

Taxanes, a group of cancer drugs that includes docetaxel and paclitaxel, have become a front-line therapy for a variety of metastatic cancers, but resistance can develop.

There are several docetaxel resistance mechanisms in prostate cancer: unfavorable tumor microenvironment, drug efflux pump, alterations in microtubule structure and/or function, and apoptotic defects (e.g. up regulation of Bcl-2 and clusterin or activation of the PTEN/PI3K/mTOR pathway or activation of the MAPK/ERK pathway).

MicroRNAs (miRNAs), small regulatory molecules, could also function as a contributor to the resistance of cancer cells to commonly used anti-cancer drugs.

Aberrant expressions of miRNAs that can act as tumor suppressors or oncogenes are closely associated with the development, invasion and metastasis of various cancers including prostate cancer. Nearly 50 miRNAs have been reported to be differentially expressed in human prostate cancer so far, but knowledge concerning the effects of miRNAs on the sensitivity to anti-cancer drugs is still limited. The author of the review focus on probable impact of miRNAs on the resistance to docetaxel and paclitaxel.

Overexpression of miR-21 increased the resistance of prostate cancer cells to docetaxel by targeting PDCD4, PTEN, RECK, and BTG2. Nevertheless, decreased expressions of tumor suppressors: miR-34a, miR-143, miR-148a and miR-200 family are involved in resistance of anti-cancer drugs by inhibition of apoptosis and activation of signaling pathways. Conclude miRNAs become very attractive target for potential therapeutic interventions.

Key words: microRNA, taxanes, chemoresistance, prostate cancer.

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Role of microRNAs in the resistance of prostate cancer to docetaxel and paclitaxel

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Introduction

The taxane class of cytotoxic chemotherapeutic agents has been shown to provide a survival benefit in advanced prostate cancer (PCa), inhibiting tumour development [1]. Taxanes (docetaxel, paclitaxel) suppress micro tubular depolymerization and attenuate the effects of bcl-2 and bcl-xL gene expression. Taxane-induced microtubule stabilization arrests cells in the G(2)M phase of the cell cycle and induces bcl-2 phosphorylation, thereby promoting a cascade of events that ultimately leads to apoptotic cell death [2]. Apart from the afore-mentioned, taxanes may affect androgen receptor (AR) signaling that they inhibit ligand-induced AR nuclear translocation and downstream transcriptional activation of AR target genes such as prostate-specific antigen [3].

Although taxanes inhibit tumour growth and improve survival in advanced PCa, development of resistance is inevitable and patients' disease eventually progresses. Resistance can be formed through a variety of mechanisms; both intrinsic to prostate cancer biology (e.g. AR up regulation, increased extragonadal androgen synthesis) and general resistance mechanisms (limited tumor/tissue penetration, inherently resistant subpopulation of cells, multidrug resistance efflux pump) [4]. Increased transport of the drug out of tumour cells by up-regulation of ATP-binding cassette transporter molecules in the cell membrane, such as P-glycoprotein, is classified as a common resistance mechanism [1].

The mechanisms of docetaxel resistance include inhibition of apoptosis and activation of the extracellular signal-related kinase (ERK)/mitogen-activated protein kinase (MAPK) or phosphatidylinositol-3 kinase/Akt survival pathways as well. Additionally, docetaxel-resistant PCa cells exhibit an epithelial-mesenchymal transition (EMT) phenotype linking docetaxel resistance with the development of metastasis, whereas up-regulation of ZEB1 transcription factor, a direct regulator of EMT, can confer docetaxel resistance [1].

MicroRNAs (miRNAs) are small (~22 nt) non-coding RNAs that regulate gene expression causing post-transcriptional inhibition or degradation of target mRNA. MiRNAs serve crucial components in the regulation of many cellular functions and biological processes [5]. Scientific studies have demonstrated that aberrant expressions of miRNAs, which can act as tumor suppressors or oncogenes, are closely associated with the development, invasion, metastasis and prognosis of various cancers including prostate cancer (PCa) [6]. The initial studies of miRNAs deregulation in prostate cancer were performed by miRNA microarray profiling and, since then, several researchers have analyzed prostate cancer specific miRNA profiles using genome-wide screenings and validation by quantitative PCR technology [7–15].

