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Emerging aspects of microRNA interaction with TMPRSS2-ERG and endocrine therapy

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ABSTRACT

Prostate cancer (PCa) is the most common malignancy detected in males and the second most common cause of cancer death in western countries. The development of the prostate gland, is finely regulated by androgens which modulate also its growth and function. Importantly, androgens exert a major role in PCa formation and progression and one of the hypothesized mechanism proposed has been linked to the chromosomal rearrangement of the androgen regulated gene TMPRSS2 with ERG. Androgens have been therefore used as main target for therapies in the past. However, despite the development of endocrine therapies (e.g. androgen ablation), when PCa progress, tumors become resistant to this therapeutic castration and patients develop incurable metastases. A strategy to better understand how patients respond to therapy, in order to achieve a better patient stratification, consists in monitoring the levels of small noncoding RNAs (microRNAs). microRNAs are a class of small molecules that regulate protein abundance and their application as biomarkers to monitor disease progression has been intensely studied in the last years. In this review, we highlight the interactions between microRNAs and endocrine-related aspects of PCa in tissues. We focus on the modulation of TMPRSS2-ERG and Glucocorticoid Receptor (GR) by microRNAs and detail the influence of steroidal hormonal therapies on microRNAs expression.

1. Introduction

Prostate cancer (PCa) is the second leading cause of death from cancer in males in western countries, after lung cancer (Siegel et al., 2015). The growth of the prostate is regulated by androgens and the androgen dependency of prostate cancer has been established over half a century ago (Huggins and Hodges, 1941). Despite the significant improvement in early cancer detection achieved by PSA testing and in spite of the development of endocrine therapy (androgen ablation), when prostate cancer progresses, tumors acquire resistance to this therapeutic castration and are therefore classified as castration-resistant prostate cancer (CRPC) (Scher and Sawyers, 2005).

The notion that those prostate cancers arising during androgen deprivation therapy are androgen independent has been reconsidered in the last years (Thompson et al., 2003). Remarkably indeed, so called “androgen independent” tumors often contain amplification of the gene encoding for the androgen receptor (AR) (Visakorpi et al., 1995) and in many cases also overexpress the AR (Linja et al., 2001; Chen et al., 2004) which is sufficient to switch the growth of PCa cells from androgen dependence to androgen independence (Chen et al., 2004). Multiple clinical trials have provided evidence that CRPCs maintain androgen responsiveness (Tran et al., 2009; Reid et al., 2010). This effect might be determined by metabolic alterations such as an activation of steroidogenic pathways, potentially via a *de novo* intratumoral biosynthesis of steroid hormones, which might facilitate tumor survival in presence of androgen deprivation therapy (ADT) (Green et al., 2012). In the past decade, one mechanism of androgens to induce PCa has been hypothesized to be due to chromosomal rearrangement of the androgen regulated gene TMPRSS2 (transmembrane protease,

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serine 2) and the ETS transcription factor ERG, which becomes also androgen regulated (Tomlins et al., 2005). This rearrangement usually occurs during cancer initiation and is also detected as early event during tumor progression and is present in up to 50–60% of all prostate tumors (Visakorpi, 2012). In addition to the involvement of androgens and AR, which fundamentally drive PCa, Glucocorticoids (GCs) are another class of steroidal hormones that recently have been shown to mediate chemotherapy resistance (Kroon et al., 2016a,b). This opened new possibilities for novel therapeutic approaches, suggesting the application of GCs and Glucocorticoid Receptor (GR) antagonism to re-sensitize resistance to taxane-based drugs in PCa (Kroon et al., 2016a,b).

Endocrine therapy has been used for decades to treat prostate cancer and its complications. An interesting aspect of the effect that this therapy can produce, is its influence on small noncoding RNA (microRNAs, miRNAs, miRs).

microRNAs are evolutionary conserved short noncoding single-stranded RNA molecules (approximately 18–22 nucleotides long) that regulates protein abundance (Ambros, 2004). miRNAs negatively regulate the translation of target mRNA by binding to their 3′ untranslated region (UTR) or, although to less extent, to their 5′UTR or coding sequence. In presence of perfect complementarity between the seed sequence of the miRNA and the mRNA, this will result in mRNA degradation; alternatively, if the binding is not perfect, it will produce translational repression (Valinezhad Orang et al., 2014). microRNAs regulate a vast variety of processes, such as cell proliferation, motility, apoptosis, maintenance of stem-like properties and have been implicated in PCa initiation, progression and metastases.

