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Chapter 22

Trithorax Genes in Prostate Cancer

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1. Introduction

For several years, prostate cancer (PCa) has been considered a genetic disease, driven by somatic mutations occurring at critical oncogenic or tumor suppressive *loci* [1]. This view has changed over the last decades, thanks to mounting evidence on the role of epigenetics in PCa initiation and progression [2]. The term "epigenetics" derives from a Greek word, and literally means "above the gene". In molecular biology, this definition includes all heritable gene expression patterns, which are not derived from an alteration of DNA primary sequence [3]. The first epigenetic alteration to be linked with cancer was DNA methylation, which occurs at 5-cytosine residues in specific genomic regions, called CpG islands [2, 4]. Cytosine methylation results in gene silencing, especially when occurring at the promoter region of a targeted *locus*. This process is mediated by enzymes called DNA methyltransferases (DNMTs) [5]. Functional studies demonstrated that, along with inactivating mutations, DNA methylation is an alternative way of tumor suppressor silencing, and that this event might even anticipate the occurrence of a genetic mutation. For example, the *PTEN* (Phosphatase and tensin homolog) gene encodes for a phosphatase which acts as a potent tumor suppressor in PCa [6]. Indeed, PTEN protein is able to inhibit AKT (protein kinase B), which in turn activates several anti-apoptotic and proliferative signals in PCa cells. In keeping with these observations, PTEN- knockout mice display an early onset of PCa [7]. *PTEN* inactivating mutations are found in approximately 20% of PCa samples, and are associated with hormone refractory disease and higher tumor stage [8]. However, *PTEN* mutation is rarely homozygous, and approximately 50% of PCa patients are PTEN-negative, even if they do not display any genetic alteration [9]. Subsequent studies found that DNA methylation is the main mechanism of *PTEN* silencing in PCa, as well as in other neoplasms [10]. This event may occur in association with mutation on the other allele [11]. DNA methylation in the *PTEN* promoter region acts as a

docking site for MeCP2 (methyl-CpG-binding protein 2), which in turn recruits several chromatin remodelling factors. Those complexes are able to turn transcriptionally active chromatin (euchromatin) into an inactive form (heterochromatin) [12]. Since then, several tumor suppressor genes were shown to be methylated in a significant fraction of PCa patients [13]. DNA methylation patterns are useful biomarkers for early diagnosis and patient stratification. Unlike genetic alterations, epigenetic changes are reversible, and thus can be targeted by specific drugs [2]. DNMT inhibition is able to reactivate silenced oncogenes, thereby inducing apoptosis and reducing treatment resistance [14]. Pharmacological inhibitors of DNMTs have been developed and tested in clinical trials, and some of them are approved for the treatment of haematological malignancies [15]. In PCa, as well as in other solid tumors, DNMT inhibitors displayed encouraging effects in pre-clinical models [14], but often failed to demonstrate clinically relevant activity [16]. One possible explanation for this discrepancy is that DNA methylation is not the key epigenetic mechanism in PCa.

As basic research on epigenetic gene regulation proceeds, it is becoming increasingly clear that gene expression regulation in human cells is finely tuned by the concurrent activity of different protein complexes. To understand the foundation of this intricate process, it is necessary to consider the tridimensional structure of chromatin [17]. The nucleosome is the basic chromatin unit. It is composed by approximately 150 bp of DNA, which are wrapped around a cylindrical protein complex (histone core) [18]. The core is an octamer composed of two copies of histone H2a, H2b, H3 and H4. Histone H1 acts as a linker between two nucleosomes. Nucleosomes can restrict the access of RNA polymerases to the DNA; thus, their local interaction with DNA is critical for gene expression control. Histones are characterized by long N-terminal tails, which mainly interact with the DNA phosphate backbone [19]. For this reason, post-translational modifications at histone tails can shape the local tridimensional structure of chromatin, thereby affecting RNA polymerase (and transcription factor) accessibility, and eventually modifying gene expression. Seminal studies revealed that the range of possible histone post-translational modifications (HPTMs) is wide, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation and ADP-ribosylation [20]. Another layer of complexity is represented by the variable number of amino-acidic residues that can be modified. In addition, some modifications may be repeated on the same residue. For example, histone H3 Lys 27 (H3K27) can be mono-, di- or tri-methylated (me) [19]. Each single modification affects gene activity, and likely interacts with others [20]. As it is easy to understand, the combinatorial complexity of those modifications is immense, and we still lack appropriate technologic tools to comprehensively investigate this phenomenon [21]. Some authors proposed the systematic discovery of the histone code, i.e. the hidden language by which HPTMs cooperate to determine local gene activity [22].

Despite this discouraging complexity, some research sheds light on the functional role of specific HPTMs. For example, it is well known that histone lysine acetylation loosens DNA-histone binding, thereby providing transcriptionally active chromatin [23]. Accordingly, histone acetylases (HATs) are a class of activating epigenetic modifiers [24]. For the same reason, histone deacetylases (HDACs) are enzymes that repress gene expression [25]. To the

contrary, histone methylation is multifaceted, since it can be associated with gene repression or activation depending on the targeted amino acid residue. For example, H3K9me and H3K27me are repressive marks, while H3K4me and H3K36me activate gene expression [26]. Interestingly, most of those HPTMs are mediated by two classes of histone modifiers, which appear to act as counteracting forces during embryonic development, and are emerging as novel oncogenes and tumor suppressor genes. The first class to be discovered was the Polycomb group (PcG) genes, which are mainly organized in multimeric Polycomb repressive complexes 1 and 2 (PRC1 and PRC2; Table 1) [27]. PRC2 catalyzes H3K27 trimethylation (me3), which acts as a docking site for PRC1. The latter complex then catalyzes histone H2aK119 ubiquitination (ub). Both modifications are repressive marks, and can be associated with DNA methylation [28]. In addition, it has been shown that PRC1 can act independently of PRC2 [29]. The function of PRCs was revealed by mutational analyses conducted on *Drosophila.* PRCs are essential for HOX (homeobox) gene silencing and tissue specification [30]. *Drosophila* PRC homologs are also expressed in human cells, where they regulate stem cell function and differentiation. Studies on human cells also revealed that PRCs can target a wider set of genes, and that they are involved in physiologic and pathologic phenomena, including cancer [31]. In PCa, both PRC1 and PRC2 display oncogenic functions, through the repression of key tumor suppressor genes. For example, PRC1 member BMI1 (B-cell-specific Moloney murine leukemia virus integration site 1) induces resistance to conventional chemotherapy (docetaxel) [32], while PRC2 member EZH2 (enhancer of zeste homolog 2) is essential for PCa cell invasion and metastatic spreading [33].

