Epigenetics of prostate cancer: beyond DNA methylation

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Abstract

Epigenetic mechanisms permit the stable inheritance of cellular properties without changes in DNA sequence or amount. In prostate carcinoma, epigenetic mechanisms are essential for development and progression, complementing, amplifying and diversifying genetic alterations. DNA hypermethylation affects at least 30 individual genes, while repetitive sequences including retrotransposons and selected genes become hypomethylated. Hypermethylation of several genes occurs in a coordinate manner early in carcinogenesis and can be exploited for cancer detection, whereas hypomethylation and further hypermethylation events are associated with progression. DNA methylation alterations interact with changes in chromatin proteins. Prominent alterations at this level include altered patterns of histone modification, increased expression of the EZH2 polycomb histone methyltransferase, and changes in transcriptional corepressors and coactivators. These changes may make prostate carcinoma particularly susceptible to drugs targeting chromatin and DNA modifications. They relate to crucial alterations in a network of transcription factors comprising ETS family proteins, the androgen receptor, NKX3.1, KLF, and HOXB13 homeobox proteins. This network controls differentiation and proliferation of prostate epithelial cells integrating signals from hormones, growth factors and cell adhesion proteins that are likewise distorted in prostate cancer. As a consequence, prostate carcinoma cells appear to be locked into an aberrant state, characterized by continued proliferation of largely differentiated cells. Accordingly, stem cell characteristics of prostate cancer cells appear to be secondarily acquired. The aberrant differentiation state of prostate carcinoma cells also results in distorted mutual interactions between epithelial and stromal cells in the tumor that promote tumor growth, invasion, and metastasis.

Keywords: DNA methylation • chromatin • transcription factor • tumor suppressor • prostate carcinoma • growth factor • tumor stroma • androgen receptor coactivator • cancer stem cell

Introduction

Prostate cancer is the most or second-most frequent non-cutaneous cancer of males in Western industrialized countries [1] and a major cause of suffering and mortality. In spite of substantial progress in research, diagnosis and treatment, the disease remains characterized by puzzles.

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The most relevant one in the clinic is its pronounced heterogeneity. Up to 40% of elderly males may harbor carcinomas in their prostate, but clinical symptoms and lethal disease develop in fewer men. This heterogeneity continues at the histological level, since one cancer can display variable degrees of differentiation and some cases are multifocal. This complicates diagnosis and therapy, because it is not sufficient to detect tumors, but also necessary to distinguish forms of the disease requiring palliative treatment, definitive therapy, or active monitoring, respectively. Moreover, since definitive therapy can be performed by surgery or irradiation and may be supplemented by antiandrogenic therapy and cytotoxic chemotherapy depending on the circumstances of each individual case, criteria for optimal selection of therapy are desirable [2].

An epidemiological puzzle is the geographical distribution of prostate cancer. Substantial differences exist in age-adjusted incidence and mortality between countries in East Asia and Northwest Europe. This difference does not derive predominantly from genetic differences between populations, indicating that environmental factors play a major role in etiology. While differences in diet and life-style are implicated, exogenous carcinogens have not been unequivocally identified [3].
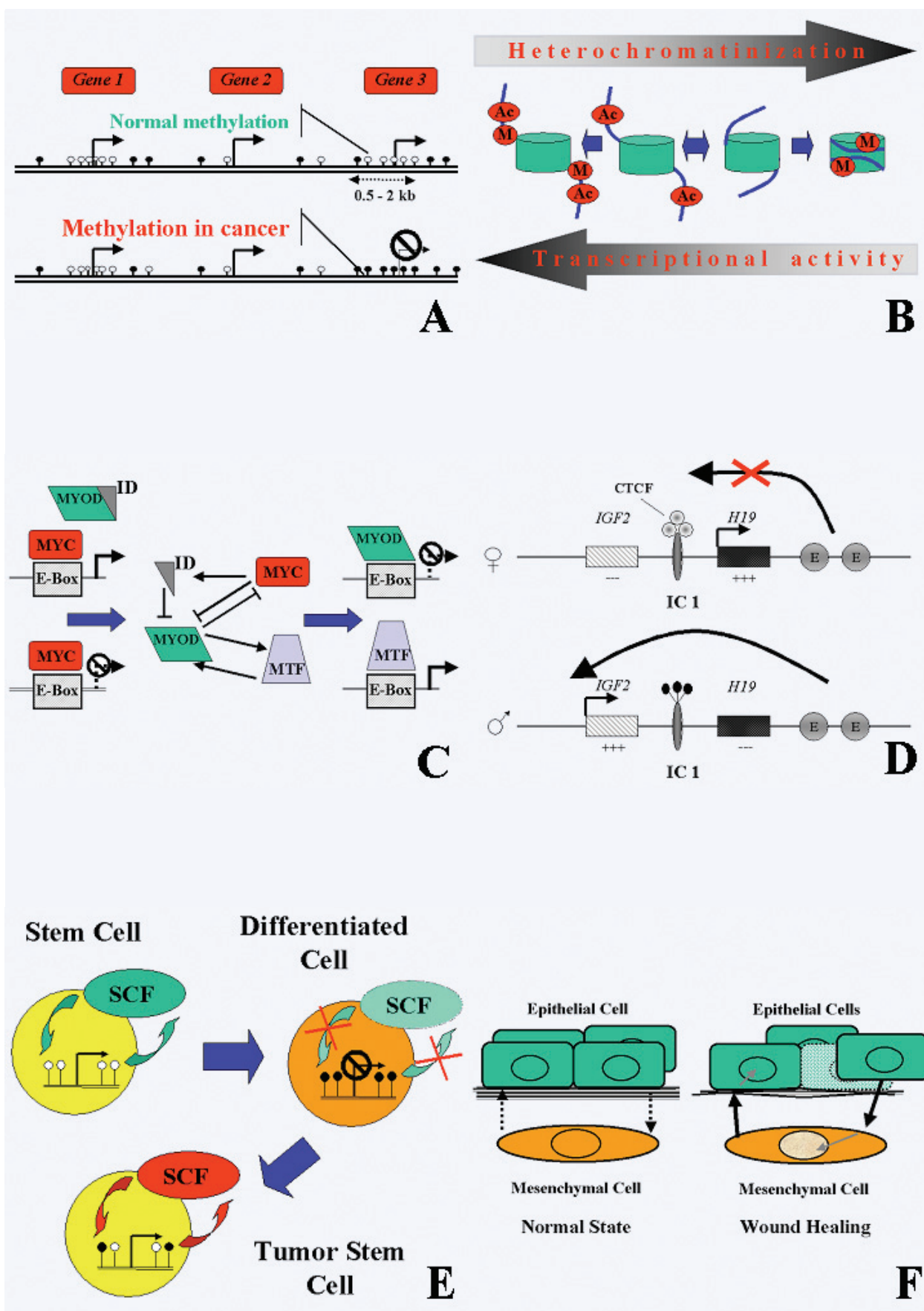
Yet another puzzle concerns heredity. Relatives of prostate cancer patients are clearly at an increased risk for the disease. This increase is now estimated as ≈4-fold for first grade relatives. Nevertheless, extensive genome-wide searches for hereditary prostate cancer genes have come up with many candidate regions and occasionally with candidate genes that confer only modestly increased risks [4, 5]. Moreover, many findings could not be replicated between populations. Accordingly, a consensus has been reached that 'hereditary' prostate cancer genes do not act as classical tumor suppressor genes like *RB1* or *APC*, but may rather modulate cancer risk in response to unknown exogenous factors. The data are most convincing for a locus termed *HPC1* (hereditary prostate cancer 1) at 1q24, most likely corresponding to the *RNASEL* gene [6]. Inactivating mutations and polymorphisms in this gene appear to increase the risk of developing an aggressive form of prostate cancer. As RNaseL acts in an intracellular pathway protecting against viral infections, its association with prostate cancer suggests a causal role for viral infections in this disease [7]. The evidence for a viral etiology is however controversial. In view of such uncertainties, genetic counseling of family members of prostate cancer patients is at present only possible in very general terms. Likewise, preventive measures for this prevalent disease remain confined to general dietary and life-style recommendations such as 'take 5' (servings of fruit and vegetables) and frequent exercise [3]. These measures certainly help to prevent cardiovascular disease and colorectal cancer and do otherwise no harm. Whether they really prevent prostate cancer is not certain. The value of chemoprevention, *e.g.* by 5α-reductase inhibitors, is under investigation [8].