Each miRNA can modulate the expression of multiple mRNAs and a single mRNA can be modulated by multiple miRNAs, leading to a biological complexity which makes miRNAs important regulators of many properties of normal and neoplastic cells. Depending on the mRNA target genes, miRNAs are classified in tumor suppressor miRs or onco-miRs and their expression can be aberrant in cancerous cells. miRNAs represent a relatively recent class of interesting molecules of potential utility as PCa biomarkers, beside the employment of proteins and mRNA measurement in the clinical practice. This also opens new possibilities for the use of miRNAs as predictive markers for endocrine therapy response.

In this review, we focus on the interactions between microRNAs and endocrine-related aspects of PCa (e.g. androgens and glucocorticoids). We specifically highlight the cross-talk between microRNAs and the androgen-regulated gene TMPRSS2-ERG and discuss the effect of endocrine therapy on microRNA expression.

2. microRNA deregulation in prostate cancer

Deregulations of microRNAs, through processes such as promoter methylation, histone modifications, genomic deletion and upstream protein alteration (Lujambio et al., 2008; Shi et al., 2008), have been documented in multiple cancers (Lu et al., 2005; Volinia et al., 2006). A significant portion of microRNAs are indeed localized in the proximity of CpG islands, which are susceptible sites of epigenetic silencing (Rauhala et al., 2010). A pattern of microRNA downregulation has indeed been documented for multiple malignancies such as colorectal (Michael et al., 2003; Cummins et al., 2006) and lung cancer (Yanaihara et al., 2006) and has also been suggested to reflect the lower differentiation stage of the tumor cells compared with normal cells (Lu et al., 2005; Porkka et al., 2007). Together, this supports the general pattern of down-regulation that has been described in prostate cancer in microRNA expression studies comparing benign vs. cancerous tissues (Porkka et al., 2007; Ozen et al., 2008; Spahn et al., 2010).

A comprehensive review by Fabris et al., has recently summarized the microRNAs that are consistently altered in PCa tissues in different studies and associated with the same trend of expression (Fabris et al., 2016). Among the most common microRNAs consistently downregulated in PCa tissues, miR-125b, miR-145 and Let-7b are associated with altered apoptosis (Porkka et al., 2007; Ambs et al., 2008; Ozen et al., 2008; Martens-Uzunova et al., 2012; Larne et al., 2013), miR-205 with cell proliferation (Porkka et al., 2007; Ambs et al., 2008) and miR-221 and miR-222 with cell cycle (Porkka et al., 2007; Ambs et al., 2008; Martens-Uzunova et al., 2012; Larne et al., 2013). On the other hand, among the most common microRNAs consistently upregulated in PCa tissues, miR-93 is associated with metastasis and miR-25 with cell proliferation (Volinia et al., 2006; Martens-Uzunova et al., 2012). Despite the large volume of information generated with expression analysis of bulk-tissues, it is becoming increasingly evident that such approaches also reduce the chances of measuring the contribution of microRNAs altered in specific subpopulation of cells (e.g. cancer progenitor/stem-like cells). Studies focused on microRNA alterations in selected metastatic Prostate Cancer Stem Cells (CSCs) have shown, for example, that a microRNA (miR-25) previously shown to be increased in PCa “bulk tissues” (Volinia et al., 2006) was significantly downregulated in PCa CSCs and restored expression of miR-25 resulted in strong reduction of distant growth of PCa cells inoculated in zebrafish embryos (Zoni et al., 2015a,b).

3. Influence of endocrine therapy on microRNAs expression

Despite big progresses in the development of new drug delivery strategies, the applicability of miRNAs as therapeutic agents is still in its infancy. This is mainly due to multiple challenges such as specificity and therapeutic delivery (Conde and Artzi, 2015). Moreover, the observation of decreased microRNAs during cancer formation and progression, has led to the concept of microRNA replacement therapy. An example of this was recently described with the AR regulating miR-34a (Ostling et al., 2011): it was shown that nanoparticle-mediated delivery of miR-34a decreased PCa cells growth in the bone (Gaur et al., 2015). However, microRNAs may have clear diagnostic and prognostic value and can be employed as predictors of therapy response and biomarkers (Junker et al., 2016).