As anticipated, trithorax group genes (TrxGs) were first discovered as PRC-counteracting forces in *Drosophila*, where their role in switching on and maintaining the activation of HOX genes is well known [30]. TrxG complex organization is more variable than what has been found for PRCs. First, TrxGs include both histone modifiers and ATP-dependent chromatin remodelling factors [34]. The first class acts by decorating histone tails with activating marks, while the latter "reads" those modifications and actively induces a tridimensional change in chromatin structure, which then becomes available for RNA polymerases and transcription factors. Since this chapter is focused on strictly epigenetic mechanisms of gene expression control, we will not discuss chromatin remodelling factors. In mammals, histone modifier TrxGs are grouped in 3 major complexes (refer to Table 1): COMPASS (complex protein associated with SET domain), COMPASS-like and ASH (absent small and homeotic discs). COMPASS contains a histone methyltransferase domain (SET), which is shared with PRC2 [35]. Unlike PRC2, COMPASS mediates H3K4me, a broad activating mark found throughout the genome. COMPASS-like complexes also display the SET domain, which is used to silence a more restricted group of genes [36]. COMPASS-like can also activate gene expression through H4K16 acetylation [34]. Depending on subunit composition, this complex is also able to demethylate H3K27me, thereby directly counteracting PRC2 [37]. Finally, ASH1 is able to catalyze H3K36me, a further activating mark. In mammals, this function is mediated by a single protein rather than a complex [34].

Along with their function in embryonic development, TrxG histone modifiers are emerging as a novel class of cancer-related genes [43]. Due to their multifaceted interaction with PcGs,

and due to the role of PcGs in PCa, it is likely that TrxGs also play a role in this neoplasm. For this reason, we decided to summarize current knowledge on the role of TrxGs in cancer initiation and progression, and to query a publically available gene expression database, to get insights into the role of those genes in PCa metastasis, which is the major determinant of death induced by this neoplasm. Based on our literature search and our results, we will propose a model to explain putative mechanisms of TrxG-dependent oncogenic, or tumor suppressive, functions.

Type	Complex	Subunits	HPTMs Catalyzed	Transcriptional Effect	References
PcG	PRC1	BMI1; RING1; RING1B; CBX	H2AK119Ub	Repression	[38-40]
	PRC2	EZH2; SUZ12; EED	H3K27me3	Repression	[38-40]
TrxG	COMPASS	SET1A,B; CXXC1; WDR82; ASH2L*; DPY30; HCF1; RBBP5; WDR5	H3K4me3	Activation	[34, 41, 42]
	COMPASS-like (A)	MLL1,2; MOF; MENIN; ASH2L; DPY30; HCF1; RBBP5; WDR5	H3K4me3; H4K16ac	Activation	[34, 41, 42]
	COMPASS-like (B)	MLL3,4; UTX; NCOA6; PA1; PTIP; ASH2L; DPY30; HCF1; RBBP5; WDR5	H3K4me3; H3K27 demethylation	Activation	[34, 41, 42]
	ASH1	ASH1L	H3K36me3; H3K27ac	Activation	[34, 41, 42]

Note : Red indicates core COMPASS subunits

Table 1. Composition and Activity of TrxG Complexes

2. Body

2.1. Overview of trithorax group activity in prostate cancer

It is now well established that TrxG counteracts PcG HPTMs to promote a transcriptionally competent chromatin state [34, 44]. An intricate regulatory network controls whether the repressive effect of PcG activity or the activating role of TrxG dominates at specific *loci* [45]. The best characterized interplay between these two families of epigenetic modifiers occurs during embryonic development. In undifferentiated cells, PcG is highly expressed and maintains lineage-specific genes in a transcriptionally incompetent state while TrxG activity is minimal [46]. In response to external differentiation cues, PcG activity is reduced while TrxG becomes functionally active. As a result, lineage-specific genes are expressed and drive the cell towards a differentiated state [46]. The classic example of TrxG and PcG interplay

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involves the regulation of the HOX *locus*(See Figure 1). First silenced by PcG in embryonic stem cells (ESCs), HOX genes are subsequently induced upon TrxG activation during differentiation [47].

The functional relationship between PcG and TrxG is not limited to development. Incorrect regulation of PcG and TrxG also plays an inherent role in cancer initiation and progression [48-51]. In cancer, many embryonic transcriptional programs are orchestrated and push tumor cells towards a more undifferentiated state [43]. This directly implicates PcG and TrxG as they regulate many common target tumor suppressor genes that inhibit differentiation, invasion, and cell cycle progression [41]. These tumor suppressor genes are turned off in cancer, correlating with increased PcG expression and H3K27 trimethylation [52]. This indicates that, in cancer cells, PcG somehow undergoes a gain of function while TrxG activity is lost at key metastasis-inhibitory *loci*. In this classical model, PcG therefore act as oncogenes while TrxG operate as tumor suppressors (See Figure 1).

