MicroRNAs and the Response of Prostate Cancer to Anti-Cancer Drugs

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Abstract: Despite considerable advances in early diagnosis, prostate cancer (PCa) remains the second leading cause of cancer-related deaths in men in western countries. In fact, although efficient therapies exist for early-stage disease, the treatment of advanced PCa remains unsuccessful mainly due to its poor responsiveness to anti-cancer agents. This evidence underlines the urgent need for the development of novel and more effective therapeutic approaches. In this context, the documented dysregulation of microRNAs (miRNAs) -which are short non-coding RNAs that regulate gene expression at post-transcriptional level- in PCa, together with their potential to simultaneously regulate multiple oncogenic/tumor-suppressive pathways, has stimulated interest in defining a functional association between altered expression of specific miRNAs and the response of PCa to anti-cancer agents. The purpose of this review is to provide an overview on PCa-related miRNAs as potential novel therapeutic targets/tools, with a special focus on the role that they may play in conditioning the responsiveness of PCa to anti-cancer drugs.

Keywords: Anti-cancer drugs, microRNA, prostate cancer.

1. AN OVERVIEW OF MicroRNAs

1.1. A Glimpse of microRNA Mechanism of Action

MicroRNAs (miRNAs) are a class of short non-coding RNAs (~22 nucleotides in length) that play a key role in modulating gene expression at post-transcriptional level, by causing either mRNA degradation or protein translation inhibition according to perfect or partial base pairing of their seed region and the target transcripts [1]. Since the discovery of the first miRNA in Caenorhabditis elegans [2], in the last two decades hundreds of miRNAs have been identified in almost all multicellular organisms, including plants, worms, flies, fish and mammals [3-6], as well as in DNA viruses [7]. To date, more than 14,000 miRNAs have been discovered in humans (annotated in miRBase database, www.mirbase.org), where they represent one of the most abundant classes of regulatory genes and are involved in the control of almost all critical biological and physiological processes, including development, proliferation, differentiation, adhesion, angiogenesis and apoptosis [8].

miRNAs biogenesis (Fig. 1) is a multi-step process that involves several key regulator factors and begins in the nucleus with the transcription of miRNA genes, which, in mammalian cells, are located within the introns of protein-coding genes, within exons/introns of long non-coding RNAs or may be present as independent transcriptional units [9]. In the first step of the biogenesis, miRNA genes are transcribed by the RNA polymerase II into 100-1000 bp-long primary transcripts (pri-miRNAs), that are characterized by a double-stranded stem-loop structure and contain a 7-methylguanosine cap at the 5’ end and a polyadenylated tail at the 3’ terminus [9]. Still in the nucleus, the pri-miRNA is processed into a 70-nucleotides long precursor (pre-miRNA) by the RNase III enzyme Drosha in association with its co-factor DGCR8 (DiGeorge syndrome critical region gene 8). Subsequently, the pre-miRNA is exported via the RanGTP nuclear receptor XPO5 (Exportin-5) into the cytoplasm where it is processed into a 21-24 nucleotide-long mature miRNA duplex (miRNA:miRNA*) by the RNase III enzyme Dicer. During the miRNA maturation process, the strand of the miRNA duplex that will become the mature, active miRNA is selected according to the thermodynamic stability of the duplex itself, which will be unwound from the more energetically favorable terminus. Once correctly selected, the mature miRNA is incorporated into a multi-component complex known as miRISC (miRNA-RNA inducing silencing complex), composed of Argonaute protein (AGO) family members and ancillary factors that mediate miRNA/mRNA recognition and subsequent target regulation [8, 10].

miRNA target sequences are mainly located in the 3’-UTR (untranslated region) of target mRNA, although they can be also found in the 5’-UTR or in the open reading frame [11]. As previously mentioned, depending on the degree of complementarity with its target mRNA, a miRNA can negatively regulate gene expression either by mRNA degradation or inhibition of the translation. However, to further complicate the intricate role exerted by miRNAs in the regulation of gene expression, recent evidence suggests that miRNAs may also target specific sequences in gene promoters, thus favoring gene transcription, a phenomenon known as RNA activation (RNAa) [11, 12]. In this context, Vasudevan and col-
leagues [13] first reported that let-7 can switch repression to activation of translation according to cell proliferating status.