Interestingly, although endocrine therapy has been used for decades, its influence on the expression of microRNAs in clinical tissue specimens has not been extensively analyzed (Lehmusvaara et al., 2013). In a recent study (Lehmusvaara et al., 2013), the expression of 723 human microRNAs was analyzed in freshly frozen specimens from PCa patients treated with goserelin and bicalutamide vs. untreated controls (Lehmusvaara et al., 2012). In this study, a significant difference in microRNAs modulation was registered upon the treatments. Among the 19 miRNAs with decreased expression, six were common to both treatments, namely miR-9, miR-492, miR-210, miR-149, miR-200a and miR-200b (Table 1). Conversely, among the 23 miRNAs with increased expression, only three were common to both treatments, namely miR-99a, miR-125b and miR-100 (Table 1). Strikingly, the majority of the microRNAs measured in this study, displayed a pattern of upregulation upon treatment. Given that the expression of microRNAs has been shown to be reduced during PCa progression (Lu et al., 2005; Martens-Uzunova et al., 2012), this suggests that, the registered pattern of expression upon treatment, might indicate a therapeutic response and a reduction of cancerous characteristics (Lehmusvaara et al., 2013). It is important to note that, the two treatments investigated in the study, differ significantly for the targeting mechanism: goserelin affects the androgen production from the testis whereas bicalutamide prevents DHT binding to the AR. Given the discrepancy in the number of miRs selectively

Table 1
Effect of (endocrine) therapy on microRNA expression.

(endocrine) treatment	Downregulated miRNAs	Upregulated miRNAs	FDA approved	References
Trichostatin A (TSA)	–	miR-9; miR-193	no	Rahuala et al., 2010
Mifepristone	–	miR-99a/100	yes	Rane et al., 2016
Goserelin + Bicalutamide	miR-9; miR-492; miR-210; miR-149; miR-200a; miR-200b	miR-99a; miR-125b; miR-100	yes	Lehmusvaara et al., 2012

modulated by one drug (and the other), this highlights that the effect of treatments targeting a common pathway seems to be quite different. Additionally, among the miRs which displayed a decrease, upon bicalutamide and goserelin treatment, miR-9 was also shown to be moderately increased upon TSA treatment (discussed in previous paragraph) (Rauhala et al., 2010). Given that miR-9 directly targets ERG (Nowek et al., 2016), these reinforce the notion that miR-9 finely modulates the balance between TMPRSS2-ERG and AR. Therefore, the decrease of miR-9 upon treatments targeting AR signaling, suggest that miR-9 might play a role during therapy resistance in castration resistant phase in PCa. These also suggest that therapeutic approaches targeting multiple pathways (e.g. ERG and AR) might be promising to improve patient's response.

On the other hand, miR-99a and miR-100 displayed an increase upon bicalutamide and goserelin treatment. Interestingly, inhibition of the GR by mifepristone (Lin et al., 1995) resulted in an enhanced miR-99a/100-mediated radiation response in patient-derived prostate cells (Rane et al., 2016). This support the notion that, targeting AR and GR pathway simultaneously, might represent a strategy to prevent resistance to chemotherapy in a later stage of the disease.

Moreover, in a miRNA library screening to identify anti-androgen bicalutamide PCa resistance-related microRNAs, miR-216a was identified as associated with endocrine resistance (Miyazaki et al., 2015). Ectopic expression of miR-216a inhibited bicalutamide-mediated growth suppression of LNCaP cells and miR-216a was upregulated upon DHT treatment. This suggests that miR-216a might be employed as marker to monitor endocrine therapy response in PCa.

Another approach to target endocrine signaling in PCa, is to interfere with AR coactivators (Culig and Santer, 2013). The transcriptional integrator p300 and its functional homologue CBP, have been shown to be involved in AR transactivation and to display acquisition of agonistic properties of hydroxyflutamide, a non-steroidal antiandrogen. Moreover, androgen ablation therapy resulted in increased expression of p300 and CBP (Debes et al., 2003; Comuzzi et al., 2004; Heemers et al., 2007). Inhibition of p300 and CBP by a newly developed molecule C646 (Bowers et al., 2010) (p300 histone acetyltransferase inhibitor) in androgen-sensitive and –insensitive cell lines reduced proliferation and invasion (Santer et al., 2011). Recently, we identified in silico a signature of 30 validated microRNAs associated with p300/CBP in the context of EMT in cancer (Zoni et al., 2015a,b). Strikingly, multiple microRNAs identified in our signature appear to be relevant for their involvement in endocrine aspects in PCa.