Nearly 50 miRNAs have been reported to be differentially expressed in human prostate cancer (about forty up- and remaining down-regulated) so far,

Table 1. Some of the miRNAs that expression is altered in prostate cancer [own elaboration]

Up- (↑) or down-regulation (↓) of miRNAs (fold change)							
Refs (year)	Method	miR-21	miR-34a	miR-143	miR-148a	miR-200b	miR-200c
[7] (2013)	PCR array		↑ (4.32)				
[8] (2012)	Micro-array	↑ (1.40)				↑ (1.54)	↑ (2.92)
[9] (2010)	Small RNA cloning*	↑ (1.73)		↓ (0.24)			↑ (4.54)
[10] (2009)	miR-MASA technique**			↓ (0.31)			
[11] (2008)	Micro-array		↓ (0.8)				↑ (1.7)
[12] (2007)	OAH***		↓		↓ (in HRPC)	↓	
[13] (2006)	Micro-array	↑ (~0.20)					

Refs – references; year – year of publication; HRPC – hormone refractory prostate cancer

* Small RNA cloning and deep sequencing

** miR-MASA technique based on liquid-phase hybridization reactions

*** OAH – Oligonucleotide array hybridization

though, knowledge concerning the effects of miRNAs on the sensitivity to anti-cancer drugs is still limited. Several groups of researches have studied whether the sensitivity to docetaxel and paclitaxel could be altered by miRNAs in prostate cancer cells. The development of chemoresistance has been attributed to alterations at the level of miRNAs. Table 1 presents the expression changes only for miRNAs involved in taxanes resistance. These results are not perfectly correlated as it could be caused by the application of different analytical methods.

miR-21 – oncomiR (role in docetaxel-resistance)

The miR-21 is one of the most commonly implicated miRNAs in cancer as its expression is highly up-regulated in a variety of solid tumors. In prostate cancer miR-21 is crucial in tumorigenesis and invasiveness (induction of tumor angiogenesis and initiation of epithelial-mesenchymal transition) by targeting PDCD4, PTEN, RECK, and BTG2. miR-21 can directly down-regulate the expression of PDCD4 (programmed cell death 4; neoplastic transformation inhibitor protein) – suppressor of tumorigenesis in PC3 cells [16]. Overexpression of miR-21 in DU145 cells increased the expression of HIF-1 α and VEGF and induced tumor angiogenesis. MiR-21 induces tumor angiogenesis through targeting PTEN, leading to activate AKT and ERK1/2 signaling pathways and thereby, enhancing HIF-1 α and VEGF expression; HIF-1 α is a key downstream target of miR-21 in regulating tumor angiogenesis [17]. Apart from, in PCa cell line, DU-145 miR-21 directly inhibits RECK, a tumor suppressor gene involved in the control of matrix metalloproteinase 9 (MMP9) [18]. Coppola *et al.* [19] investigated whether down-regulation of the basal protein B-cell translocation gene 2 (BTG2) is implicated in prostate cancer transformation and progression. It was shown that BTG2 loss can shift normal prostate basal cells towards luminal markers expression, a phenotype also accompanied by the appearance of epithelial-mesenchymal transition (EMT) traits. Additionally, the research proved that

the overexpression of miR-21 suppresses BTG2 levels and promotes the acquisition of luminal markers and EMT in prostate cells.

Using microarrays Shi *et al.* [20] found that a number of miRNAs were significantly altered in the docetaxel-resistant PC3 cells (PC3R). miR-21, one of the miRNAs identified by microarrays, was up-regulated in PC3R cells. Ectopic expression of miR-21 increased the resistance of PC3 to docetaxel in the wild type cells. In contrast, silencing of miR-21 with transient transfection of its inhibitors led to sensitize the cells to docetaxel. These findings suggest that miR-21 contributes to the resistance of PC3R cells to docetaxel. Researchers found that miR-21 can directly down-regulate the expression of PDCD4 – suppressor of tumorigenesis. Silencing of PDCD4 expression increased the cell viability and resistance to docetaxel in PC3 cells suggesting that PDCD4 is a functional target for miR-21 induced chemoresistance to docetaxel. MiR-21 functions as a significant regulator of prostate cancer cell resistance to docetaxel, which provides new evidence that miRNAs may be involved in the tumor resistance to chemotherapy.