The conundrums complicating diagnosis, treatment, and prevention of prostate cancer are mirrored in puzzling observations at the molecular level. For instance, alterations of the androgen response are widespread in prostate cancers, but appear quite paradoxical [9]. A subgroup of prostate cancers contain an hyperactive androgen receptor (AR) as a consequence of point mutations or amplification of the *AR* gene at Xq12. Conversely, in up to 30% of advanced tumors the AR is downregulated and in a few the gene is even silenced by promoter hypermethylation [10].

Tumor suppressors in prostate cancer also appear to behave atypically [11–13]. Several well-characterized tumor suppressor genes, *e.g. RB1* and *PTEN*, are implicated in prostate cancer as in other cancers, while others are specific, prominently *NKX3.1* at 8p21 (see below). However, while allelic loss of these tumor suppressor genes is frequent in prostate cancer, inactivation of both alleles by mutation, homozygous deletion, or promoter hypermethylation is rare and restricted to very advanced cases. A reasonable explanation for these observations is haploinsufficiency for tumor suppressors in prostate cancer, at least during its initial stages.

The difficulties encountered in molecular analyses of prostate cancer are illustrated by the case of chromosome 8, a commonly altered

Gene 1   Gene 2   Gene 3

Normal methylation

0.5 - 2 kb

Methylation in cancer

A

Heterochromatinization

Ac
M
M
Ac
Ac
M
M

Transcriptional activity

B

MYOD   ID

MYC
E-Box

MYC
E-Box

ID

MYC

MYOD

MTF

MYOD
E-Box

MTF
E-Box

C

CTCF

IGF2   H19
---   +++

E   E

IC 1

IGF2   H19
+++   ---

E   E

IC 1

D

Stem Cell   Differentiated Cell

SCF

SCF

SCF

Tumor Stem Cell

E

Epithelial Cell

Mesenchymal Cell

Normal State

Epithelial Cells

Mesenchymal Cell

Wound Healing

F

chromosome in this cancer type. Alterations are found on both arms, but several regions on each arm are affected concomitantly or separately. Losses are most frequent at 8p12-21 and 8p23, and gains at 8q21-22 and 8q24. All published studies concur that alterations of chromosome 8 are associated with a more aggressive behavior of prostate cancers. However, the various studies disagree on several issues, *i.e.* at which stage of cancer development these changes arise, and with which clinical parameter which particular chromosomal alteration is associated (refs. [11, 13, 14] and references therein).

The most common alteration at the DNA level in prostate cancers is an epigenetic change. The CpG-rich promoter of the *GSTP1* gene is hypermethylated in the great majority of cases [15]. Therefore, *GSTP1* hypermethylation assays are being developed for prostate cancer detection. The great promise of these assays tends to obscure how strange *GSTP1* hypermethylation is. In other cancers, the glutathione transferase GSTP1 is typically upregulated, often contributing to drug resistance. Moreover, the consistency with which this gene and a number of others are hypermethylated in prostate cancer is singular. This is one of several observations indicating that epigenetic changes are particular important in prostate cancer.

Epigenetic mechanisms allow the stable inheritance of properties of cells and organisms without changes in DNA sequence or DNA content. The modern, more precise definition of epigenetics considers only nuclear processes such as DNA methylation, certain histone modifications, and transcription factor networks [16], a wider definition – in keeping with the initial notion of C. H. Waddington [17] - additionally encompasses regulatory circuits at the cellular or even tissue level (Fig. 1). Epigenetic processes are responsible for the regulation of individual genes, *e.g.* by parental imprinting, of whole chromosomes, *e.g.* by X-chromosome inactivation, of cells, *e.g.* during cell differentiation or maintenance of stem cells, and of tissues, *e.g.* in homeostatic epithelial-mesenchymal interactions.

Epigenetic changes, including aberrant DNA methylation and histone modification, are observed in almost all cancer types and typically interact with genetic changes such as point mutations or chromosomal aberrations in bringing about the cancer phenotype. However, cancer types differ in the relative contribution of genetic and epigenetic alterations. For instance, the chronic phase of chronic myelogenic leukemia is dominated by a genetic change, *i.e.* the chromosomal translocation generating the *BCR-ABL* fusion oncogene. Epigenetic alterations such as

Fig. 1 Examples of epigenetic processes. A: DNA methylation: methylated and unmethylated CpG-sites are symbolized by black and white circles, respectively. Note hypermethylation and hypomethylation in cancer. B: Histone modification: M stands for activating (left) or inactivating (right) methylation, *e.g.* at K4 or K9 of histone H3; Ac: acetylation, *e.g.* at K9 of histone H3. Acetylation is not usually regarded as an epigenetic process, since it is probably not inherited through replication and cell division. C: A transcription factor network: MYOD, MYC, ID proteins, and a number of myocyte transcription factors (summarized as MTF) interact during myocyte differentiation to regulate genes responsible for proliferation (top) or differentiation (bottom) *via* E-boxes. D: Imprinting: The current model for parental-specific expression of the IGF2/H19 double locus at 11p15. Differential expression of the maternal and paternal alleles is caused by differential methylation of a boundary element regulating enhancer access to the promoters. It is bound by the insulator protein CTCF only in the unmethylated state. E: Stem cell determination: Stem cell maintaining factors (SCF, not necessarily the growth factor with that name) are expressed from hypomethylated genes in stem cells to (directly or indirectly) regulate chromatin structure and DNA methylation patterns maintaining their expression. In adult somatic cells, downregulation of these factors is ensured by DNA methylation of these genes. Hypomethylation in tumor cells could allow re-expression conferring stem cell properties. F: Epithelial-mesenchymal interaction: Epithelial and mesenchymal cells communicate with each other *via* growth factors. In normal tissue (left), these are expressed at low levels or cannot pass through the intact basement membrane. Wounding increases growth factor production and/or mobility thereby eliciting signals in mesenchymal and epithelial cells to mutually stimulate further growth factor synthesis, cell activation and proliferation, but also differentiation and repair of the basement membrane which reestablishes the initial steady state. Obviously, this regulatory system is disturbed in tumors. Note that only A-D are epigenetic processes in the stricter definition and that all representations are very simplified.

hypermethylation of *CDKN2A*/p16^{INK4A} and the remaining intact *ABL* gene become more important during progression, *i.e.* accelerated phase and blast crisis [18]. We argue here that in prostate cancer epigenetic mechanisms - in the narrower and the wider sense of the definition – are important throughout the course of the disease. We will therefore discuss in turn altered DNA methylation, chromatin structure, transcription factor networks, stem cell maintenance, and stroma-epithelial interactions, but omit altered imprinting, for which the evidence in prostate cancer is scarce [19].

This emphasis on epigenetic changes should not be interpreted as negating the importance of genetic alterations in prostate cancer. Most prostate cancers harbor multiple chromosomal alterations and point mutations. Their effects synergize with and are propagated by epigenetic mechanisms as discussed in detail below. Typically, genetic changes initiate chains of events maintained by epigenetic mechanisms that amplify and diversify their impact. The longer and the more branched these chains are, the more difficult it becomes to relate properties of an individual cancer to a particular genetic change. In this fashion, the prevalence of epigenetic alterations in prostate cancers may underlie some of the puzz-ling features of the disease.

To limit the number of references, throughout this paper, we have usually cited reviews rather than original articles when dealing with matters that are well established or of a more general nature.