The previously discussed miR-9 was one of the 30 miRs identified in our signature and was shown to target p300 (Grimson et al., 2007). Moreover, in a screening of 1129 miRNAs to identify microRNAs regulating the AR at protein level, miR-9 was identified as direct targets the 3′-UTR of AR (Ostling et al., 2011). This reinforce the identification of miR-9 as a promising marker for endocrine therapy response in PCa. Additionally, in the same study, miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-34c, miR-371-3p, miR-421, miR-449a, miR-449b, miR-634 and miR-654-5p were identified as direct binding partners of the 3′UTR of AR (Ostling et al., 2011). The assessment of the levels of these microRNAs, could therefore represent a promising measurement to address the microRNAs response upon endocrine therapy. Furthermore, miR-26b, miR-182 and miR-200b were also previously reported to interact with p300/CBP (Mees et al., 2010). These three miRs are associated with ERG modulation, TMPRSS2-ERG correlation and endocrine treatment respectively. Together these highlight the potential of monitoring the levels of these miRs during endocrine therapy and the relevance of targeting p300/CBP in PCa.

4. microRNA interaction with GR & glucocorticoids

GCs are steroidal hormones that have been used in the treatment of prostate cancer, typically in combination with docetaxel and abiraterone acetate in the castration-resistant phase of the disease, reviewed in (Montgomery et al., 2014). The rationale for the administration of GCs is basically related to slow disease progression, improve pain control and reduce the side effects of chemotherapy (Piccart et al., 1997; Attard et al., 2012). GCs can suppress androgen synthesis through inhibition of the hypothalamic/pituitary axis, which results in suppression of testicular and adrenal androgen production (Alesci et al., 2001). However, GCs usage remains controversial as both pro- and antitumor effects have been documented (Montgomery et al., 2014). Additionally, GR expression is enhanced in PCa patients who received docetaxel and in docetaxel-resistant cell lines (Kroon et al., 2016a,b). Moreover, GR antagonism by RU-486 and cyproterone acetate (CPA), has been shown to revert docetaxel resistance in human PCa, opening new possibilities for the clinical utility of the GR antagonists in the management of patients with advanced PCa (Kroon et al., 2016a,b).

To date, only one report has highlighted the functional connection between miRs and GR in the context of human prostate cancer (Rane et al., 2016). Rane et al., have shown that inhibition of the GR by mifepristone (Lin et al., 1995) results in an enhanced miR-99a/100 expression (Table 1) and increased radiation response in patient-derived prostate cells (Rane et al., 2016). miR-99a and miR-100 have been shown to be significantly suppressed in prostate stem-like cells ($CD133^+$, $\alpha_2\beta_1^{hi}$ cells) compared to their differentiated progeny committed basal cells ($CD133^-$, $\alpha_2\beta_1^{lo}$) (Rane et al., 2015). $CD133^+$ cells have been reported to be tumorigenic *in vivo* after fractionation of heterogeneous bulk samples (Maitland et al., 2011). Strikingly, the similar pattern of downregulation in microRNA expression during PCa progression, documented in a remarkable number of studies, is also observed in tumorigenic prostate cancer stem-like cells vs. more differentiated cells (i.e. miR-99a and miR-100 are decreased in prostate stem-like cells vs. committed basal cells). Thus, during progression towards a more aggressive state, there seems to be a general tendency to reduce the expression levels of tumor suppressor microRNAs.

Interestingly, TMPRSS2-ERG has been reported to be expressed in the stem-like compartment ($CD133^+$, $\alpha_2\beta_1^{hi}$ cells) enriched for $CD44^+$ cells (Birnie et al., 2008). Together, this suggest that miR-99a/100 present in fusion-positive prostate cancer stem-like cells might represent interesting target molecules in combination with endocrine-therapy aimed to inhibit GR.

5. microRNA interactions with TMPRSS2-ERG

The chromosomal rearrangement resulting in the formation of the fusion gene between TMPRSS2 and the transcription factor ERG, is detected in approximately 6% of benign prostatic hyperplasia (BPH) and 50–60% of all PCa (Tomlins et al., 2005; Clark et al., 2007; Visakorpi, 2012). It was reported that the frequency of the TMPRSS2-ERG fusions in high-grade PIN lesions and localized PCa, is about 15% and 50% respectively (Clark et al., 2008; Mosquera et al., 2008), suggesting that this event either occurs after cancer initiation, or alternatively predisposes to clinical progression (Shen and Abate-Shen, 2010), althought this is not cancer restricted. It has also been proposed that the formation of this chromosomal rearrangement might be controlled by androgens; AR binding in LNCaP androgen responsive PCa cells resulted in juxtaposition between the AR regulated promoter of TMPRSS2 and ERG (Lin et al., 2009). Moreover, androgen signaling might recruit topoisomerase II inducing double strand breaks even in absence of stress (Haffner et al., 2010; Kolar et al., 2014).