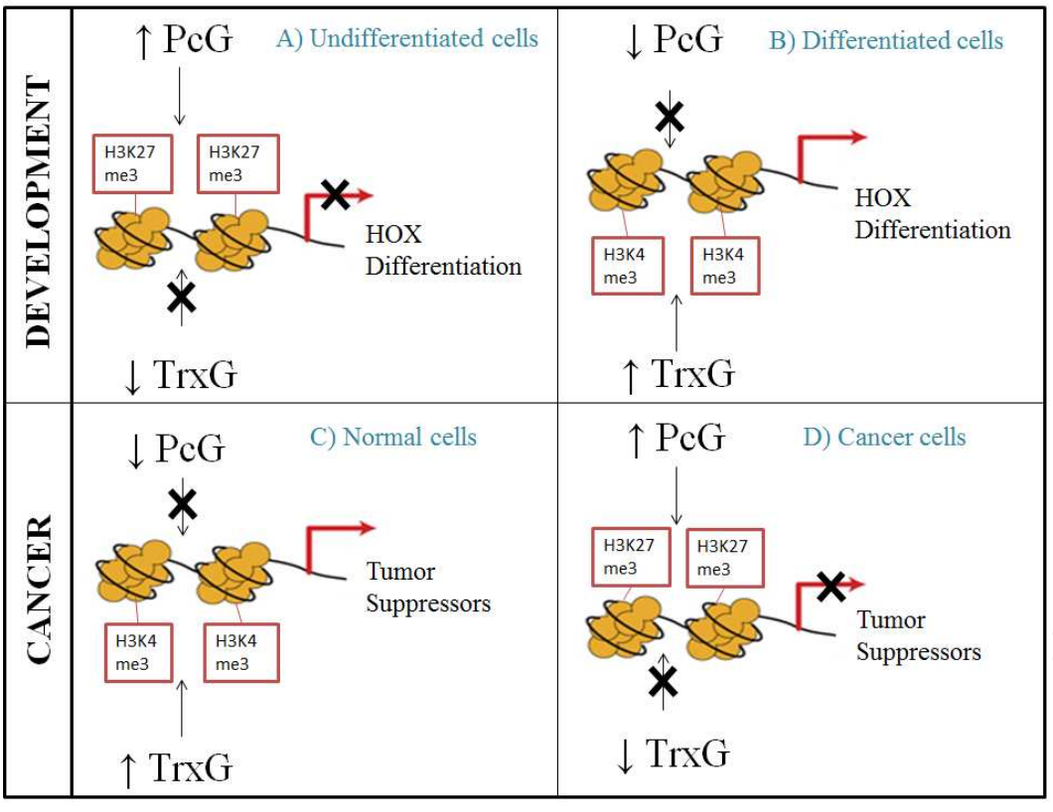

Figure 1. Classical Model of the PcG-TrxG Interplay in Development and Cancer

However, this model does not explain all the data regarding TrxG in cancer as the expression of individual TrxG subunits is highly heterogeneous across, and within, different tumor types. According to the classical model, TrxG genes act as tumor suppressors and should therefore be consistently downregulated in malignant cells. In fact, the expression of some TrxG genes increases in cancerous tissues, suggesting an oncogenic role for these particular TrxG genes [52, 53]. This indicates that there must exist an additional level of complexity

which regulates not only the expression of individual TrxG genes, but also the activity and sequence specificity of TrxG complexes. Since TrxG proteins function as multimeric structures, their activity is highly context-dependent [54]. Many factors need to be taken into consideration when trying to assess the molecular function of TrxG complexes in a given temporal and spatial context. First of all, what is the relative expression of the individual subunits present within the TrxG complex? If many subunits are overexpressed or underexpressed, the composition of the complex changes, which might lead to functional differences. Second, which coregulators of these complexes are present? For example, a corepressor could bind to a given TrxG complex and inhibit its H3K4 methyltransferase ability. Another possibility is that a transcription factor expressed specifically in cancer cells binds to a TrxG complex and recruits it to a normally untargeted loci. Finally, how is TrxG activity regulated by PTMs of its individual subunits? Every TrxG complex is composed of multiple proteins, all of them able to be chemically modified at multiple residues. Each PTM potentially affects the activity of the complex and the additive effect of all these possible PTMs accounts for an astronomical number of possible transcriptional outcomes [21]. In summary, although the traditional model by which TrxG simply opposes PcG functions in cancer still represents a good approximation, it remains incomplete as additional factors regulate TrxG activity.

Even though the epigenetic landscape of PCa remains quite complex, interesting links can be found between histone modifiers and the metastatic process. PcG members EZH2 and BMI1 are both overexpressed in PCa and their elevated expression correlates with metastasis and poor prognosis [55-57]. Their importance in PCa progression is reflected by the numerous studies that explored the possibility of targeting them pharmacologically [58-61]. While the role of PcG has been extensively investigated, few studies directly assessed the role of TrxG in PCa. Our analysis revealed that although no individual TrxG genes shows consistently significant up- or downregulation, a very high proportion of metastatic prostate tumors contain at least one TrxG gene whose expression is deregulated. The accumulated evidence suggests that TrxG does not act only as traditional tumor suppressors which counteract PcG activity. In fact, individual TrxG genes can interact with other complexes to either promote or repress progression to metastasis. To account for this functional heterogeneity, we will review the current literature for individual TrxG gene previously associated with cancer and then discuss expression data from a publicly available PCa database. We will finish by proposing putative mechanisms of TrxG misregulation in PCa, with a focus on the metastatic process.

3. Literature review – Individual TrxG genes

3.1. ASH2L

ASH2L is the human homologue of *Drosophila* ASH2 (absent small homeotic 2) and represents a core member of the COMPASS and COMPASS-like complexes. Through interactions with WDR5 (WD-repeat protein 5) and RBBP5 (retinoblastoma binding protein 5), ASH2L activates SET1 domain-containing proteins (SET1A, SET1B and mixed lineage leukemia

(MLL)1-4) which subsequently catalyze H3K4 trimethylation [54]. The presence of ASH2L is essential for optimal H3K4 trimethylation as knockdown of ASH2L led to a genome-wide decrease in H3K4me3 [62]. Since COMPASS and COMPASS-like complexes are required for the transcriptional activation of numerous differentiation genes such as the HOX family, defects in ASH2L activity result in developmental defects [42, 63]. In mice, homozygous knockdown of ASH2L with gene-trap technology resulted in early embryonic lethality [64]. ASH2L also promotes differentiation in muscle during later developmental stages. Through an interaction with ASH2L, PAX7 (paired box 7) recruits the WDR5-ASH2L-MLL2 complex to myogenic gene promoters and promotes trimethylation of H3K4 at these sites [65]. MEF2D (myocyte enhancer factor 2D) is a transcription factor downstream of the p38 MAPK (mitogen-activated protein kinase) that also directs ASH2L-containing complexes to MyoD (myoblast determination protein)-bound genes in myoblasts [66]. At specific *loci*, MyoD, PAX7, and ASH2L cooperate to induce a transcriptional program that leads to myogenic differentiation [67].