DNA methylation

Hypermethylation of *GSTP1* is found in the great majority of prostate cancers and in a considerable fraction of high-grade prostate intraepithelial neoplasias (HGPIN), a likely precursor [15]. This is however not the only gene hypermethylated in such a large fraction of these tumors (Table 1). The singular consistency with which hypermethylation of several genes is found in prostate cancers independent of tumors stage and grade argues for a coordinated hypermethylation event closely associated with the emergence of actual prostate carcinoma [20]. Most likely this 'epigenetic catastrophe' [21] takes place during the progression of precursors like HGPIN to actual carcinoma. Two important conclusions follow. First, assays detecting hypermethylation of one or preferably several genes are well-suited to detect prostate carcinoma in biopsies, fluids derived from the prostate (ejaculate, urine, prostate massage urine), or serum. They exhibit high specificity and their sensitivity is essentially limited only by sampling bias, *i.e.* the actual presence of DNA from prostate cancer cells in the sample. Second, understanding of the trigger eliciting this coordinate hypermethylation ought to yield a clue to the elusive etiology of prostate cancer. For instance, the aberrant hypermethylation of genes like *GSTP1* could be a consequence of the aberrant proliferation state of prostate cancer cells (Fig. 2). *GSTP1* is normally expressed only in the basal cells of the glandular prostate epithelium [22] and its transcription is downregulated in terminally differentiated secretory cells. However, although the gene is inactive, these cells do not proliferate and the gene is never replicated in an inactive state. In contrast, in prostate cancer, cells with an inactive *GSTP1* gene traverse S-phase, when DNA methyltransferases are most active. This hypothesis obviously implies that prostate cancer cells are derived from cells at an advanced stage of differentiation.

While it seems likely that the coordinated hypermethylation and silencing of several genes is caused by a single mechanism, perhaps in response to a key genetic alteration, an open question is whether one of the hypermethylated genes is the main tumor suppressor in prostate carcinogenesis, whose inactivation is mechanistically linked to that of 'passenger' genes, or whether hypermethylation at large is a side effect of the crucial carcinogenic alteration in prostate cancer. Among the genes affected by hypermethylation are several that are reasonable candidates for tumor suppressors, prominently *RARB2* encoding a retinoic acid receptor regulating differentiation of epithelial cells [23] and *RASSF1A* whose product counteracts stimulation of cell proliferation by RAS-linked pathways [24]. Hypermethylation of *APC* affects only one of its two promoters [25] and does not as a rule lead to complete gene silencing. Typical tumor suppressor genes important in other human cancers such as *PTEN*, *RB1*, and *TP53* are not hypermethylated in prostate cancer, although allelic loss and point mutations are found, predominantly in advanced stage cases [11–13]. Thus, there may be further,

Table 1 Some genes hypermethylated in prostate cancer §

Hypermethylation	Genes	Remarks
almost consistently (70-95%)	*GSTP1, RARB2, RASSF1A, APC, COX2, MDR1, TIG1, HIC1*	*HIC1*, *MDR1*, and *RASSF1A* also methylated in preneoplastic tissues
in many cases (20-70%)	*CD44, ESR2, CDH1, SFRP1, TMS1, ETNRB, CDKN1C, MGMT, RUNX3, THBS1, CDH13, 14-3-3 σ*	Data on several of these genes extremely discrepant (*e.g. CDH1*)
in few cases (<20%)	*AR, CDKN2A, CDKN1B, DAPK, ESR1, NKX3.1*	Data on several of these genes extremely discrepant (*e.g. AR*)

§ More genes have been reported as hypermethylated in prostate cancer tissues and particularly in cell lines, but the number of cases studied was small or the reports were not confirmed independently. Further candidates have been identified in survey studies, but the frequency of hypermethylation in prostate cancer is not defined. See refs. [51, 71, 72] for comprehensive lists and more original references.

hitherto unknown tumor suppressor genes affected by hypermethylation. Determining comprehensively all genes hypermethylated in prostate cancer remains an important object of research.

A number of genes have been reported as frequently hypermethylated in prostate cancer, but not with the consistency of *GSTP1* and its likes (Table 1). It is not clear whether these genes too become hypermethylated during initial stages of prostate cancer development or subsequently during progression. In any case, these hypermethylation events define subgroups of prostate cancers potentially differing in the natural course of disease and response to certain therapies. Indeed, several hypermethylation events have been proposed as potential prognostic markers. Hence, prostate cancer might be classified by a panel of methylation markers.

Such a panel would presumably also include a number of hypomethylation markers. In advanced stage prostate cancers and metastases, the overall methylcytosine content is decreased in spite of locally increased methylation at several genes. This is due to the diminished methylation of abundant repetitive sequences that are densely methylated in normal cells, such as LINE-1 retrotransposons [26]. In addition, selected single copy genes, such as *CYP1B1* [27] and *HPSE* (heparanase) [28] are also likely to become hypomethylated and induced. Others, such as the *XIST* gene, are hypomethylated

approximately in parallel to repeat sequences, but are not significantly overexpressed [29]. Many genes hypomethylated in other cancers are involved in adaptive responses to altered microenvironments and may be particularly important during tumor invasion and metastasis [30].

As for hypermethylation during the initial stages, a straightforward mechanistic explanation for hypomethylation in high stage prostate cancers is lacking. As in other cancers, hypomethylation is associated with chromosomal instability, but it is not entirely sure, whether one causes the other or both are a consequence of other alterations, such as a disturbance of cell cycle checkpoints that control S-phase and mitosis, but also coordinate DNA methyltransferases with DNA replication. It is almost certain, though, that genome-wide and local alterations in DNA methylation interact with changes at the chromatin level [30–32], which are also abundant in prostate cancer.

Chromatin changes

In a fashion exemplary for epigenetic mechanisms, DNA methylation and chromatin structure are interdependent at several levels [33, 34]. DNA methyltransferases interact with his-
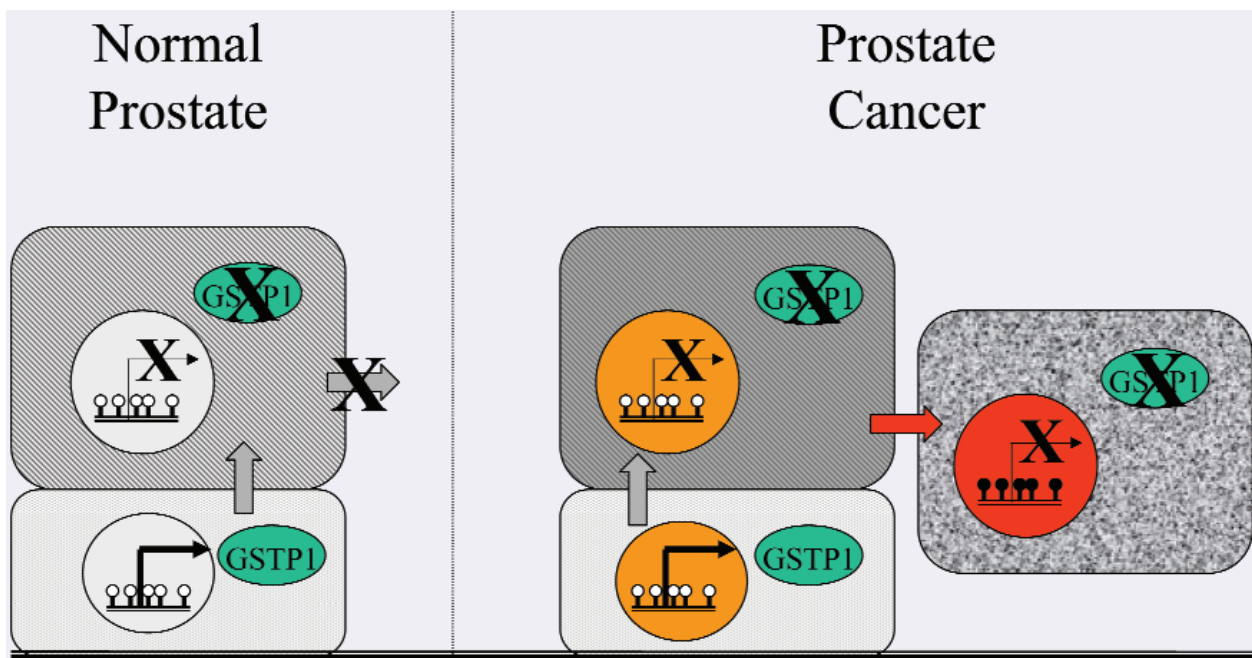
Fig. 2 A hypothesis explaining GSTP1 hypermethylation in prostate cancer. Left: GSTP1 is normally expressed only in basal cells of prostate epithelium and downregulated in terminally differentiated secretory cells. As these cells do not proliferate, the inactive gene is never replicated. Right: In prostate cancer, cells with an inactive *GSTP1* gene traverse S-phase, when DNA methyltransferases are most active.

tone-modifying enzymes such as histone deacetylases and histone methylases. Methylcytosine in DNA is recognized by specific proteins modulating chromatin structure, including histone deacetylases and chromatin remodeling complexes. The methylcytosine-binding proteins MECP2 and MBD2 are normally crucial for repression of hypermethylated genes in human cancers [32]. A report on a lack of these proteins in prostate cancer is therefore highly unexpected [35], but no refutation has been published. In some organisms, DNA methylation has been shown to become in turn promoted by certain histone modifications, particularly by inactivating histone methylation, such as trimethylation of H3 at K9 [36]. Conceivably, mammalian DNA methyltransferases also react to such modifications. Altered expression and activity of histone methyltransferases could therefore elicit local and genome-wide changes in DNA methylation, which could in turn promote altered chromatin structure.