Although extensive research, (reviewed in (Tomlins et al., 2009; Visakorpi, 2012; Adamo and Ladomery, 2016; Archer et al., 2016)) has been performed in the last years, related to ERG-induced oncogenesis in PCa, following the chromosomal rearrangements with TMPRSS2, only few studies have functionally investigated the reciprocal influences between microRNAs and TMPRSS2-ERG fusion gene.

miR-221 is one of the microRNAs that was shown to be progressively downregulated in aggressive prostate cancer in hormone naïve tumors and it has been proposed as novel prognostic biomarker and therapeutic target in high-risk prostate cancer, being an effective predictor of clinical recurrence (Spahn et al., 2010). Recently, miR-221 was shown to regulate prostate cancer cell growth, invasiveness, and apoptosis via direct inhibition of SOCS3 and IRF2, two oncogenes that negatively regulate the JAK/STAT signaling pathway (Kneitz et al., 2014) (Fig. 1). Interestingly, Gordanpour et al., have shown that miR-221 is downregulated in prostatic tumors bearing TMPRSS2-ERG fusion transcripts (Table 2) (Gordanpour et al., 2011), providing the first published evidence for miRNA associations in prostate cancer that overexpress the ERG oncogene from the TMPRSS2-ERG fusion transcript. However, it is important to highlight that in PCa the situation seems to be more complex, because Sun et al., (2014), have shown that miR-221 expression levels are increased in tissue derived from bone metastasis of CRPC, which suggests a specific function of miR-221 in the development of androgen resistance. miR-221 could indeed abolish proliferation in the SOCS3-positive and androgen independent PC3 and Du145, but not in the SOCS3-negative and androgen dependent LNCaP cells (Kneitz et al., 2014). Together this supports the notion that in PCa the sensitivity against androgen signaling depends on SOCS3 expression (Neuwirt et al., 2007) and that miR-221 might play a pivotal role in the regulation of the androgen-independent growth.

Remarkably, the mechanism of pathogenesis for fusion−-negative tumors compared to TMPRSS2–ERG positive ones, is still not entirely elucidated (Borno et al., 2012). Interestingly, distinct epigenetic mechanisms distinguish TMPRSS2–ERG fusion-positive and -negative prostate cancers (Alumkal and Herman, 2012) and, as previously discussed, DNA methylation and histone modifications are two epigenetic mechanisms responsible for the de-regulation of microRNAs expression (Lujambio et al., 2008). Interestingly, one of the highly upregulated genes during prostate cancer progression is the human homologue of the Drosophila protein Enhancer of Zeste 2 (EZH2), which belongs to the group of polycomb proteins and is involved in silencing of homeobox genes through methylation (Pirrotta, 1998; Hoffmann et al., 2007).