1.2. miRNAs in Cancer

Alterations of physiological miRNA expression levels can lead to a number of human diseases, including cancer [9]. The first evidence of a direct involvement of miRNAs in human cancer was reported in 2002 by Calin and colleagues during the characterization of the 13q14.3 deletion, the most frequent chromosomal anomaly associated to human B-cell chronic lymphocytic leukemia (CLL) [14]. The vain attempt to identify a protein-coding gene causal for leukemia predisposition and residing in the deleted 30-Kb region led the researchers discover two miRNA genes, namely, miR-15a and miR-16-1, located within the locus. Accordingly, both miRNAs are absent or down-regulated in most CLL patients [14]. Successively, Cimmino et al. [15] demonstrated that the anti-apoptotic protein Bcl-2 is a direct target of miR-15a and miR-16-1, thus suggesting that the loss or down-modulation of such miRNAs may contribute to malignant transformation by up-regulating Bcl-2.

Since then, hundreds of miRNAs have been reported to play a key role in tumor initiation and progression [8]. The development of different high-throughput technologies has indeed permitted to analyze the miRNA global expression profiles in pathological vs normal tissues [16]. In this context, growing evidence indicates that tumor tissues show typical miRNA signatures (i.e., miRNome, miRNA fingerprints) -made of both up- and down-regulated miRNAs- that have been proven to be a useful tool to discriminate between normal and tumor tissues and to identify different cancer subtypes [17]. In addition, in the context of specific cells/tissues, the analysis of the miRNome has allowed to identify miRNAs as markers of prognosis and as indicators of treatment response [17]. The aberrant miRNA expression levels in tumors vs normal counterparts can be dependent on several mechanisms, including gene deletions, amplifications, point mutations, dysregulations (gain or loss) of transcription factors, changes in epigenetic regulatory mechanisms (such as DNA methylation and histone modifications), as well as perturbations in the machinery responsible for miRNA biogenesis and maturation [18].

Deregulated miRNAs in tumors trigger a global perturbation in the expression and/or function of their downstream targets. In fact, a single miRNA can target many different mRNAs and a single mRNA can be targeted by several miRNAs. However, since the expression patterns of miRNAs and their respective targets may differ among tissues and differentiation states, miRNAs may act as tumor suppressors or oncogenes (oncomir) depending on the cell/tissue context and the presence of relevant targets [19]. Moreover, given that cancer-associated miRNAs often affect factors involved in the response of cells to anti-cancer drugs or radiation (such as drug resistance-associated proteins, apoptosis-associated and DNA repair factors), miRNAs have been suggested as key determinants of chemo- and radio-resistance [17].

2. ROLE OF miRNAs IN THE RESPONSE OF PROSTATE CANCER TO ANTI-CANCER DRUGS

Prostate cancer (PCa) is the most prevalent tumor in the male population and the second leading cause of cancer-related death in man in western countries [20]. Organ-confined disease is successfully treated with radical prostatectomy or radiotherapy [21]. Androgen deprivation therapy is currently used to treat patients with metastatic PCa [22]. However, the efficacy of such a therapeutic option is time-limited and most patients develop a castration-resistant disease [22]. Castration-resistant PCa continues to rely on the androgen receptor for growth and progression, as recently demonstrated by the impact exerted on patient overall survival by two drugs, abiraterone acetate and enzalutamide, that ultimately function by decreasing the androgen receptor signaling [23]. The acquisition of the castration-resistant
phenotype represents one of the key events in the progression of PCa, which, at this stage, becomes also refractory to chemotherapeutic agents, although taxanes, such as docetaxel and, more recently, cabazitaxel, have demonstrated overall survival benefit [23].