Several histone-modifying enzymes and other chromatin proteins have been reported to be altered in prostate cancer (Table 2). The polycomb protein EZH2 is an essential component of a protein complex catalyzing methylation of histone H3 at K9 which contributes to transcriptional repression of a large number of specific genes. EZH2 is overexpressed in most prostate cancers, with moderate increases in localized tumors, and higher expression in metastatic cases [37]. The cause of EZH2 overexpression in general is not understood, as an increased dosage of the gene at 7q36 is only found in selected cases. Since EZH2 expression is regulated by RB1 in a cell-cycle related fashion [38], part of the increase may reflect increased cell proliferation in advanced prostate cancers. EZH2 overexpression may lead to the stable downregulation of approximately 100 genes and increased expression of a smaller number [37]. This is an example of an epigenetic chain of events typical of prostate cancer. Presumably, dosage changes in *RB1* (or other genetic or epigenetic alterations acting upon RB1) increase the expression of the epigenetic regulator protein which affects a number of downstream target genes. As some

Table 2 Some chromatin protein changes in prostate cancer [§]

| Factor	Function	Change in PCa	Remarks	Reference(s)
BRG1	remodeling	mutation (in one cell line, but not in tissues)		[174, 175]
CTCF	transcriptional control; boundary function	Mutation (few cases)		[176]
CTCFL/BORIS	transcriptional control, epi-genetic reprogramming	increase (likely small)		[177] Hoffmann *et al.* submitted
EZH2	histone methylase, repression	Increase	gradual increase with progression	[37]
HMG-I(Y)	architectural, recombination	Increase	parallels chromosomal instability	[49, 178]
LSD1	histone demethylation, coac-tivator	Increase	in androgen-refractory cancer	[43]
MBD2/MECP2	methylation-dependent repression	lack of protein expression	mRNA present	[35]
MTA1	remodeling, deacetylation, repression	Increase	gradual increase with progression	[50]
SOX7	transcriptional activation	Increase (few cases)		[179]
YY1	repressor or activator	Increase	highest levels in localized cancers	[45]

[§] The compilation does neither consider DNA methyltransferases nor histone deacetylases nor many further coactivators and corepressors of the AR.

EZH2 targets regulate the cell cycle and proliferation, this chain of events could come full circle to establish altered proliferation of prostate (cancer) cells. EZH2 is also suppressed by activated TP53 [39].

As local hypermethylation elicits nucleosome repositioning and histone deacetylation, hypomethylation of abundant repeat sequences ought to be associated with large-scale alterations in chromatin structure and histone modifications. Recent evidence indicates that this is indeed the case in prostate cancer [40] and in human cancers in general [41]. Specifically, the activatory histone modifications H3 K18 acetylation and H4 R3 dimethylation have been reported to occur in many cases of prostate cancer and to be associated with higher grades and a worse prognosis [40]. One wonders how these changes are related to the hypomethylation of widespread repeat sequences like LINE-1 retrotransposons that are also typical of more aggressive prostate cancers.

Recently, histone methylation has been found to be reversible not only by degradation of the modified histone, but also by selective demethylation. Demethylation at the K9 site of histone H3 is catalyzed by the monoamine oxidase LSD1 [42]. As K9 methylation helps to suppress transcription, this instance of demethylation is associated with derepression. LSD1 is targeted to chromatin by DNA-binding transcription factors, acting thus as a coactivator. Intriguingly, one of the transcription factors interacting with LSD1 is the androgen receptor [43] and LSD1 may be overexpressed in cases of prostate cancer refractory to anti-androgenic treatment.
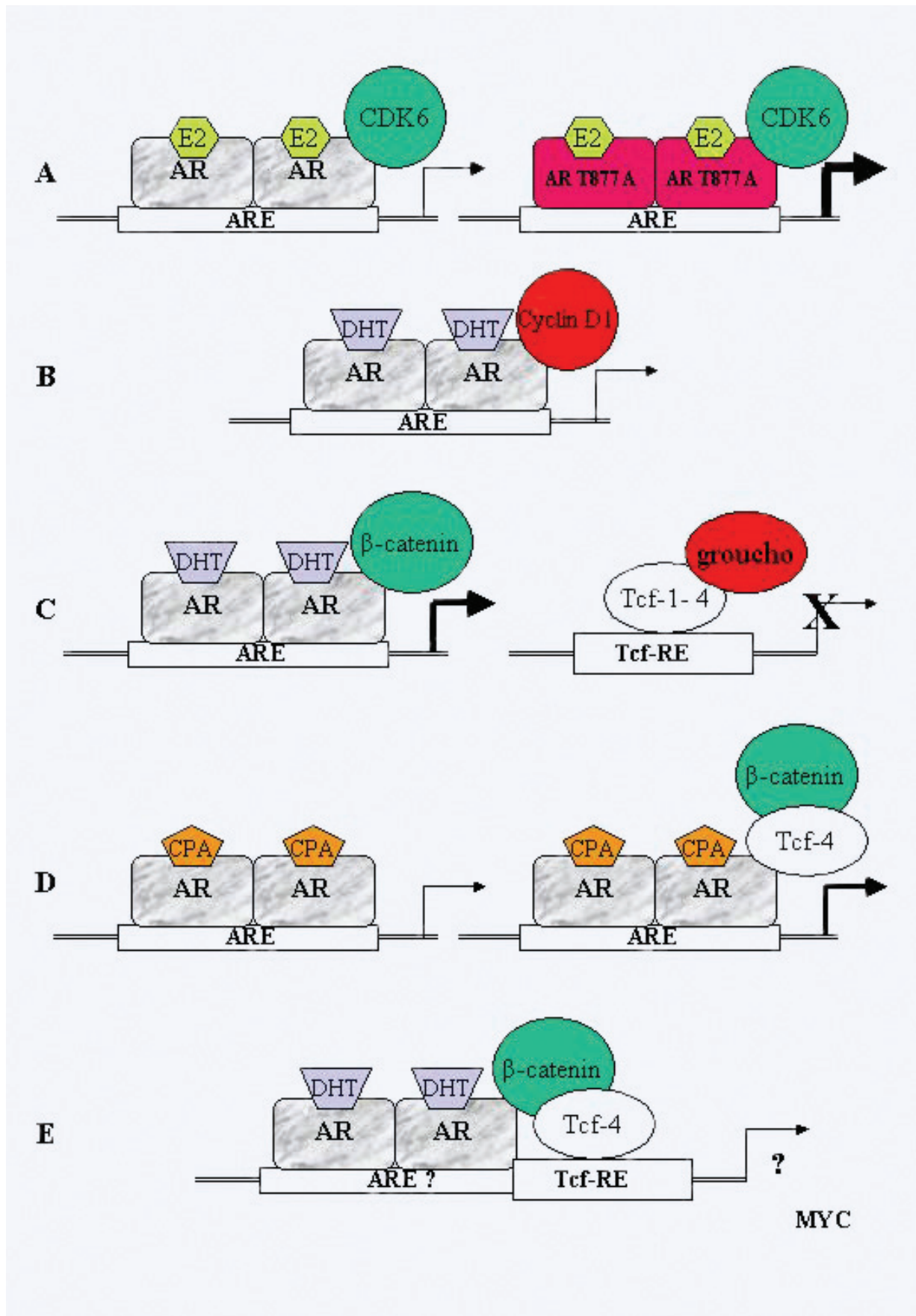
Further chromatin proteins, for which alterations in prostate cancer have been reported, are listed in Table 2. Very few of them have been investigated as intensely as EZH2 and the reports are often based on few cancer tissues or even on cell lines only. Thus, the mechanisms leading to altered expression of these chromatin proteins are poorly understood. Point mutations have been observed in rare cases, and gene dosage changes have been observed in others. As with EZH2, changes in expression of these epigenetic regulators may often be caused themselves by epigenetic mechanisms. Likewise, the consequences of these changes are poorly understood in the context of prostate cancer, but some may interact mutually with alterations of DNA methylation. For instance, YY1 is a major regulator of endogenous retroelements [44, 45] that are hypomethylated in advanced stage prostate cancers [46]. CTCF and CTCFL binding at many sites depends on DNA methylation and the factors have been postulated to influence local methylation patterns in turn [33, 47]. HMGIY is presumably located at matrix-attachment sites [48] where LINE retrotransposons are enriched. Like hypomethylation of LINEs [46], HMGIY overexpression has been shown to be associated with chromosomal instability [49]. Clearly, elucidation of the precise relationships seems worthwhile.