In addition to its role in development, ASH2L is also involved in tumor initiation. While ASH2L mRNA levels remain normal in human cancers, ASH2L protein levels increase dramatically in malignant cells, suggesting an oncogenic function for ASH2L [68]. Supporting this hypothesis, ASH2L was also identified in complexes containing MYC (myelocytomatosis viral oncogene homolog) oncogene [68]. Since MYC activity increases in many types of cancers, the interaction between ASH2L and MYC suggests that ASH2L potentially adopts an oncogenic function [69]. Indeed, ASH2L transforms primary rat embryo fibroblasts (REFs) through cooperation with H-Ras (Harvey rat sarcoma viral oncogene homolog) [68]. As expected from an oncogene, knockdown of ASH2L reduces cell proliferation and inhibits transformation of REFs by MYC and H-RAS [68]. A recent study revealed that ASH2L might affect PCa progression by acting as a co-activator of the androgen receptor (AR) [70]. Co-immunoprecipitation experiments showed that AR interacts with ASH2L [70]. Importantly, TrxG genes MLL1 and MLL2 also interact with AR [70], suggesting that ASH2L function in PCa results from association with complexes having H3K4 methyltransferase activity (See Figure 2A). Furthermore, siRNA (small interfering RNA) silencing of MLL or ASH2L significantly repressed AR signalling [70]. However, pathways underlying the oncogenic nature of ASH2L remain poorly characterized. An important question that needs to be addressed is whether ASH2L promotes tumorigenesis through the same pathways in all tumor types or if its activity depends on the availability of other context-specific coregulators.

3.2. MENIN

MENIN (protein encoded by multiple endocrine neoplasia 1 gene – *MEN1*) represents an integral subunit of the COMPASS-like complex that contains MLL1-2, MOF (MYST family histone acetyltransferases), and core COMPASS proteins that trimethylate H3K4 [42]. In contrast to ASH2L, whether MENIN acts as an oncogene or a tumor suppressor highly depends on the specific tissue. Inherited mutations inactivating the *MEN1* gene lead to a condition called multiple endocrine neoplasia type 1, in which the patients develop neoplasias in endocrine organs such as the parathyroid gland, the pituitary gland, and the pancreas [71,

72]. In endocrine organs, MENIN functions as a tumor suppressor and its role has been well characterized [73]. MENIN induces the transcription of cyclin-dependent kinase inhibitors p18 and p27 [74]. A mutated *MEN1* gene therefore leads to a decrease in p18 and p27 expression, which accelerates cell-cycle progression. Loss of MENIN also promotes tumorigenesis by releasing the inhibition of the oncogenic transcription factor JUN D (jun sarcoma virus 17 oncogene homolog) [75], which subsequently induces the expression of genes responsible for proliferation [76]. In summary, mutation of the *MEN1* gene leads to neoplasm formation in endocrine organs, which signifies that MENIN acts as a tumor suppressor in these tissues. However, studies in hematopoietic malignancies containing MLL fusion proteins suggest an oncogenic role for MENIN [77]. In this context, MENIN binds to the MLL fusion protein and the complex activates the expression of key oncogenes which drive leukemogenesis [78]. Since MLL fusion proteins do not possess a SET domain, it is important to note that the oncogenic function of MENIN does not implicate H3K4 methylation [78]. Misregulation of MENIN activity also induces the formation of some solid tumors, although its mechanism of action varies considerably with the tumor type. For example, MENIN has been described as a tumor suppressor in non-small cell lung carcinomas (NSCLC) [79]. MENIN function can also be observed in other solid tumors. In breast cancer, MENIN represents a transcriptional coactivator of ERα (estrogen receptor alpha). [80]. In MCF7 breast cancer cells, MENIN co-localizes with ERα and activates ERα transactivation in a ligand-dependent manner [81]. Interestingly, MLL2 was also independently shown to associate with ERα, suggesting that MENIN's oncogenic function requires the methyltransferase activity of its associated TrxG proteins [82]. Furthermore, ER-positive breast cancer samples highly expressing MENIN had a worse outcome than those with low levels of MENIN after tamoxifen treatment [80]. These findings support the idea that MENIN overexpression promotes the progression to a malignant phenotype in mammary tumors. As in breast cancer, MENIN seems to function as an oncoprotein in PCa [53]. Significant upregulation of MENIN has been described in metastatic prostate tumors in comparison with their non-metastatic counterparts [83]. Copy number gains for *MEN1* represent frequent events in PCa and correlate with an increase in MENIN levels [83]. Depletion of MENIN also significantly suppresses proliferation of DU145 PCa cells, in addition to increasing the levels of Integrin-β1, CASPASE8, and p53 tumor suppressor [53]. Interestingly, MLL and MLL2 interact with AR. Since MENIN associates with MLL and MLL2, it is possible that its oncogenic function stems from cooperation with AR [70]. Given these findings, we propose that MENIN promotes tumorigenesis in PCa.