EZH2 is a target of the TMPRSS2–ERG gene fusion, and

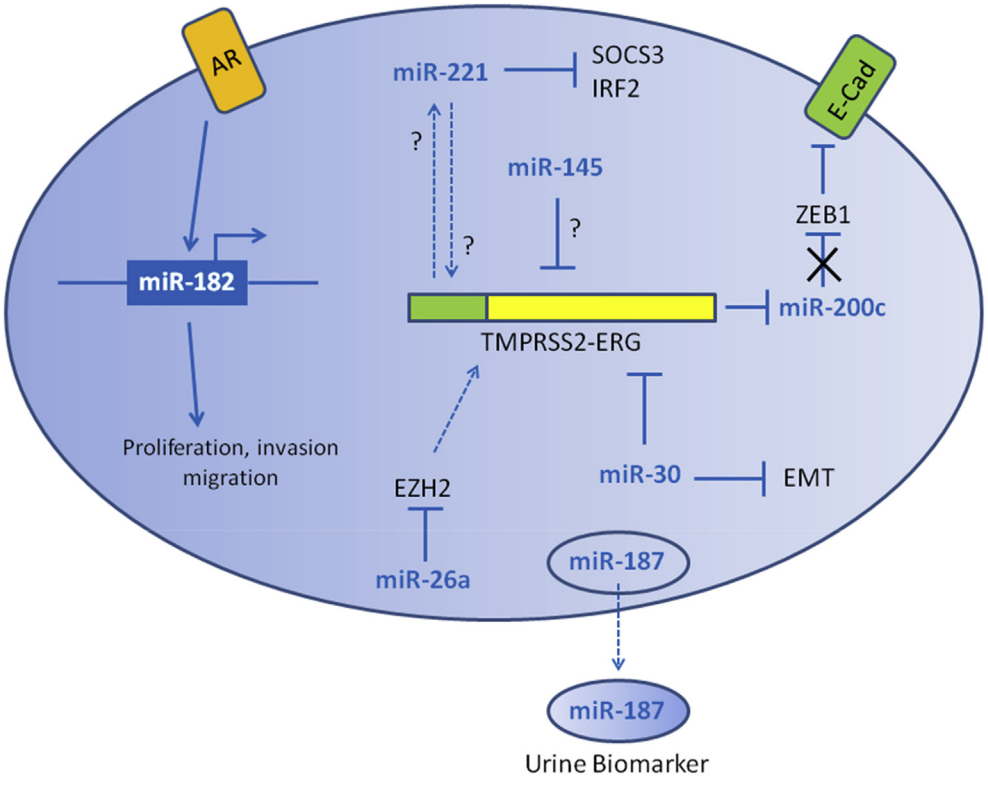

Fig. 1. Schematic representation of most important and functionally relevant microRNA in ERG-positive PCa cell.

Table 2
microRNAs expression modulation in TMPRSS2-ERG positive cells vs. TMPRSS2-ERG negative.

microRNAs	TMPRSS2-ERG Positive (vs. Negative)	Sample	p-value	References
miR-221	Downregulated	153 samples of radical prostatectomy	$p < 0.01$	Gordanpour et al., 2011
miR-26a	Upregulated	51 prostate cancer samples	$p < 0.05$	Borno et al., 2012
miR-200c	Downregulated	15 ERG + vs. 14 ERG − Pca samples	0.002	Kim et al., 2014
miR-145	Downregulated	26 corresponding pairs of non-malignant prostate tissue and PCa	0.0013	Hart et al., 2013
miR-187	Downregulated	273 paraffin embedded PCa samples	4.94E-06	Casanova-Salas et al., 2014
miR-182	Upregulated	273 paraffin embedded PCa samples	1.63E-06	Casanova-Salas et al., 2014

TMPRSS2—ERG and EZH2 cooperate in the regulation of shared target genes, including AR (Yu et al., 2010). Aberrant DNA methylation associated with altered EZH2 expression correlates with PCa progression and has been proposed as an early event in tumorigenesis (Varambally et al., 2002). Interestingly, a recent report has documented the global epigenetic alterations in fusion—negative tumors, providing a mechanistic explanation for the formation of these cancers (Borno et al., 2012). In this report, it has been proposed that hypermethylation of miR-26a is an alternative way to activate EZH2 in an ERG rearrangement-independent manner. miR-26a directly targets EZH2 and it is suppressed in fusion-negative prostate cancers (Table 2, Fig. 1) (Borno et al., 2012). This suggests that a suppression of miR-26a caused by a hypermethylation leads to a decreased inhibition of EZH2, leading to perturbations to the global DNA methylation profile, providing a new mechanistic model for fusion-negative tumors (Borno et al., 2012).

Given the established notion that ERG is overexpressed in a high proportion of the prostate carcinomas (Tomlins et al., 2005), a relatively large part of the scientific work performed in the past decade has focused on the molecular characteristics of these fusion-positive tumors. The overexpression of ERG represents one of the key factors in the switch from confined to metastatic disease (Hagglof et al., 2014) and is accompanied by loss of E-cadherin expression, increased cell mobility and invasion (Leshem et al., 2011). Additionally, is has been proposed that TMPRSS2-ERG promotes epithelial-to-mesenchymal transition (EMT) through the ZEB1/ZEB2 axis in PCa (Leshem et al., 2011). Interestingly, it was recently shown that docetaxel resistant human PCa cells display EMT features and properties of tumor-initiating cells (Puhr et al., 2012) and experimental work revealed that these cells display upregulation of ZEB1 and downregulation of miR-200c. Intriguingly, miR-200c has been recently identified as downstream target of ERG and miR-200c expression in tissues from patients with ERG-positive PCa was significantly lower compared with ERG-negative tumors (Table 2), supporting the notion that ERG directly represses miR-200c (Kim et al., 2014). These data together indicate that ERG might be involved in the acquisition of chemotherapy resistance and suggest that monitoring of ERG, miR-200c and ZEB1 in PCa patient's tissues might be relevant to predict the outcome of chemotherapy (Culig, 2014) (Fig. 1).