Like altered DNA methylation, changes in the expression of chromatin proteins and post-translational histone modifications can be used for prostate cancer detection and classification. For instance, MTA1 overexpression [50] is associated with more aggressive cases. Moreover, these proteins are interesting targets for therapy, particularly those exhibiting enzymatic activity. At present, clinical application of histone deacetylase (HDAC) inhibitors is most advanced. They exhibit excellent efficacy in preclinical models of prostate cancer [51] and have yielded promising results in initial clinical trials for several cancer types [52]. Inhibitors of DNA methyltransferases that are less toxic than 5-aza-deoxycytidine (decibitine) already approved for clinical use, are being developed [53] and are envisaged for prostate cancer treatment. Presumably, they will be combined with drugs targeting other chromatin proteins or proteins induced by demethylation.

Transcription factor networks in prostate differentiation and tumorigenesis

DNA methylation, histone modification, and non-histone chromatin proteins interact with transcriptional activators and repressors to establish stable lineage-specific gene expression. In this fashion, proliferation and differentiation of prostate epithelial cells are regulated by an interlinked network of transcriptional factors. Some of these transcription factors are expressed in many cell types, while others are more or less specific to the prostate epithelium. Several of these are implicated in prostate carcinogenesis, as oncogenic or tumor suppressing factors.

By far the most studied transcriptional activator in prostate cancer is the AR. As quite a number of reviews exist on this topic [9, 54–57], only selected pertinent points will be discussed here. First, alterations of AR activity in prostate cancer are caused by genetic as well as epigenetic mechanisms. Genetic mechanisms comprise point mutations in the transcriptional activation or the ligand-binding domains and gene amplification. Epigenetic mechanisms comprise altered

Fig. 3 Novel coregulators of androgen receptor transactivation. A: CDK6 as a AR coactivator. CDK6-mediated coactivation greatly enhances the activation of the T877A-mutated AR by non-androgen steroids. B: Cyclin D1 as a general, dominant corepressor of the androgen receptor. C: β-Catenin associated with the ligand-activated androgen receptor acting as its strong coactivator and sequestered from genes, whose activation relies on β-Catenin-TCF4 interaction. These are repressed by Groucho corepressors. D: AR binding cyproterone acetate, a partial agonist/antagonist cannot interact with β-Catenin. Overexpressed TCF4 can recruit β-Catenin to the AR for transactivation. E: Certain genes are activated by DNA-bound AR and TCF4, which cooperatively recruit β-catenin. Such complexes were identified at the *MYC* promoter. AR probably recognizes a nonconsensus motif in this case.

AR - androgen receptor; ARE - androgen response element; CPA - Cyproterone acetate; DHT - dihydrotestosteron; E2 - 2-β-estradiol; TCF-RE - TCF response element

regulation of AR activity through gene hypermethylation and more commonly altered expression and activity of coactivators and corepressors, which respond to intracellular regulatory circuits and exogenous signaling by growth factors and cytokines (see below). Secondly, the AR interacts with several prostate-specific transcription factors, regulating their transcription, as in the case of NKX3.1 and HOXB13, or through physical interaction, as in the case of HOXB13 and PDEF. Thirdly, the AR shares cofactors with other transcription factors, allowing competition or coregulation. For instance, FOXO1 is repressed by FHL2, a coactivator of the AR [58]. Of note, not only the AR, but also other members of the steroid hormone receptor superfamily play a role in normal prostate function and tumorigenesis. For instance, the *RARB2* gene encoding the retinoic acid receptor β is almost consistently hypermethylated in prostate cancer [23], and the *ESR1* and *ESR2* genes encoding the estrogen receptors, ERα and ERβ, are hypermethylated [51] at lower frequencies (Table 1). The *VDR* gene encoding the receptor for calcitriol (1α,25-dihydroxy-vitamin D3) is not hypermethylated, but the SMRT corepressor associated with histone deacetylase activity is overexpresssed in prostate cancer cells [59].

The mutual interactions between transcription activators and cofactors are extremely complex, as illustrated by the plethora of interactions involving the AR. Recently, even cell cycle regulators have been identified as transcriptional cofactors (Fig. 3). Cdc25B [60] and CDK6 [61] were identified as coactivators, whereas Cyclin D1 exhibits a dominant corepressor function [62]. Cdc25B is a rather promiscuous coactivator for nuclear receptors, whereas CDK6 seems to be more specific. Likewise, the corepressor function of Cyclin D1 may be essentially restricted to the AR, since for ERα Cyclin D1 acts as a potent ligand-independent coactivator, at least in breast cancer cells [63]. The CDK6-assisted AR bearing a T877A mutation in the ligand-binding domain behaves as a promiscuous "super-receptor", whose activating potency exceeds that of the wild type AR. As it responds not only to dihydrotestosterone, but to a range of other steroids including cortisol, estradiol, progesterone and even the androgen antagonist flutamide, this interaction might be crucial in the development of certain therapy-resistant tumors [61].

The corepressor function of Cyclin D1 is more ambiguous. Apparently, in normal prostate epithelial cells the induction of D-Cyclins mediates the mitogenic action of androgens and growth factors by activating CDK4 and CDK6 [64]; the AR corepressor function of Cyclin D1 could represent a feedback mechanism limiting proliferation. Cyclin D1 is overexpressed in up to 30% of prostate cancers [65, 66]. In these cases, Cyclin D1 would be expected to drive cell cycle progression, but also to suppress AR activity. These tumors should therefore be less dependent on androgens or at least be subject to strong selective pressure for true hormone independence. One genetic change likely causing a transition to complete androgen independence is MYC overexpression due to amplification of the gene at 8q24 [67]. Interestingly, the chromatin remodeling protein MTA1 overexpressed in prostate cancers may be an essential mediator of MYC action [68]. Of note, amplifications at 8q24 usually activate several genes [69].

Both *CCND1* (Cyclin D1) and *MYC* are target genes of the Wnt/β-Catenin pathway [70], which regulates proliferation and differentiation in many tissues. In normal epithelial cells, β-Catenin, the key protein of this pathway, is localised in two cellular compartments – at the cell membrane in a complex with E-Cadherin and in the cytoplasm in a complex with GSK3β, Axin, and the tumor suppressor APC. Activation of the pathway allows β-Catenin to translocate to the nucleus and to activate transcription through the TCF4 transcription factor. Whereas in colorectal cancers, constitutive activation of the pathway is caused by loss of APC function or activating point mutations in β-Catenin, these are relatively rare in prostate cancer [11–13]. Instead, in many prostate cancers, the *CDH1* gene encoding E-Cadherin is hypermethylated [51, 71, 72]. The consequent decrease in E-Cadherin expression not only diminishes cell-to-cell adhesion facilitating the epithelial-to-mesenchymal transition associated with tumor invasion, but also changes the intracellular β-Catenin distribution.