Anti-cancer drug resistance is a multifactorial phenomenon involving several major mechanisms. In recent years, a considerable effort has been made to characterize specific alterations associated to the poor responsiveness of PCa to anti-cancer agents, with the aim to identify new targets to be used for the development of innovative therapies [24]. In this context, a few published studies have been focused on the identification of deregulated miRNAs and the evaluation of the consequences of their modulation with the dual aim to produce a direct anti-cancer effect and to increase PCa sensitivity to anti-cancer agents.

A variety of experimental approaches have been used to artificially modulate miRNA expression in PCa models. Specifically, the inhibition of the expression of up-regulated oncogenic miRNAs has been accomplished through the use of “antisense” oligonucleotides carrying chemical modifications to increase stability and resistance to nucleases. The most widely used “antisense” oligonucleotides are antagonirs -which contain a phosphorothioate linkage and are conjugated with a cholesterol moiety- and locked nucleic acids (LNAs) [25, 26]. To transiently restore the expression of down-regulated miRNAs, miRNA mimics (small, chemically modified double-stranded RNAs that mimic endogenous miRNAs) are largely used. An alternative approach to stably over-express down-regulated miRNAs is accomplished by cloning miRNA genes into plasmid or viral vectors [25, 26].

2.1. miRNAs as Determinants of Resistance to Anti-Cancer Drugs and Targets for Treatment Response Modulation

Studies carried out in in vitro and in vivo models led to the identification of a small panel of miRNAs whose experimental modulation was able to modify the responsiveness of PCa to a variety of anti-cancer drugs (Table 1).

miR-21. miR-21 -which is located on chromosome 17- is one of the most studied miRNAs thus far [27]. Although still debatable [19], miR-21 has been found widely over-expressed in multiple cancers and is thought to play a pivotal role in tumorigenesis [28-36]. Specifically, it has been demonstrated to counteract the expression of a plethora of target proteins [37-39], thus resulting a key player in the regulation of basic hallmarks of cancer. Several preclinical studies have demonstrated that inhibition of miR-21 affects tumor cell growth and survival, invasion and migration as well as the response of cancer cells to conventional anti-cancer therapies. Altogether, these observations have contributed to identify miR-21 as an oncomiR and as a suitable therapeutic target for anti-cancer interventions [17].

To date, scanty and contradictory information is available concerning the role of miR-21 in PCa. In fact, it was initially described as one of the six miRNAs the over-expression of which may represent the signature for solid tumors, including PCa [40]. Successively, Ribas et al. [41] found that miR-21 levels are frequently elevated in early grade PCa samples in comparison to adjacent normal tissue. They also demonstrated that miR-21 over-expression stimulates the growth of both castration-sensitive and -resistant PCa cell lines, enhances tumor xenograft growth and promotes androgen-independent proliferation following surgical castration [41, 42], thus supporting an oncogenic role for miR-21. By contrast, Hulf et al. [43], using an integrative approach that combines primary transcription and genome-wide DNA methylation with miRNA expression, found miR-21 to be epigenetically repressed in PCa cells, thus suggesting that it may instead act as a tumor suppressor gene. We found miR-21 expression levels to be similar in tumor and matched non-neoplastic tissues from PCa patients who underwent radical prostatectomy and failed to appreciate differences in the miRNA expression levels as a function of nodal status, extraprostatic extension of the disease and Gleason score [19]. In addition, in our hands LNA-mediated miR-21 knockdown did not affect the proliferative and invasive capabilities, as well as the responsiveness to anti-cancer drugs (cisplatin and paclitaxel) and ionizing radiation of castration-resistant PCa cell lines [19]. Conversely, Shi and colleagues [44] recently found a relationship between miR-21 expression levels and the responsiveness of PCa cells to docetaxel, showing that miR-21 ectopic reconstitution or deletion resulted in increased resistance or sensitivity, respectively, to the taxane.