3.3. MLL

MLL is a H3K4 methyltransferase and its role has been well characterized in certain types of leukemia where it is frequently involved in translocations [84]. Five MLL family members, MLL1-5 are encoded in the mammalian genome [42]. MLL and MLL2 can associate with MENIN, MOF and core TrxG subunits to form a complex with H3K4 and H4K16 methyltransferase activity [54]. MLL3 and MLL4, on the other hand, can only be constituents of TrxG complexes that contain UTX and therefore possess H3K27 demethylase activity [45]. MLL5 does not directly associate with core TrxG members and there is still no evidence that

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it has H3K4 methyltransferase activity [85]. The oncogenic role of MLL in leukemia arises through a translocation that removes its SET domain responsible for H3K4 methylation [84]. However, the role of MLL in PCa tumors has not been fully studied yet. Recent reports indicate that MLL enhances androgen signalling by directly interacting with AR and trimethylating H3K4 at AR target genes [70]. In accordance with an activating role of MLL on AR signalling, RNAi-mediated depletion of MLL significantly decreases Prostate-Specific Antigen (PSA) levels [70]. MLL expression is induced by SOX4 (Sex-determining region Y-box 4), a transcription factor that also activates epidermal growth factor receptor (EGFR), Integrin αv, Ras-related C3 botulinum toxin substrate 1 (Rac1), and ADAM metallopeptidase domain 10 (ADAM10) [86]. The pathways influenced by MLL activity suggest that MLL plays a role in promoting tumorigenesis. As is the case with MLL, MLL2 has also been shown to interact with AR. Although the role of MLL2 remains unclear in PCa, it seems to function as an oncoprotein in breast cancer [87]. By acting as a coactivator, MLL2 stimulates the transcription of estrogen receptor (ER) target genes in ER^+ breast tumors [88]. Amplification of MLL2 has also been recorded in many solid malignancies including breast, pancreatic, brain, and ovarian tumors [89]. In summary, it seems that the H3K4 methyltransferase activity of MLL1 and MLL2 mediates an oncogenic function in solid tumors.

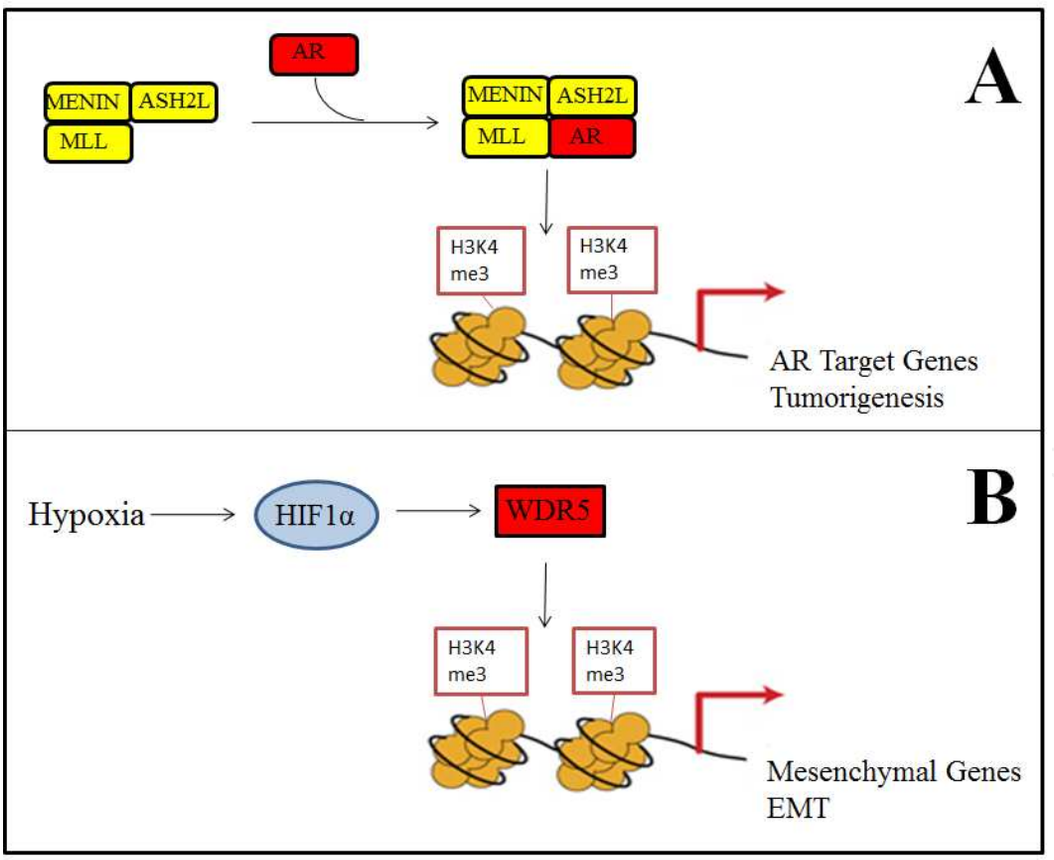

Figure 2. Putative Mechanisms of Oncogenic TrxG Genes in PCa

3.4. MOF

The acetyltransferase MOF (males absent on the first) associates with MENIN, MLL or MLL2, and the core COMPASS proteins (ASH2L, DPY30, HCF1, RBBP5, and WDR5) to form a distinct TrxG complex [90]. MOF specifically acetylates H4K16, a HPTM linked to transcriptional activation [91]. In cancer cells, loss of H4K16ac represents a common event and correlates with general hypomethylation of repetitive DNA sequences [92]. This suggests that MOF activity is inhibited in cancer cells and that MOF therefore functions as an oncosuppressor. Many important growth-regulatory pathways are regulated by MOF, some of which do not require the H3K4 methyltransferase ability of COMPASS-like complexes. First of all, MOF inhibits cancer progression by cooperating with forkhead box protein P3 (FOXP3) [93]. FOXP3 recruits MOF and the H3K4 methyltrasferase complex close to the transcription start site of tumor suppressors [93]. The synergistic effect of H3K4 trimethylation by MLL1-2 and of H4K16 acetylation by MOF results in transcriptional activation of target *loci*. In addition to its regulatory function in transcription, MOF also plays an important role in the DNA damage response (DDR), more specifically in the repair of double-stranded breaks (DSBs) [94]. In response to ATM (ataxia telangiectasia mutated) pathway activation, MOF gets recruited to chromatin where it acetylates H4K16 near DSBs [95]. At sites of DSBs, MOF stimulates the activity of DNA-dependent protein kinases (DNA-PKcs), a critical component of non-homologous end-joining (NHEJ) [96]. Interestingly, studies demonstrated that MOF inhibition also affects homologous recombination (HR) in addition to NHEJ [96]. In short, depletion of MOF leads to a reduction in H4K16 acetylation and is associated with defective DNA repair and chromosomal aberrations following ionizing radiation [97]. MOF also plays another critical role in DDR and apoptosis induction by acetylating the DNA-binding domain of p53 at lysine 120 [98]. This modification leads to increased p53 stability and triggers p53-mediated apoptosis through the upregulation of pro-apoptotic genes [99]. In summary, MOF acts as an important tumor suppressor in PCa through three distinct mechanisms: 1) cooperating with FOXP3 to induce the expression of oncosuppressors 2) recruiting DDR proteins at DSBs by acetylating H4K16 and 3) acetylating p53 on lys120, leading to the expression of pro-apoptotic genes (See Figure 3A).