Surprisingly, prostate cancer cells do not seem to engage the canonical pathway of β-

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Catenin action. Instead, β-Catenin acts primarily as an AR coactivator [73]. The AR and β-Catenin proteins appear to interact already in the cytoplasm and the ligand-activated AR promotes the nuclear translocation of β-Catenin. This mechanism ensures a sequestration of β-Catenin to AR-containing transcription activation complexes and selective repression of TCF-regulated genes [74, 75]. The AR/β-Catenin cooperation may have clinical consequences as β-Catenin enhances AR-mediated transactivation by certain steroids other than dihydrotestosterone [76]. Interestingly, TCF4 may also interact directly with the AR in a β-Catenin-independent manner (Fig. 3). This AR-TCF4 complex influences certain TCF target genes, including *MYC* [77]. It is interesting in this context that HOXB13 (discussed below) is a TCF4 repressor [78] as well as an AR corepressor [79].

The coactivator protein Tip60 constitutes another nexus between androgen signaling and β-Catenin. Tip60 acts as an AR coactivator, probably by means of its histone acetyltransferase activity. Its expression, nuclear accumulation and activity are increased upon androgen withdrawal and in relapsed hormone refractory tumors. Conversely, high level nuclear accumulation of Tip60 in tumors at diagnosis is significantly negatively correlated with the presence of distant metastases [80]. This finding can be explained by the involvement of Tip60 in activation of certain metastasis suppressor genes, like *KAI1*. This function is counteracted by the β-Catenin–Reptin complex, which represses the *KAI1* promoter by displacing Tip60 [81]. This mechanism explains how *KAI1* becomes downregulated by epigenetic mechanisms in prostate cancer progression, albeit not hypermethylated [82, 83].

The prostate-specific transcription factor, NKX3.1, was discovered only in the late 1990s, but has received considerable attention because of its location at 8p21.2 and its largely prostate-specific expression [84]. This factor belongs to a homeobox protein subfamily involved in the regulation of cell differentiation. NKX3.1 itself is induced by androgens and promotes ductal morphogenesis, epithelial cell differentiation and secretory cell function in the prostate. Accordingly, mice lacking one or two alleles of

the gene develop hyperplasia and dysplasia of the prostate epithelium and are prone to prostate cancer development upon introduction of further genetic alterations, *e.g. Pten* or *Cdkn1b/p27^{Kip1}* deficiency [85–87], or upon serial tranplantation of tissue recombinants of prostatic epithelium from mutant mice and urogenital sinus mesenchymal cells [88].

Nkx3.1 shows haploinsufficiency in the animals, *i.e.* not only homozygotes, but also the heterozygotes display defects. However, at least in the *Nkx3.1 / Pten* double knockout model the haploinsufficiency at the *Nkx3.1* locus (*Nkx3.1* $^{+/-}$) is accompanied by total loss of Nkx3.1 protein expression in tumors, despite the retention and continued transcription of the wild type allele [85]. Human prostatic carcinomas present, depending on dosage of the *NKX3.1* locus, a continuum of NKX3.1 protein expression [89]. Common losses at 8p thus lead consistently to decreased NKX3.1 expression but rarely to complete loss, in keeping with a 'true' haploinsufficiency. The consequences of such quantitative changes in the level of a transcription factor await further study. Conceivably, genes containing high-affinity binding sites will be less severely affected than those with low-affinity binding sites. A prostate-specific *Nkx3.1* knockout mouse model indeed displays a nearly continuous distribution in expression change of downstream genes depending on *Nkx3.1* dosage. In heterozygous mutant prostates, some genes (*e.g.* Probasin) are barely affected while others (*e.g.* Intelectin) are almost completely silenced. Moreover, the expression level seemed to parallel the proportion of cells expressing a particular downstream gene at a largely unchanged level, rather than a quantitative change in transcription throughout all cells [90]. This suggests that NKX3.1 might function as a 'promoter switch factor' on some genes deciding whether they are expressed or switched off. Accordingly, in prostates with diminished NKX3.1 expression, some downstream genes will be completely silenced in some cells and these could represent the actual tumor precursors.

Dosage effects hinting at haploinsufficiency have not only been detected for NKX3.1, but also for PTEN [91] and *CDKN1B* encoding the CDK inhibitor p27^{KIP1} [87, 91], which is regulated by

PTEN, in animal experiments and in human tumors. Loss of one *PTEN* allele at 10q and downregulation are quite frequent in human cancers, while mutational inactivation and promoter hypermethylation are restricted to rare advanced cases. Like PTEN loss, low p27^{KIP1} expression is a good predictor of prostate cancer progression [92–94]. Typically, *CDKN1B* expression is downregulated but not lost completely and changes in the gene are rare, even hypermethylation [95]. The 'two-hit' hypothesis so useful in understanding other cancers should therefore be applied with due caution in prostate cancer.

Like NKX3.1, HOXB13 is a homeobox transcription factor, but belongs to the canonical family of homeobox proteins [96]. Accordingly, the gene is localized at the edge of a homeobox gene cluster at 17q21. Unlike the other genes in the *HOXB* cluster, it is apparently not involved in establishing the segmentation of the embryo, but is expressed in a restricted pattern, prominently in the prostate epithelium [97]. NKX3.1 and HOXB13 are coinduced by androgens during puberty and their functions are similar and partly overlapping [98]. While they do not seem to interact with each other [97], HOXB13 acts as a repressor of AR activity [79], perhaps to prevent overstimulation of proliferation by androgens and ensure terminal differentiation. Moreover, HOXB13 also represses TCF4, a transcriptional activator mediating WNT/β-Catenin signaling, independent of its influence on androgen signaling [78, 99]. Such properties make *HOXB13* a similarly likely candidate for a tumor suppressor gene in the prostate as NKX3.1. There are indeed indications from microarray expression analyses that *HOXB13* expression is altered in prostate cancers, but surprisingly, most cancers appear to overexpress the gene [100]. Indeed, gains as well as losses have been reported for the chromosomal region where the gene resides [11]. Interestingly, in breast cancer *HOXB13* overexpression is discussed as a prognostic marker [101].

Considerable interest was elicited by a report that *KLF6* is mutated in 60% of prostate cancers with allelic loss at 10p15 [102]. Later, this high mutation frequency was disputed [103, 104]. Krüppel-like transcription factors (KLF) regulate cell differentiation and KLF6 is the main member of this family in the prostate. It could thus promote terminal differentiation of prostate epithelial cells. Meanwhile, altered splicing has been proposed to diminish KLF6 function in prostate cancer [105]. This is in many cases a consequence of a germ-line polymorphism in the gene, although no association of this polymorphism with prostate-cancer risk has been found. Alternative splicing is thought to produce dominant-negative proteins inhibiting the tumor-suppressive function of KLF6. Interestingly, KLF6 is also regulated by protein acetylation [106]. The related KLF5 may also be downregulated in prostate cancer as a consequence of allele loss of its gene at 13q21 [107].

Although the KLF6 issue is still open, it illuminates several issues. First, mutations in transcription factors are good candidates for events initiating epigenetic chains of events prevalent in prostate cancer. For instance, inactivation of an essential transcription factor could elicit target gene hypermethylation. Accordingly, point mutations have recently been reported in the transcription factor gene *ATBF1* located at 16q22, a region more commonly subject to allelic loss than 10p [108]. Secondly, while altered splicing is a regular observation in human cancers, its causes and consequences are understood in very few cases. The case of KLF6 suggests that it deserves more attention. Thirdly, one wonders how the alterations in different transcription factors interact with each other. Are they complementary, additive, synergistic, or even antagonistic? Can changes in one factor explain altered expression levels of others?