Based on aforementioned information, a consensus on miR-21 as a modulator of PCa to specific anti-cancer drugs has not yet been achieved, owing to large incongruities in the experimental data collected so far.

miR-34a. The three members of miR-34 family are produced from two transcriptional units: miR-34a is transcribed from chromosome 1, whereas miR-34b and miR-34c share a common primary transcript obtained from a region on chromosome 11 [45]. Often deregulated (lost or reduced) in tumors [46-50] -wherein its expression has been shown to be dependent on the tumor suppressor p53 [45]-, miR-34a has been demonstrated to control the expression of several target proteins involved in cell proliferation, cell cycle progression, apoptosis, invasion and migration [45].

Results from miRNA profiling analysis indicated that miR-34a expression levels are reduced in PCa compared to normal prostate tissues [51, 52]. miR-34a expression levels are also higher in p53 wild-type (LnCAP) than in p53-null (PC3) or p53-mutated (DU145) PCa cells [53], suggesting that reduced miR-34a expression levels in PCa could be ascribable, at least in part, to p53 defects. Such a hypothesis was corroborated by the evidence that ectopic expression of p53 in p53-defective PC3 cells increased miR-34a expression [53]. Transfection of p53-null PCa cells with miR-34a synthetic precursor caused cell growth inhibition and the concomitant appearance of cells with a senescence-like phenotype [53].

miR-34a expression is down-regulated in CD44+ cells -which are characterized by enhanced clonogenic, tumor-initiating and metastatic capacities- purified from PCa xenografts and primary tumors. Enforced expression of miR-34a decreased clonogenic expansion and tumor regeneration in both bulk and purified CD44+ PCa cells [54]. Moreover, systemically delivered miR-34a reduced lung metastasis and
Table 1. Summary of miRNAs whose modulation modifies the responsiveness of PCa cells to specific anti-cancer drugs.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression*/Role in PCa</th>
<th>Cell Model</th>
<th>Approach Used for miRNA Modulation</th>
<th>Effect on the Sensitivity to Anti-Cancer Drugs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>=</td>
<td>DU145</td>
<td>Transfection with LNAs</td>
<td>Cisplatin, paclitaxel (=)</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>↑ / Oncogene</td>
<td>PC3</td>
<td>Transfection with antisense oligonucleotides</td>
<td>Docetaxel (↑)</td>
<td>[44]</td>
</tr>
<tr>
<td>miR-34a</td>
<td>↓ / Tumor Suppressor</td>
<td>Docetaxel-resistant PC3 and 22Rv1</td>
<td>Transient transfection with miRNA mimics</td>
<td>Paclitaxel, Daunorubicin, Etoposide (↑)</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paclitaxel-resistant PC3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parental PC3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-125b</td>
<td>↑ / Oncogene</td>
<td>LnCAP-eds PC-346C</td>
<td>Transfection with antisense oligonucleotides</td>
<td>Cisplatin (↑)</td>
<td>[72]</td>
</tr>
<tr>
<td>miR-143</td>
<td>↓ / Tumor Suppressor</td>
<td>DU145 PC3</td>
<td>Transient transfection with miRNA mimics</td>
<td>Docetaxel (↑)</td>
<td>[84]</td>
</tr>
<tr>
<td>miR148a</td>
<td>↓ / Tumor Suppressor</td>
<td>Parental PC3 PAclitaxel-resistant PC3</td>
<td>Transient transfection with miRNA mimics</td>
<td>Paclitaxel (↑)</td>
<td>[87]</td>
</tr>
<tr>
<td>miR-200b</td>
<td>↓ / Tumor Suppressor</td>
<td>DU145 PC3</td>
<td>Transient transfection with miRNA mimics</td>
<td>Docetaxel (↑)</td>
<td>[92]</td>
</tr>
<tr>
<td>miR-205</td>
<td>↓ / Tumor Suppressor</td>
<td>DU145 PC3</td>
<td>Transient transfection with miRNA mimics</td>
<td>Cisplatin, doxorubicin (↑)</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WPE1-NA22 WPE1-NB26</td>
<td>Stably-transfected clones with a precursor sequence cloned into a viral vector</td>
<td>Cisplatin, docetaxel (↑)</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DU145</td>
<td>Stably-transfected clones with a plasmid</td>
<td>Cisplatin (↑)</td>
<td>[112]</td>
</tr>
</tbody>
</table>