Another microRNA relevant in the context of EMT and TMPRSS2-ERG positive tumors is miR-30 (Kao et al., 2014). Kao et al., have demonstrated that ERG is a direct target of miR-30 and increased expression of miR-30 in VCaP and PC3 prostate cancer cells resulted in reduction of EMT phenotypes and abolished migration and invasion (Kao et al., 2014) (Fig. 1). miR-30 has been shown to be significantly downregulated in tumor vs. benign tissue and in hormone-refractory prostate cancer (Porkka et al., 2007). Interestingly, administration of selective inhibitors of Src-family tyrosine kinases resulted in a strong upregulation of miR-30 and decreased ERG expression at mRNA and protein level. Given that specific Src kinase inhibitors have been tested in Phase I, II and III clinical trials (in combination with docetaxel (Araujo et al., 2013)) for the treatment of PCa patients, this suggests the employment of Src inhibitors for especially targeting ERG-positive castration-resistant tumors. However, the role of EMT in the context of androgens and androgen-regulated genes seems to be more complex: AR splice variants appears to contribute to PCa aggressiveness and EMT induction (Kong et al., 2015), however ADT has been shown to generate EMT in normal and neoplastic prostate in animal models (Sun et al., 2012).

miR-145 is a direct regulator of ERG and shown to be consistently downregulated in prostate cancer (Hart et al., 2013). Although no association between miR-145 and ERG mRNA expression is reported, a negative correlation between miR-145 and ERG protein was demonstrated (Table 2) (Hart et al., 2013). Interestingly, the documented reduction of miR-145 in PCa might support the elevated expression of multiple ERG isoforms, all detected ERG variants display, indeed, the miR-145-responsive 3′ UTR seed sequence (Hart et al., 2013) (Fig. 1).

Finally, miR-187 also displayed a strong pattern of downregulation during PCa progression in a cohort of 50 PCa samples vs. 10 normal tissues (Casanova-Salas et al., 2014). These results were validated in an independent cohort of 273 paraffin embedded PCa samples and displayed an inverse correlated with TMPRSS2-ERG expression (Table 2) (Casanova-Salas et al., 2014). Notably, in the same study, a positive correlation between miR-182 and TMPRSS2-ERG was detected and miR-182 was shown to be significantly associated with progression free survival and revealed to be a significant independent predictor of worse outcome for biochemical progression free survival (defined as PSA 0.4 ng/ml or greater during follow-up) but not for progression free survival (defined as local, lymph nodes or distant metastasis growth) (Casanova-Salas et al., 2014). Therefore, miR-187 and miR-182 have been proposed as biomarkers of early diagnosis and prognosis in PCa patients (Fig. 1). Interestingly, miR-182 has been shown to be directly regulated by the AR and to promote prostate cancer cell proliferation, invasion and migration and inhibit apoptosis (Yao et al., 2016). Accordingly, miR-182 expression is increased in AR-positive cell lines, such as LNCaP, 22RV1 and C4-2, and reduced in the AR-negative cell line DU145 (Yao et al., 2016). The association of miR-182 with AR reinforce its positive correlation with TMPRSS2-ERG in patient's specimens.

6. microRNA and ERG based therapy

As previously discussed, ERG has been extensively shown to be implicated in PCa initiation and progression and to be involved in multiple processes such as EMT, invasion and metastasis. ERG based therapy options have been recently comprehensively reviewed by Adamo et al. (Adamo and Ladomery, 2016). In this paragraph we highlight the connection between ERG-linked molecules, currently targeted by ERG based therapy and clinically relevant microRNAs in PCa.

Poly(ADP-Ribose) Polymerase (PARP) is a DNA repair protein which interacts with ERG through a DNA-independent mechanism (Sebastian de Bono et al., 2011). Inhibition of PARP reduces the aggressiveness of ERG-positive PCa cells. High levels of miR-182 in