Factors such as NKX3.1, HOXB13, and KLF6 appear to promote differentiation of prostate epithelial cells rather than proliferation, and the AR one or the other depending on its cofactors. Proliferation of prostate epithelial cells may be controlled to a substantial extent by members of the ETS transcription factor family. Several family members including the prototypic ETS1 [109] and the more prostate-specific PDEF [110], also an AR coactivator, are overexpressed in prostate cancer. Very recently, two further members of the family, ERG and ETV1, have been reported to be alternatively overexpressed in a large proportion of prostate cancers by an unexpected mechanism, *i.e.* by

chromosomal translocations [111]. In each case, overexpression of the transcription factors is brought about by apposition of the coding regions of the respective genes at 21q22 and 7p21 to the promoter of the *TMPRSS2* gene at 21q22 which is androgen-inducible. In this fashion inappropriate expression of the ETS transcription factors could be elicited in differentiated AR-positive prostate epithelial cells and maintain them in a proliferation-competent state. It remains to be seen whether the reported high frequency of these translocations holds up in follow-on investigations.

Epithelial-mesenchymal interactions

Another dimension of epigenetic regulation is provided by interactions between epithelial and mesenchymal compartments (Fig. 1). Such mutual and reciprocal interactions are crucial in normal organogenesis, including that of the prostate [112]. In tumor progression, the supportive or even inductive role of cancer-associated fibroblasts is increasingly appreciated [113, 114]. In prostate cancer, this issue was highlighted by the demonstration that prostatic cancer-associated fibroblasts but not normal fibroblasts promote tumorigenic progression of nontumorigenic SV40 Tag-immortalized, but not of normal prostatic epithelial cells [115]. Interestingly, cell lines derived from such tumors are tumorigenic without assistance by cancer-associated fibroblasts [116]. Conversely, fibroblasts associated with benign prostatic hyperplasia or normal prostate, exert a suppressive effect on telomerase-immortalised nontumorigenic prostate carcinoma-derived cells. This effect disappears with increasing age of fibroblasts donors, perhaps explaining the pronounced age dependency of prostate cancer. The age-dependent loss of growth suppression was linked to increasing expression of the stromal-derived factor-1 (SDF-1) [117]. Importantly, the interactions between epithelial and fibroblastic cell populations are bi-directional. For example, transformed but not normal prostate epithelial cells induce local invasion of urogenital sinus-derived (*i.e.* normal) mesenchymal cells in tissue recombinants [86, 88].

Changes in the expression of oncogenic transcription factors, *e.g.*, ETS factors, may promote tumorigenesis by acting on epithelial or stromal cell populations or both. Overexpression of ETS1 induces matrix metalloproteinases MMP1, MMP3, MMP13, and integrins in different cell types in the tumor stroma like vascular endothelial cells and fibroblasts, too [118, 119]. ETS1 expression levels therefore determine the extent of angiogenesis and remodeling of the extracellular matrix during tumor invasion. Of note, moderate overexpression or downregulation of ETS1 leads to disproportional changes in the expression of some of its target genes [119]. ETS1 expression and activity itself is regulated by signaling cascades stimulated by growth factors, *e.g.* by VEGF in endothelial cells [120]. Its activation in tumor stromal cells therefore is a consequence of altered growth factor signaling, *e.g.* of VEGF overexpression by hypoxic carcinoma cells and by activated stromal cells. Moreover, in a transgenic model transformed prostatic epithelium was shown to exert a selective pressure for loss of *Tp53* function in the stroma, contributing to fibroblast hyperproliferation. In late-stage tumors, clusters of epithelial cells adjacent to Tp53-negative hyperproliferative mesenchyme also lose Tp53, initiating another round of this reciprocal crosstalk [121]. These examples illustrate how changes in carcinoma cells can initiate a chain of events affecting other cell types in the tumor to cause changes that further promote cancer growth.

Specific and elaborate tumor-stroma interactions occur also in bone metastases, which develop in up to 80% of advanced prostate carcinomas and crucially contribute to morbidity and mortality. Like the epithelial-mesenchymal interactions in the primary tumor, the interactions with bone cells have many facets and are reciprocal. Metastasizing prostate carcinoma cells adapt their gene expression profile to the osseous microenvironment, a phenomenon named osteomimicry [122]. As in the primary tumor, carcinoma cells induce epigenetic and morphologic changes and favor genetic alterations in cells derived from the bone [122, 123]. In the bone environment, metastasizing prostate cancer cells dynamically impinge on the differentiation of osteogenic progenitors to either

osteoclasts or osteoblasts. Stimulation of osteoclasts increases bone resorption and likely facilitates the colonization of bone matrix by invading prostate cancer cells. In established metastases, stimulation of osteoblasts predominates. Osteoblasts provide important survival and growth factors to metastasizing prostate carcinoma cells that contribute to the resistance against androgen ablation therapy. Targeting the prostate cancer-associated osteoblasts may therefore constitute an alternative treatment for metastatic hormone-refractory tumors [124].

It follows that a crucial part of the epithelial-mesenchymal crosstalk in the course of prostate carcinogenesis and metastasis represent paracrine hormonal interactions, *i.e.* the coordinate expression of growth factors and their receptors in different subpopulations of a complex tumor tissue. Several growth factor families and their respective receptors are involved in the paracrine interactions between prostate carcinoma cells and stromal cells in the prostate or bone [123, 125, 126]. They include prominently FGFs and FGFRs, IGFs and IGFRs as well as IGF binding proteins (IGFBP), HGF and its receptor MET, the related TGFβ and BMP peptides and their receptors, endothelin-1 and its receptors, and the EGF family and its receptors. Moreover, the processing, storage, and turnover of these growth factors are also altered through changes in the composition of the extracellular matrix and altered protease activity in the primary tumor and at metastatic sites. For example, osteoblasts may promote the expression of the urokinase-type plasminogen activator by metastasizing prostate carcinoma cells, leading to proteolytic degradation of IGFBP-3 and subsequent local increase in IGFs [124]. These changes have been excellently reviewed by others [123, 125, 126]. In the present context, two aspects are particularly important. First, many of these changes are caused by epigenetic mechanisms or moderate dosage changes rather than by point mutations or gene amplification. Second, the mutual interactions between stromal fibroblasts and epithelial carcinoma cells create vicious cycles that are a caricature of the 'epigenetic' (in the Waddington sense) homeostatic interactions between epithelial and mesenchymal cells.

Stem cell maintenance

As stem cells contain the same amount and sequence of DNA as more differentiated cells, their distinctive properties must be bestowed by epigenetic mechanisms. These comprise cell-autonomous mechanisms interacting with external signals. In embryonic stem cells, the ability to proliferate indefinitely and to maintain pluripotency is controlled by an interdependent network of transcription factors [127, 128]. Most of these factors, including OCT3/4 (now also designated POUF5), SOX2, and Nanog, are almost exclusively expressed in early embryonic and developing germ cells. Downregulation in adult tissues occurs by epigenetic mechanisms, including promoter DNA methylation. There are hints that expression of OCT3/4 and other factors is maintained in a small fraction of adult tissue cells, presumably stem cells.

Genes conferring stem cell properties to early embryonic or germ cells are aberrantly re-expressed in human cancers, particularly in germ cell-derived cancers and certain hematopoetic and pediatric cancers. It is not entirely clear to what extent carcinomas harbor a smaller fraction of 'cancer stem-cells' [129]. In prostate cancer, this question is of particular interest in the context of anti-androgenic therapy. Anti-androgenic treatment of prostate cancer often diminishes the tumor load, but the cancers recur and become refractory to the therapy. Prostate cancers refractory to anti-androgenic therapy present a wide variety of genetic and epigenetic alterations that are responsible for their resistance to therapy, although most of them express the AR. These alterations may already have been present in a subset of cancer cells at the start of therapy. These could represent cancer stem cells that do not depend on androgens [9, 12, 54, 55–57, 112].