*expression in PCa vs normal cells/tissues (=, unchanged expression levels; ↑, up-modulated; ↓, down-modulated)
(↑), increased responsiveness to anti-cancer drugs
(=), unmodified responsiveness to anti-cancer drugs

Prolonged survival of human PCa-bearing mice as a consequence of the down-regulation of CD44, a direct target of the miRNA [54].

In the attempt to identify potential clinically relevant biomarkers of docetaxel-resistance, Corcoran and colleagues [55] performed a global profiling of intracellular and extracellular (cell-derived exosomes) miRNAs in castration-resistant PCa cell lines with acquired resistance to docetaxel (PC3RD, DU145RD and 22Rv1RD) and their parental cell lines (PC3, DU145 and 22Rv1). Such an analysis revealed that four miRNAs (miR-598, miR-34a, miR-146a, miR-148a) are significantly decreased in cells and exosomes of two of the three docetaxel-resistant variants [55]. Functional studies showed that mimicked expression of miR-34a in docetaxel-resistant PC3RD and 22Rv1RD cell lines caused a significant increase in the anti-proliferative effect of docetaxel [55]. Similarly, the ectopic expression of miR-34a attenuated the resistance of established paclitaxel-resistant PC3 (PC3PR) cells and concomitantly increased their sensitivity to drugs with different mechanism of action, such as daunorubicin and etoposide [56]. In addition, transfection of parental PC3 cells with the miR-34a precursor significantly enhanced their sensitivity to camptothecin as a consequence of an enhanced apoptotic response [53]. The described chemo-sensitizing effects are ascribable, at least in part, to the miR-34a-dependent suppression of SIRT1 and Bcl-2 [53, 55, 56]. Altogether, these data support miR-34a reconstitution as a promising strategy to counteract the drug-resistant phenotype of castration-resistant PCa cells, irrespectively of p53 status.

A different scenario would appear to arise for castration-sensitive PCa. In fact, Rokhlin and colleagues [57] showed that inhibition (or forced expression) of either miR-34a or miR-34c was not able to modulate doxorubicin-mediated apoptosis in p53 wild-type LNCaP cells. However, simultaneous over-expression of both miRNAs in the same experi-
mental model resulted in improved p53-mediated apoptotic response following doxorubicin treatment, whereas their concomitant inhibition resulted in the suppression of doxorubicin-mediated apoptosis [57]. Although based on a single cell model, these findings suggest that modulation of miR-34a alone is not sufficient to produce a chemosensitizing effect, and corroborate the notion that the role exerted by a specific miRNA is highly dependent on the specific cellular context.

**miR-125b.** MiR-125b belongs to the miR-125 family which is composed of three homologs: miR-125a, miR-125b-1 and miR-125b-2 [58]. MiR-125a is located at 19q13, while miR-125b is transcribed from two loci located on chromosomes 11q23 (miR-125b-1) and 21q21 (miR-125b-2) [59]. All members of the family play crucial roles in a variety of cellular processes, including cell differentiation, proliferation and apoptosis [59]. Aberrant expression of miR-125b has been reported to occur in several solid [60-64] and hematologic [65-67] malignancies, where it has been found to act alternatively as an oncogene or a tumor suppressor depending on the tumor type [59].