In another study [21], miR-21 was up-regulated in docetaxel-resistant prostate cancer PC3R cells as well. Similarly to Shi's *et al.* [20] results, ectopic expression of miR-21 increased the resistance to docetaxel in PC3 wild-type cells, while silencing of miR-21 in PC3R cells sensitized the cells to docetaxel. The authors [21] state that miR-21 is not per se a central player in the onset of PCa and that its single hitting does not represent a valuable therapeutic intervention in such a disease. Their findings contribute to support the theory that the oncogenic properties of miR-21 and generally speaking that of any miRNAs could be cell and tissue dependent and its potential role as a biomarker or therapeutic target should be put in the context of a given disease.

Additionally, Zhang *et al.* [22] discovered that serum miR-21 levels were higher in hormone refractory prostate cancer (HRPC) patients than those with androgen depen-

dent prostate cancer (ADPC) and localized PCa. Serum miR-21 levels were higher in the HRPc patients resistant to docetaxel – based chemotherapy, when compared to those sensitive to chemotherapy. As a result, levels of serum miR-21 correlated to levels of serum PSA in patients with metastatic PCa. Their results have indicated that miR-21 may be a useful biomarker for patients with PCa during disease progression.

miR-34a – tumor suppressor (role in paclitaxel- and camptothecin-resistance)

The expression levels of miR-34a were markedly decreased in androgen-refractory PC3 and DU145 cells compared to androgen-sensitive LNPCa and normal prostate epithelial cells. Furthermore, miR-34a expression depends on the p53 activity in prostate cancer cell lines and appears to be completely absent in p53-null PC3 cells. The expression level of silent mating type information regulation 2 homolog 1 (SIRT1) was up regulated in p53-defective PC3 and DU145 cell. SIRT1 deacetylates pro-apoptotic proteins such as p53 and promotes cell survival under genotoxic and oxidative stress. The anti-apoptotic activity of SIRT1 is implicated in tumorigenesis. In addition, SIRT1 is suggested to be involved in resistance to anticancer drug. In p53-null PC3 cells, introduction of p53 increased miR-34a expression. Ectopic expression which in turn reduced SIRT1 expression. It is therefore presumed that miR-34a can inhibit cell growth and enhance chemosensitivity to camptothecin [23].

Kojima *et al.* [24] have explained miR-34 action. The miR directly and indirectly via regulating HuR expression acts on the 3'-UTR of SIRT1 and Bcl2 mRNAs and suppresses their expression. Decreased expression of miR-34a leads to the up-regulation of SIRT1 and Bcl2, resulting in resistance to apoptosis caused by paclitaxel.

MicroRNA profiling of DU14-TXR and PC3-TXR cells and prostate cancer tissue from the patients done by Singh *et al.* [25] showed decreased expression of miR-34a. Experimental replenishment of miR-34a in cultured cells prevents metastasis and invasion. They concluded that chemoresistance to paclitaxel in DU145-TXR and PC3-TXR cells is possibly regulated by miRNAs, which are differentially expressed when the paclitaxel sensitive cell line is transformed to a resistant phenotype.

miR-143 – tumor suppressor (role in docetaxel-resistance)

Xu *et al.* [26] observed an inverse correlation of expression between miR-143 and KRAS protein (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) in prostate cancer cell lines. Oncogene KRAS is the key molecule of EGFR/RAS/MAPK signaling way which regulates a variety of biological activities, including cell proliferation, migration and chemosensitivity. In addition to KRAS, ERK5 (extracellular signal-regulated kinase 5) becomes also a target of miR-143. Ahmad's *et al.* [27] findings show a significant correlation between low miR-143 and elevated ERK5 levels in primary human prostate cancers. MiR-143 contributes to suppressing tumor cell growth. Over-expression of

miR-143 strikingly inhibited migration of prostate cancer cells *in vitro*. MiR-143-treated prostate cancer cells showed higher chemosensitivity to docetaxel, proving that miR-143 enhanced their response to docetaxel [26].