Theoretically, cancer stem cells could be derived from tissue precursor cells that maintain their stem cell properties or from more differentiated cells that secondarily acquire relevant properties of stem cells, such as the ability to proliferate indefinitely. Stem cell properties could be conferred by genetic or epigenetic deregulation of endogenous stem cell factors or of signaling pathways defining stem cell niches. Constitutive activation of Hedgehog and

WNT/β-Catenin pathways in basal cell carcinoma and colorectal carcinoma, respectively, may act in this fashion [130, 131]. Indeed, activation of SHH signaling has been reported recently in prostate cancer, although the reports disagree substantially on at which stage of progression it occurs [132−135]. All reports agree however that growth of prostate cancer cell lines depends on Hedgehog signaling and that the pathway is activated in metastatic cases. Activation of Hedgehog signaling in prostate cancer is the more plausible, as it is important during prostate organogenesis [136–138]. Typical of prostate cancer, activation seems rarely to occur by mutations of pathway components, but by an autocrine mechanism, overproduction of Sonic Hedgehog and perhaps Indian Hedgehog. The primary cause of overproduction is still unknown. Since Hedgehog signaling has proved an excellent target for antitumor therapy in basal cell carcinoma and medulloblastoma, it is obviously of great interest to determine which prostate cancers might be suitable for anti-hedgehog therapy [139]. Moreover, if activation of the pathway should really be restricted to metastatic cases, proteins or RNAs induced by hedgehog signaling might provide excellent markers for prognostic purposes.

One target of hedgehog signaling is BMI1, a polycomb protein like EZH2, but a subunit of a distinct polycomb protein complex. The relative expression of these complexes and their composition change during development and cell differentiation and are thought to function in the epigenetic determination of cell fate [140]. A complex containing BMI1 allows indefinite proliferation of hematopoetic and leukemia stem cells by suppressing p16^{INK4A} [141] and likely inducing telomerase [125]. Bioinformatic evidence for BMI1 activity in prostate cancer metastases has indeed be extracted from published microarray gene expression studies [143]. Obviously, the precise functions of BMI1 and hedgehog signaling in prostate development, stem cell regulation, and carcinogenesis deserve detailed investigation.

Tissue stem cells in the prostate have received considerable interest over the last few years [144–146]. Most commonly, they are assumed to represent a subfraction of the basal cells in the glandular epithelium, from which intermediary and terminally differentiated cells, and perhaps even neuroendocrine cells in the glands originate. Several stem cell markers have been proposed, including the cell surface proteins Sca-1 (a member of the LY6 glycoprotein family) [147] and CD133 [148, 149], as well as the nuclear proteins p63 [150, 151] and the audaciously named prostate stem cell antigen (PSCA) [152–154]. Prostate cells with a particular pattern of cytokeratin expression (CK5/CK14) also exhibit properties of a precursor population [155]. None of these proteins is still thought to be directly responsible for stem cell determination and all are expressed in a fraction of prostate epithelial cells that seems too large for a true stem cell population.

In fact, prostate cancer cells exhibit a curious mixture of basal cell and secretory cell markers. For instance, most express the AR and lack GSTP1 like secretory cells, but contain high levels of BCL2 which is restricted to basal cells in the normal epithelium. Strikingly, prostate cancers lose the basal cell marker p63 so consistently that its absence can be used for diagnostic purposes [156]. Similarly, the frequently hypermethylated *MDR1* [21, 157] and *CD44* [158–160] genes are normally expressed in basal cells. Like *GSTP1* hypermethylation (Fig. 2), hypermethylation of these genes could therefore be interpreted as indicating the origin of prostate cancer cells from a differentiated stage. On the other hand, a substantial fraction of prostate cancers retain PSCA1, often as a consequence of 8q gain, where the gene is located [152, 161]. A combination of CD44, high $\alpha_2\beta_1$ integrin and CD133$^+$ characterizes cells with stem cell characteristics in prostate cancers suggesting a close relationship between a normal and a tumor stem cell population [162]. However, in work submitted for publication, our group has demonstrated that typical factors involved in maintaining 'stemcellness' in early embryos and germ cells remain expressed at low levels and their gene promoters remain densely methylated in primary prostate cancers. This finding fits better with evidence suggesting that prostate cancers are derived from a more mature population that reacquires certain stem cell properties rather from tissue stem cells.

Significance of epigenetic alterations in prostate cancer

Several of the puzzling features of prostate cancer mentioned in the introduction may relate to the particular importance of epigenetic mechanisms in this tumor type. As emphasized throughout this article, a characteristic of prostate cancer is the prevalence of chains of events stabilized by epigenetic mechanisms. Although the events initiating such chains are usually difficult to ascertain, they are likely set into motion by genetic changes such as allelic losses of tumor suppressors or mutations activating or inactivating transcription factors. Following amplification and ramification by epigenetic mechanisms, these changes appear to lock prostate carcinoma cells into an abnormal differentiation state and establish aberrant, but unfortunately productive interaction between epithelial cells and the tumor stroma.

The amplification effect of epigenetic mechanisms could explain in particular, how dosage changes of genes like *NKX3*.1 or *PTEN* promote prostate carcinogenesis without complete loss of function, why dosage gains of growth factor and growth factor receptor genes have disproportionate effects, and how various assortments of changes at chromosome 8 can exhibit variable effects. Genetic alterations in a few genes, even minor dosage changes, would result in alterations of chromatin structure and ultimately DNA methylation that perpetuate active or inactive states of many other genes and stabilize the aberrant state of prostate cancer cells.

This argument predicts that aberrant DNA methylation and chromatin structure develop gradually during carcinogenesis in the prostate. While such gradual changes have been observed in preneoplastic prostate tissue [15, 20, 51], it remains possible that major changes in chromatin structure or DNA methylation are elicited by catastrophic events, *e.g.* in response to infectious agents [7].

The prevalence of epigenetic mechanisms might also account for some of the puzzling observations regarding prostate cancer epidemiology. Both genetic predisposition and environmental factors influencing prostate cancer risk might not act upon the initiating genetic events, but influence the epigenetically controlled amplification chains. This would explain the lack of high-risk gene mutations predisposing to this cancer, the non-canonical behavior of hereditary prostate cancer genes, and the failure to identify exogenous mutagenic carcinogens. For instance, the second allele of *HPC1/RNASEL* is almost never inactivated in prostate cancers [5, 6]. A common interpretation is that it is a predisposition gene. Its (partial) deficiency becomes relevant only during carcinogenesis by an exogenous agent against which RNaseL protects. An alternative interpretation, then, is that a partial deficiency in RNaseL function could add to other genetic and epigenetic defects accumulating during prostate cancer development. Similarly, hormonal factors implicated in prostate carcinogenesis, including endogenous androgens and exogenous estrogens, might exert their effects through epigenetic mechanisms by altering the balance of the transcription factor network maintaining homeostasis in the prostate epithelium.

As discussed in detail by others [163], a basic difference between epigenetic and genetic alterations in carcinogenesis is their dynamics. Thus, epigenetic alterations are in principle reversible and allow a higher plasticity in the phenotype than mutations. Reversibility provides the basis for epigenetic therapy, *e.g.* by inhibitors of DNA methyltransferases and of histone deacetylases. On the other hand, reversibility and plasticity facilitate the adaptation of cancer cells to the changing environment during tumor progression, especially during metastasis, but also during therapy. Accordingly, several genes, including *CDH1* (E-Cadherin) [164], *ESR2* (estrogen receptor β) [165, 166], and *CAV1* (caveolin) [167, 168] have been reported to be expressed in a dynamic pattern during prostate cancer progression. They are downregulated in localized cancers, but re-expressed in metastases. These changes seem to be associated with changing patterns of promoter hypermethylation. Conversely, a novel marker of prostate cancer, Hepsin, is strongly upregulated in primary prostate cancers, but expression decreases in metastases [169–171]. Accordingly, Hepsin has been alternatively reported to stimulate or inhibit prostate

cancer growth [172, 173]. Neither the mechanisms of its initial upregulation nor of later downregulation are understood, but they are likely epigenetic. Thus, during the natural course of the disease epigenetic variability may be exploited to select tumor clones with optimally adapted gene expression levels. In an analogous fashion, epigenetic variegation may underlie the selection of resistant tumor cell clones during therapy. Thus, in prostate cancer, resistance to anti-androgenic and cytotoxic chemotherapy may as often be caused by epigenetic overexpression and downregulation as by gene mutation and amplification.

In conclusion, we argue that in order to understand prostate carcinoma and to develop better means for its prevention, diagnosis, and therapy, the particular importance of epigenetic alterations in this cancer must be taken into consideration.

Acknowledgements

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