As far as PCa is concerned, although an early investigation reported that miR-125b was down-regulated in 5 of 9 tumors compared to 4 benign prostate samples [68], several subsequent studies demonstrated that miR-125b is over-expressed in PCa clinical samples and cell lines compared to normal prostate tissues [69-71]. The enforced expression of miR-125b also stimulates the androgen-independent PCa growth both in vitro and in vivo, where it promotes tumor growth in both intact and castrated male nude mice [59, 69]. Based on such evidence, in PCa miR-125b is viewed as an oncogene. Indeed, miR-125b inhibits the intrinsic apoptotic pathway and promotes the proliferation of PCa cells through the regulation of p53, PUMA, Bak1 and p14ARF [69, 72, 73]. Moreover, miR-125b knock-down in castration-sensitive PCa cells reduces cell proliferation and sensitizes LNCaP cells significantly to growth both in vitro and in vivo, whereas their concomitant inhibition resulted in the suppression of doxorubicin-mediated apoptosis [81, 82]. A recent study showed that loss of miR-143 expression in PCa correlates with up-regulation of ERK5, favoring cell proliferation, survival, and invasion, and leading to the development of more aggressive forms of PCa [83]. Transfection of castration-resistant PCa cells with miR-143 mimic abrogated cell growth both in vitro and in vivo [81, 83, 84] and inhibited migration [84]. Notably, over-expression of miR-143 enhanced the responsiveness of DU145 and PC3 cell lines to docetaxel by targeting EGFR/RAS/MAPK pathway [84], thus supporting the reconstitution of miR-143 as an attractive therapeutic strategy to inhibit PCa growth and modulate its responsiveness to taxanes.

**miR-148a.** Members of the miRNA-148/152 family (miR-148a, miR-148b, and miR-152), are implicated in the pathogenesis of several diseases, including cancer [85]. Growing evidence indicated that the expression levels of miR-148/152 family members significantly decreased in tumors compared to normal tissues, thus suggesting that they may act as tumor suppressors [85]. The functional role of miR-148/152 family members in PCa is largely unknown. However, miR-148a expression has been reported to be lower in aggressive PCa (Gleason score 8) than in normal adjacent tissues, as well as in castration-resistant PCa than in benign prostatic hyperplasia [68, 86]. In addition, Fujita et al. [87] demonstrated that the levels of miR-148a were significantly reduced in castration-resistant PCa cells with respect to both normal prostate epithelial cells and castration-sensitive PCa cells. Transfection of castration-resistant PCa cells with miR-148 precursor induced inhibition of cell growth, migration and invasion, indicating that dysregulation of miR-148a expression could contribute to the metastatic potential of PCa cells [87]. In addition, ectopic expression of miR-148a sensitized PC3 cells and attenuated the resistance of paclitaxel-resistant PC3 cells to paclitaxel by regulating MSK1 expression [87].

**miR-200 family.** The miRNA-200 family consists of five members (i.e., miR-200a, miR-200b, miR-200c, miR-429, and miR-141) which can be divided into two clusters: the miR-200a/429 cluster containing miR-200a, miR-200b, and miR-429 (located on chromosome 1), and the miR-200c/141 cluster, which contains miR-200c and miR-141 (located on chromosome 12) [88]. The role of miR-200 family members as tumor-suppressors has been extensively demonstrated in several human malignancies [88]. However, there are only few reports linking miR-200 family members with PCa, wherein they have been recognized to be down-regulated along tumor progression and to play a critical role in the suppression of epithelial-to-mesenchymal transition (EMT), tumor cell invasion and metastasis [89-92]. Accordingly, over-expression of miR-200b in castration-resistant PCa cells significantly inhibited their proliferation and reduced the formation of tumors s.c. upon xenotransplantation into nude mice [93]. Moreover, in an orthotopic model, miR-200b reconstitution was found to counteract spontaneous metastasis [93]. Such an effect was mainly due to the reversal of EMT [93]. Ectopic expression of miR-200b in castration-resistant PCa also suppressed cell proliferation and migration [92].