3.5. UTX

UTX, also called KDM6 (histone lysine demethylase 6), associates with complexes containing the H3K4 methyltransferases MLL3 or MLL4 [42]. UTX possesses H3K27 demethylase activity and therefore plays a prominent role in the balance between PcG-mediated repression and TrxG-mediated activation [100]. The role of UTX has been well characterized in HOX gene regulation during embryonic development [101]. When a cell receives a differentiation signal, UTX promotes HOX gene expression in two ways: 1) It interacts with MLL3 or MLL4, which catalyze the trimethylation of H3K4 at HOX loci and 2) It demethylates H3K27me3, a chemical modification associated with transcriptional repression [101]. Aside from its role in development, UTX has also been linked to cancer where it functions as a tumor suppressor [102]. The demethylase activity of UTX seems particularly relevant to PCa as PRC2 gain of function and H3K27 trimethylation represent common hallmarks of aggres-

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sive solid tumors [103].This global increase in H3K27me3 implies a loss of function for UTX in PCa progression. UTX also counteracts PcG-mediated silencing by stimulating the ubiquitination of H2A, a HPTM associated with transcriptional activation [104]. Moreover, UTX further antagonizes PcG function by interacting with BRM (ATP-dependent helicase brahma) and subsequently recruiting CBP (CREB-binding protein), which catalyzes H3K27 acetylation. The added acetyl group restricts the access to PRC2 at the modified sites and therefore inhibits PcG-induced silencing [37]. UTX also plays an important role in repressing cellular proliferation through the regulation of RB levels [105]. It promotes cell cycle arrest by upregulating RB, a commonly altered tumor suppressor that inhibits the transcription of genes responsible for G1/S transition [106]. In summary, UTX represses many molecular processes associated with PCa initiation and progression (See Figure 3B). The tumor suppressive role of UTX has been validated in other tumor types. Systematic sequencing of renal carcinomas, multiple myelomas, medulloblastoma, and different types of leukemias all revealed inactivating mutations in a significant number of patients [107-111] Furthermore, UTX downregulation correlates with poor clinical outcome in breast cancer [112]. Given the prominence of PcG in PCa, inactivation of UTX most likely represents a critical event in the progression to metastasis.

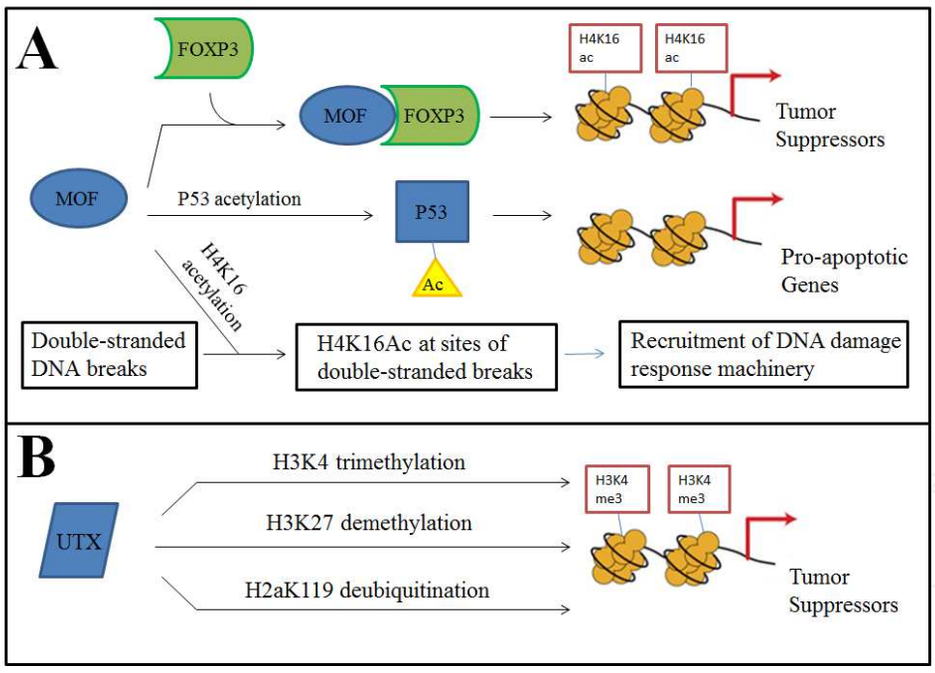

Figure 3. Putative Mechanisms of Oncosuppressive TrxG Genes in PCa

3.6. WDR5

WDR5 represents a core member of the COMPASS and COMPASS-like complexes whose functional role in cancer remains unclear [113]. To date, very few studies have focused solely on the link between WDR5 and oncogenesis. However, WDR5 appears to have a promi-

nent role in embryogenesis. In ESCs, WDR5 interacts with the transcription factors OCT4 (octamer-binding transcription factor-4), SOX2, and NANOG to induce the expression of genes necessary for pluripotency and self-renewal [114]. This transactivational ability correlates with H3K4 trimethylation at the target *loci*. Furthermore, somatic cell reprogramming and formation of induced pluripotent stem cells (iPSCs) also requires the presence of WDR5 [114]. WDR5 has been shown to be essential for proper HOX gene activation as *Xenopus Laevis* tadpoles exhibit a wide range of developmental defects upon WDR5 depletion [115]. Moreover, WDR5 cooperates with the canonical Wnt pathway to induce osteoblast and chondrocyte differentiation [116]. WDR5 is expressed upon bone morphogenetic protein (BMP) signalling, another pathway associated with differentiation [117]. In fact, WDR5 was initially called ''BMP-2-induced gene 3 kb'' and subsequently changed to its current name [118].