breast cancer increased the sensitivity to PARP1 inhibitors and antagonizing of miR-182 resulted in the opposite effect (Moskwa et al., 2011). Given the increased levels of miR-182 in AR and TMPRSS2-ERG positive cells (Casanova-Salas et al., 2014), this reinforces the use of PARP1 inhibitors to target ERG-positive PCa cells. Administration of PARP inhibitor, rucaparib, to ERG-positive and PTEN-negative PCa cells sensitized the cells to low-dose radiation which possibly generate DNA DSB (Chatterjee et al., 2013). Interestingly, two mechanisms for PARP inhibition have been proposed: 1) inhibition of the PARP1-ETS complex, a key component for the ERG-mediated invasion and cell growth; 2) lethality following the accumulation of DNA DSB (Brenner et al., 2011), which supports the described effect with radiations. Interestingly, gain-of function screen of 1129 miRNAs in a panel of human PCa cell lines, identified and validated miR-9 as direct regulator of AR in PCa (Ostling et al., 2011). Moreover, in ovarian cancer, miR-9 was shown to increase cellular sensitivity to the same PARP1 inhibitor (Sun et al., 2013). In another report, Nowek et al. demonstrated that miR-9 directly targets ERG (Nowek et al., 2016). Together this suggest that miR-9 might exerts a synergistic effect with ERG-targeted therapy in human PCa. miR-9 was also moderately upregulated in a panel PCa cell lines upon administration of trichostatin A (TSA), and HDAC inhibitor, which reduces growth of ERG-positive PCa cells and deceases the expression of TMPRSS2-ERG (Table 1) (Rauhala et al., 2010). However, in the same study, miR-193 displayed a significantly higher induction upon TSA treatment and was shown to be downregulated in PCa samples vs. BPH, suggesting a tumor suppressive role for this microRNA (Table 1) (Rauhala et al., 2010). Another microRNA interesting for its pattern of expression during disease progression is miR-1296. This microRNA is downregulated in PCa tissues compared to BPH and directly targets the minichromosome maintenance gene 2 (MCM2) (Majid et al., 2010). MCM2 is involved in DNA replication, is upregulated in PCa and correlates with poor survival (Meng et al., 2001; Majid et al., 2010). Interestingly, TSA treatment in PCa cell lines PC3 and LNCaP cell resulted in decreased MCM2 protein expression (Majid et al., 2010), suggesting that miR-1296 might represent another molecule with synergistic properties to reduce growth of ERG-positive PCa cells.

Finally another strategy for ERG-based therapy is its direct targeting with molecules promoting degradation. USP9X is a ubiquitin-specific peptidase which stabilizes ERG and knockdown of USPX9 resulted in increased ERG degradation, following ubiquitination (Adamo and Ladomery, 2016). Inhibition of USP9X with direct inhibitor WP1130, resulted in ERG degradation and inhibited growth of ERG-positive tumors *in vivo* (Wang et al., 2014). Interestingly, miR-26b has been shown to directly target USP9X (Shen et al., 2014) and is markedly downregulated in PCa tissues (Kato et al., 2016). This suggests that miR-26b replacement therapy might inhibit USP9XP, leading to ERG degradation and inhibition of ERG-positive PCa. Given the positive outcome of preclinical studies which employed non-toxic liposomal nanovectors to selectively knockdown TMPRSS2-ERG fusion (Shao et al., 2012), this opens new scenarios for microRNA replacement therapies and for the delivery *in situ* of miRNA mimics capable of interfering with ERG signaling, in combination with ERG-targeted therapy.

7. Conclusion and perspectives

In this review, we discussed and summarized the interactions between microRNAs and endocrine-related aspects of prostate cancer. We discussed multiple aspects of androgen- and glucocorticoid-targeted therapy and the influences of these treatments on the modulation of microRNA expression. We specifically highlighted the different approaches to investigate the connections between microRNAs and the androgen-regulated gene TMPRSS2-ERG and also discussed novel insights into the effect of GR targeting in advanced prostate cancer and microRNAs.

The diagnostic and prognostic value of microRNAs is nowadays clear. Non-coding RNAs can be employed as biomarkers and predictors of therapy response. However, the applicability of miRs as therapeutic agents in oncology is still in its developmental phase, mainly due to therapeutic delivery challenges. The intrinsic aspecificity that microRNAs present is one of the biggest challenge in the achievement of anti-tumor response with acceptable side effects. Additionally, the microenvironment and tissue dependent "dual" function of microRNAs as tumor suppressors or oncogenes is one of the main challenges for a therapeutic approach.

We believe that miRNA targeted therapy will be attempt, once that a selective therapeutic delivery system will be developed to specifically target cancerous cells. Meanwhile, the employment of microRNAs as predictors of therapeutic outcome is one of the strategies for better patient stratification and treatment response in the perspective of a personalized medicine era.

Conflicts of interest

The authors have nothing to disclose.

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