Puhr and colleagues [94] first reported a relationship between the docetaxel-resistant phenotype and the appearance of EMT in castration-resistant PCa sublines (DU145-DR and PC3-DR), with experimentally induced resistance to taxane. Specifically, performing a screening for key regulators of an epithelial phenotype, the authors found a significantly reduced expression of miR-200c (and miR-205) in docetaxel-
resistant compared to parental cells. Transfection of resistant cells with both miRNAs resulted in re-expression of E-cadherin and down-regulation of ZEB1 and ZEB2, accompanied by increased apoptosis, thus suggesting that reduced miR-200c (and miR-205) levels during chemotherapy are crucial for cancer cell survival and drug resistance [94]. Unfortunately, the authors did not address the impact of miRNA restoration on the sensitivity profile of PCa cells to docetaxel. However, Yu et al. [92] reported that transfection of DU145 and PC3 cells with another member of the miR-200 family, miR-200b, improved their sensitivity to docetaxel by regulating Bmi-1. Although a possible correlation between the expression levels of miR-200-family members and responsiveness of PCa cells to docetaxel is envisaged, further investigation is warranted.

miR-205. To date, there is much but controversial information regarding the role of miR-205 in cancer. In fact, it was found to be either up- or down-regulated in tumors compared with normal tissues [95-98]. With regard to PCa, miR-205 has been extensively reported to be down-regulated in PCa compared to adjacent non-neoplastic tissue [99-104], as well as in PCa cell lines, irrespective of their androgen responsiveness, compared with normal cells [99, 103-105]. Recent studies have also shown that miR-205 expression is inversely correlated with total/free PSA levels and tumor stage [104, 105-108]. Moreover, miR-205 levels were found to be significantly decreased in primary tumors from patients with lymph node dissemination compared to those from node-negative patients [99]. In addition, miR-205 expression is down-regulated in lymph node metastasis compared to the primary tumor [103], suggesting that miR-205 is critically involved in distinct processes leading to migration, invasion and/or homing of metastatic PCa cells.

We previously reported that miR-205 acts as a tumor suppressor in human PCa, as its restoration in castration-resistant PCa cells resulted in cell rearrangements consistent with a mesenchymal-to-epithelial transition (i.e., up-regulation of E-cadherin, reduction of cell locomotion/invasion, increased sensitivity to anoikis) [99]. Over-expression of miR-205 was also shown to cause cell proliferation decline, cell cycle perturbation and apoptosis induction in both castration-sensitive and -resistant PCa cells, by inhibiting specific targets such as Bcl-2, Bcl-w and c-SRC [102, 103, 105]. In addition, we demonstrated that miR-205 reconstitution triggers the reactivation of basement membrane deposition thus allowing a 3D organization of PCa cells into normal-like acinar structures [109] and interrupts the pro-invasive circuitries engaged by reactive stroma [110].

Since EMT is known to play an important role in treatment resistance [111, 112] and miR-205 is able to counteract EMT in PCa, it has been hypothesized that its restoration could represent a new therapeutic approach to sensitize PCa to drugs with different mechanism of action. Accordingly, Verdoot et al. [102] and Bhatnagar et al. [105] reported that over-expression of miR-205 sensitized PCa cells to cisplatin, docetaxel and doxorubicin by enhancing the apoptotic response as a consequence of the inhibition of anti-apoptotic members of the BH3 family (i.e., Bcl-2 and Bcl-w). In this context, we recently demonstrated that the enhancement of cisplatin cytotoxic activity in castration-resistant PCa cells following miR-205 reconstitution is only partially dependent on apoptosis induction and mainly relies on miRNA-dependent impairment of the autophagic flux [113], thus suggesting a tight interconnection between the mesenchymal phenotype and autophagy, an evolutionarily conserved mechanism that traffics materials from cytoplasm to lysosomes via autophagosomes [114]. Specifically, miR-205 reconstitution led to a marked down-regulation, among others, of LAMP3 and RAB27A genes, that are specifically associated with lysosome function and trafficking, thus resulting in a constraint on the autophagic flux [113]. Altogether these findings indicate miR-205 reconstitution as a promising approach to improve the response of PCa to anti-cancer drugs.