Recently, a study demonstrated that WDR5 is induced under hypoxic conditions and is required for epithelial-mesenchymal transition (EMT) [119]. Hypoxia activates the expression of WDR5 and HDAC3. WDR5 and H3K4 methyltransferase complexes are then recruited to promoters of mesenchymal genes to activate their transcription [119]. In parallel, HDAC3 removes pre-existing acetyl groups from H3K4 to potentiate WDR5 action. HDAC3 also removes histone acetylation marks from promoters of epithelial genes, further pushing the cell towards a mesenchymal phenotype [119]. EMT represents an essential step for tumor metastasis [120-122]. Since WDR5 is required for EMT, WDR5 could potentially act as an oncogene by promoting metastasis of primary prostate tumors (Figure 2B). Although the oncogenic role of WDR5 has not been tested in PCa, studies in head and neck squamous cell carcinoma showed that coexpression of HIF-1α, WDR5, and HDAC3 is associated with metastasis and poor prognosis [119]. These results suggest that WDR5 functions as an oncoprotein by triggering EMT. However, further studies are needed to assess the consequences of WDR5 expression in PCa.

4. Expression data analysis and putative mechanisms of TrxG function in malignant progression

As summarized in previous sections, epigenetic gene regulation plays a crucial role in PCa. In particular, HPTMs mediated by TrxG genes are emerging as novel drivers of tumor progression, or as mediators of tumor suppressive functions. Although these genes have been extensively investigated in hematological neoplasms, their roles in solid tumors such as PCa have not been completely elucidated. As demonstrated for other epigenetic players, it is likely that The functioin of TrxG members is dependent on tissue type, tumor stage, as well as on overlooked or uncharacterized determinants [123]. To gain insights into the possible role of TrxG genes in PCa progression, we conducted an analysis of their expression in primary *vs.* metastatic samples. To this aim, we exploited a publically available database (http://www.cbioportal.org/public-portal/) [124]. Our results are summarized in Table 2. At first glance, it is evident that each TrxG member represented in the table shows up- or down-regulation in a relevant fraction (16-53%) of metastatic PCa cases. This indicates that

aberrations in TrxG activity are likely to play an important role in the progression to metastasis.

TrxG	Non-metastatic: 71/131 = 54%	Metastatic: 18/19 Cases = 95%
ASH1L	↓ in 12/131 and ↑ in 1/131 = 10%	↓ in 5/19 = 26%
ASH2L	↓ in 13/131 and ↑ in 3/131 = 12%	↓ in 7/19 = 37%
WDR5	↓ in 3/131 and ↑ in 5/131 = 6%	↓ in 9/19 and ↑ in 1/19 = 53%
MEN1	↓ in 5/131 and ↑ in 19/131 = 19%	↓ in 10/19 = 53%
HCFC1	↓ in 11/131 and ↑ in 14/131 = 19%	↓ in 4/19 and ↑ in 1/19 = 26%
MLL	↓ in 18/131 = 14%	↑ in 6/19 = 32%
MLL2	↓ in 8/131 and ↑ in 12/131 = 15%	↓ in 4/19 and ↑ in 2/19 = 32%
MLL3	↓ in 6/131 and ↑ in 3/131 = 7%	↓ in 2/19 and ↑ in 1/19 = 16%
MLL4	↓ in 10/131 and ↑ in 10/131 = 15%	↓ in 6/19 = 26%
MLL5	↓ in 6/131 = 5%	↓ in 1/19 and ↑ in 2/19 = 16%
UTX	↓ in 2/131 and ↑ in 6/131 = 6%	↑ in 5/19 = 26%

Table 2. CBio portal-derived gene expression data in primary *vs.* metastatic PCa. Arrows pointing up or down indicate increased or decreased expression, respectively. The percentage indicates the fraction of alterted (up- or down-regulated) genes.

In the following paragraphs, we will briefly discuss our findings and conciliate them with published data on each TrxG memember.

1. **ASHL:** although ASH2L has been described as an oncoprotein [68], we found that ASH-1L and -2L expression is reduced in metastatic PCa samples. This discrepancy might be explained by the evidence that ASH2L protein levels rise in cancer, but mRNA level does not increase [68]. This implies additional regulation at the translational level, most likely relating to a defect in proteasomal degradation.

2. **MEN1:** MENIN can function as an oncogene [53, 78] and as a tumor suppressor [73] depending on tissue specificity. MENIN interacts with nuclear proteins like estrogen- and vitamin D-receptor [80, 125], thereby stimulating their transactivation. Since other members of the COMPASS-like complex interact with AR [70], we propose that the oncogenic function of MENIN might result from its association with, and subsequent stimulation of, AR transactivation ability through H3K4 trimethylation (See Figure 2). Since most metastatic PCas are androgen-independent, while almost all primary tumors display an active AR signaling [126], MENIN action is likely required in early tumor stages. This explains the preferential up-regulation of MEN1 in non-metastatic (likely androgen dependent) PCa samples (Table 2).

3. **MLL:** There is no documented role for MLL in PCa. Data from the cBio database shows that MLL expression is increased in metastatic *vs.* primary PCa samples. Therefore we

propose that MLL acts as a metastasis-driving oncogene in PCa. MLL is known to interact with AR [70]. Since metastatic PCa cells are usually AR-independent, the mechanism of MLL action in the metastatic process is likely androgen-independent too. Interestingly, MLL homologs are often down-regulated in metastatic PCa (Table 2), suggesting that they might counteract its oncogenic function.

