found.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

3' UTR = 3' Untranslated Region
ABC = ATP Binding Cassette Transporters
AD = Androgen Dependent
AI = Androgen Independent
AP-2α = Activator Protein 2α
AR = Androgen Receptor
azaC = 5-aza-2'-Deoxycytidine
CDC25 = Cell Division Cycle 25 – Phosphatise Activating CDK1
CDK = Cyclin Dependent Kinase
cMET = Hepatocyte Growth Factor Receptor
CSC = Cancer Stem Cells
DNMT = DNA Methyltransferase
EMT = Epithelial-to-Mesenchymal Transition
EZH2 = Enhancer of Zeste Homolog 2
GCP = Isoflavone Enriched Fermentation Product
HAT = Histone Acetyltransferase
HDAC = Histone Deacetylation
HDMT = Histone Demethylase
HMT = Histone Methyltransferase
IMP-1 = Insulin-Like Growth Factor mRNA Binding Protein 1
IPA = Ingenuity Pathway Analysis
MDR1 = Multi-Drug Resistance-1
MET = Mesenchymal-to-Epithelial Transition
MMPs = Matrix Metalloproteinases
MT = Metallothionein
MTF-1 = Metal Regulatory Transcription Factor 1
Pcα = Prostate Cancer
PcG = Polycomb Group
P-gp = P Glycoprotein
PLZF = Promyelocytic Leukaemia Zinc Finger
PNI = Perinuclear Invasion
PSA = Prostate Specific Antigen
Rb = Retinoblastoma
RISC = RNA-Induced Silencing Complex
RREB-1 = Ras Responsive Element Binding Protein-1
RTK = Receptor Tyrosine Kinase
ZIP = Zrt-Ir Like Protein or Zinc Iron Permease
ZNF = Zinc Finger
ZnT = Zinc Transporters

REFERENCES