miR-148a – tumor suppressor (role in paclitaxel-resistance)

Fujita *et al.* [28] demonstrated that miR-148a is down-regulated in prostate cancer PC3 cells and DU-145-hormone refractory prostate cancer cells. A direct target of miR-148a in PC3 cells is mitogen- and stress-activated kinase 1 (MSK1). Ectopic expression of miR-148a decreased expression of MSK1 and inhibited growth, migration and invasion of PC3 cells. MiR-148a functions as a tumor suppressor. In paclitaxel-resistant cell line from PC3 cells (PC3PR), miR-148a attenuated the resistance to paclitaxel. The authors concluded that miR-148a is a promising therapeutic target for hormone-refractory prostate cancer especially for drug resistance prostate cancer.

miR-200 family – tumor suppressors (role in docetaxel- and paclitaxel-resistance)

Members of the miR-200 family could inhibit epithelial-mesenchymal transition (EMT) and suppress cancer invasion by the direct repression of the translation factors zinc-finger E-boxbinding homeobox 1 and 2 (ZEB1 and ZEB2). MiR-200b is identified as a critical regulator of tumor invasion, metastasis, and chemosensitivity [29].

miR-200b was down regulated in clinic prostatic tumors and in PCa cell lines. Enforced miR-200b expression suppressed PCa cell proliferation and migration and enhanced chemosensitivity to docetaxel by targeting B-cell-specific Moloney murine leukemia virus insertion site 1 (Bmi-1). Bmi-1 was detected at higher levels in PCa and knockdown of Bmi-1 showed similar effects as miR-200b overexpression in PCa cells [30].

Screening for key regulators of an epithelial phenotype revealed a significantly reduced expression of miR-200c in docetaxel-resistant cells. A prolonged treatment with miRNAs resulted in elevated E-cadherin protein levels and an increase in the percentage of apoptotic cells [31].

MiR200c maintains 'epithelialness' of cancer cells by preventing endothelial mesenchymal transition and the assumption of an aggressive chemoresistant mesenchymal phenotype [25].

miR-205 and miR-31 – tumor suppressors (role in docetaxel-resistance)

Bhatnagar *et al.* [32] demonstrated that miR-205 and miR-31 are down-regulated in advanced prostate cancer cells. They identified Bcl-w as the potential target for miR-205, and E2F6 as the potential target for miR-31. Bcl-w is an antiapoptotic member of the Bcl-2 family proteins, whereas E2F6 inhibits UV- and hypoxia-induced apoptosis. The antiapoptotic properties of Bcl-w and E2F6 make them interesting targets for miR-205 and miR-31. Overexpression of miR-205 and miR-31 down-regulated Bcl-w and E2F6, respectively. Conversely, transfection of WPE1-NA22 cells with anti-miR miRNA inhibitors specific to miR-205

and miR-31 increased the protein levels of Bcl-w and E2F6, respectively. The anti-miR-205 and anti-miR-31 inhibitors also blocked docetaxel-induced apoptosis in WPE1-NA22 cells. Having been treated with docetaxel or cisplatin, cells stably expressing Bcl-w were significantly resistant to drug-induced apoptosis, comparing with empty vector-transfected cells. Similarly, cells stably expressing E2F6 were also resistant to docetaxel and cisplatin.

Let-7c

The let-7 family represents complex group of miRNAs that act as tumor suppressors. Let-7 a, b, c, d, f, g were downregulated in prostate cancer and let-7 b, d, i – upregulated [33].

Nadiminty's *et al.* [34, 35] data showed that the levels of let-7c were lower in the castration-resistant cell lines C4-2B, LNCaP-s17 and LN-IL6. Let-7c suppresses prostate tumor growth by several pathways including regulation of IL-6, Myc, Lin28 and the AR.

Downregulation of let-7 leads to the activation of protein synthesis of IMP-1 (insulin-like growth factor mRNA binding protein 1) which increases levels of MDR1 (multi-drug resistance-1) protein in cancer cells. MDR1 acting as a transporter of microtubule targeting drugs (anthracyclines and taxanes) from intra- to extra-cellular spaces, is involved in chemoresistance [36].

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