4. **MOF:** MOF was not included in the cBIO database, but based on its regulation of growth suppressive pathways (See Figure 3), we propose that MOF acts as a tumor suppressor and therefore we expect to see its expression downregulated in PCa. However, since MOF is required for optimal DNA damage response to double-stranded breaks [96], reduced MOF expression could be a predictor of good response to radiotherapy or to chemotherapy agents that induce dsDNA breaks.

5. **UTX:** The protein encoded by this gene possesses H3K27 demethylase activity, which counteracts the repressive effect of PRC2-catalyzed H3K27me3. Due to the preponderance of PRC2 activity in PCa, UTX loss of function appears to be a critical event in the progression to metastasis. UTX also interacts with other histone-modifying complexes that catalyze HPTMs associated with transcriptional activation (See Figure 3). Despite this evidence, we found an increased rate of UTX upregulation in metastatic *vs.* non metastatic PCa samples (Table 2). Those data counteract the common view that UTX acts as a tumor suppressor, at least in PCa. A possible explanation derives from the recent finding that *UTX* is frequently mutated in metastatic PCa [70]. It is worth noting that all experiments on the oncosuppressive role of UTX have been performed on the wild-type gene. We do not know whether the mutated protein simply loses its tumor-suppressive activity, or if it acquires oncogenic features. In the latter case, the upregulation reported in metastatic PCa might even drive tumor progression.

6. **WDR5:** Although no studies have directly assessed the role of WDR5 in PCa, data from ESC suggest that WDR5 might promote metastasis due to its implication in EMT. During EMT, WDR5 promotes the expression of mesenchymal genes by stimulating H3K4 methylation at target *loci* [119]. Since WDR5 triggers EMT, we would expect its expression to increase in metastatic samples. However, in the MSKCC database, WDR5 expression is reduced in metastatic tumors. This could be explained by the fact that only a subset of PCa cells acquires epigenetic alterations in response to cues from the extracellular environment (niche) which predisposes them to metastasis. Since only a minority of the tumor bulk acquire invasive and migratory potential, the elevated expression of WDR5 in those cells would not be detected by micro-array as the levels of WDR5 in non-invasive cells would dominate.

The reader is cautioned that it is necessary to consider that studies comparing metastatic and primary tumors might oversimplify the complex nature of the metastatic process. First, those studies show expression levels of target genes at 2 specific time points, while the metastatic process occurs over several years in the clinical setting [58]. Second, molecular mechanisms of regulating metastasis are complex: if EMT is required as an early step, the opposite (mesenchymal-to-epithelial transition) is needed during metastatic cell homing [127]. Thus, a gene required during early metastatic steps might even be silenced at later stages. These

considerations underscore the fact that our conclusions are limited, and need to be complemented by functional and clinical studies. However, results shown in Table 2 indicate that at least some TrxG genes are likely involved in PCa metastasis and thus are candidate therapeutic targets or prognostic factors.

5. Conclusion

While for many years cancers were thought to arise as a result of genetic alterations, an increasing number of studies report that in fact epigenetic misregulation primarily drives PCa progression and metastasis [13, 128]. PcG proteins EZH2 and BMI1 are overexpressed in PCa, an event that correlates with increased metastatic spreading and poor prognosis [57]. Since TrxG antagonizes PcG action, we explored the possibility that aberrant TrxG signalling could also represent a key factor in PCa metastasis. Since PcG is overactive in PCa and TrxG counteracts PcG activity, TrxG were historically thought to be oncosuppressive [41]. Analysis of expression databases revealed that almost all metastatic prostate tumors show deregulated expression of at least one TrxG gene. Interestingly, an in-depth literature review combined with an analysis of expression data indicated that aberration in TrxG complexes impacts PCa progression in a way that goes beyond their anticipated roles as classical tumor suppressors. In fact, some TrxG genes show elevated expression in metastatic PCas and have been shown to interact with, and enhance the activity of, known oncogenes such as AR, c-MYC, h-RAS [68, 70]. The finding that TrxG genes can act as either oncogenes or tumor suppressors implies that the regulation of TrxG activity highly depends on the cellular context [68, 129]. Changes in individual TrxG gene expression, availability of coregulators, as well as post-translational modifications on both individual TrxG subunits and coregulators all regulate the functional output of TrxG complexes. These multiple levels of regulation account for the highly diversified spectrum of molecular processes affected by TrxG activity, and explain why some TrxG genes can act as oncogenes and others as tumor suppressors.

Since it is becoming increasingly clear that misregulated TrxG activity represents a key driver of PCa progression, an important question arises: How can TrxG complexes be targeted clinically? Inhibiting core TrxG subunits like MLL, ASH2L, and WDR5 does not represent a suitable strategy. TrxG complexes play many important physiological roles [130] and therefore disrupting these core TrxG proteins would result in high toxicity. In fact, it is important to recognize that TrxG activity is highly context-dependent and is controlled by many coregulators. This context-dependency can be exploited in the search for new drug targets. An interesting strategy to adopt would be to identify TrxG coregulators that are overexpressed in PCa only. Inhibiting these coregulators would impair TrxG function in PCa cells specifically while leaving normal cells unaffected. Since TrxG complexes can be oncogenic or tumor suppressive, two types of coregulators should be targeted clinically. The first represents coactivators of oncogenic complexes and second, corepressors of oncosuppressive complexes. Pharmacologic disruption of both of these proteins would in theory limit the tumorigenic potential of aberrant TrxG signalling. To date, no such coregulators have been described in PCa. The link between TrxG and PCa remains poorly characterized and many

more studies are required to understand the impact of dysregulated TrxG on PCa progression. Nonetheless, the implication of TrxG in PCa supports the idea that epigenetic alterations represent key drivers in the progression to metastatic disease.

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