2.2. Clinical Relevance of miRNAs as Predictive Biomarkers of Treatment Response

Due to their presence and stability in body fluids (such as blood and urine), miRNAs represent perhaps the best opportunity for developing novel non-invasive approaches for predicting and monitoring tumor response to treatment [115]. Thus far, the possible relevance of circulating miRNAs as biomarkers of response to chemotherapy in PCa has been investigated in two studies. In the first one, Zhang et al. [116] evaluated the expression levels of miR-21 in serum samples from 10 hormone-refractory PCa patients who underwent docetaxel-containing treatment. Study results indicated that miRNA-21 levels were significantly higher in patients who were resistant to docetaxel-based chemotherapy when compared to those sensitive to treatment.

More recently, Li et al. [117] performed a global miRNA profiling in docetaxel-sensitive and-resistant cell lines to identify candidate miRNAs and then evaluated the expression levels of the best 46 candidate miRNAs in plasma/serum samples collected before and after docetaxel treatment from 97 castration-resistant patients. Non-responders to docetaxel and patients with shorter survival showed high pre-treatment levels of miR-200 family members or decrease/unchanged post-treatment levels of miR-17 family members. In addition, in multivariable analysis, pre-treatment miR-200b levels were found to be an independent predictor of overall survival.

Although these studies results have envisaged the possibility of establishing a correlation between the expression of specific miRNAs and docetaxel chemotherapy outcome, further investigation in clinical trials is warranted.

CONCLUDING REMARKS

Results obtained in PCa experimental models through the use of different miRNA-modulating approaches have provided evidence of a direct involvement of specific miRNAs in the responsiveness of PCa to anti-cancer drugs, thus revealing a new mechanism by which tumor may be refractory to chemotherapy. miRNAs, such as miR-205, miR-200b and miR-34a, each regulating at least a couple of important players in different chemotherapy-relevant pathways, could prove particularly useful targets for developing strategies aimed at positively modulating chemosensitivity in PCa. However, since the role of these miRNAs has been mainly investigated in cell models, their actual involvement in the
chemosensitivity profile of PCa need to be properly defined in preclinical models, including orthotopic and patient-derived xenografts, which better recapitulate clinical PCa features.

Although further improvement in miRNA delivery strategies, in terms of increased efficiency and specificity of delivery to the target organ/tissue, is still necessary, the applicability of strategies aimed to modulate miRNA expression in the clinical setting is currently under investigation as the first liposome-formulated mimic of the tumor suppressor miR-34a (MRX34) recently entered Phase I clinical trial for patients with unresectable primary liver cancer and advanced or metastatic cancer with liver involvement (ClinicalTrials.gov Identifier: NCT01829971).

At present, nothing can be concluded concerning the possible relevance of miRNAs as novel biomarkers of treatment response. However, the possibility to carry studies encompassing large case series of patients, homogeneous for clinicopathological characteristics will be instrumental for the identification and validation of specific miRNAs as predictive biomarkers for more precise stratification of patients according to their probability to respond to specific treatments.

LIST OF ABBREVIATIONS
AGO = Argonaute
CLL = B-cell chronic lymphocytic leukemia
DGCR8 = DiGeorge syndrome critical region gene 8
EMT = Epithelial-to-mesenchymal transition
miRISC = miRNA-RNA inducing silencing complex
miRNA = microRNA
pri-miRNA = microRNA primary transcript
pre-miRNA = microRNA precursor
RNAa = RNA activation
XPO5 = Exportin 5

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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