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REVIEW

Specific changes in the expression of imprinted genes in prostate cancer—implications for cancer progression and epigenetic regulation

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Epigenetic dysregulation comprising DNA hypermethylation and hypomethylation, enhancer of zeste homologue 2 (EZH2) overexpression and altered patterns of histone modifications is associated with the progression of prostate cancer. DNA methylation, EZH2 and histone modifications also ensure the parental-specific monoallelic expression of at least 62 imprinted genes. Although it is therefore tempting to speculate that epigenetic dysregulation may extend to imprinted genes, expression changes in cancerous prostates are only well documented for insulin-like growth factor 2 (IGF2). A literature and database survey on imprinted genes in prostate cancer suggests that the expression of most imprinted genes remains unchanged despite global disturbances in epigenetic mechanisms. Instead, selective genetic and epigenetic changes appear to lead to the inactivation of a sub-network of imprinted genes, which might function in the prostate to limit cell growth induced *via* the PI3K/Akt pathway, modulate androgen responses and regulate differentiation. Whereas dysregulation of IGF2 may constitute an early change in prostate carcinogenesis, inactivation of this imprinted gene network is rather associated with cancer progression.
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EPIGENETIC DYSREGULATION IN PROSTATE CANCER

Like other cancers of old age, prostate cancer develops over many years. Multiple pathogenic events adding on each other drive initially the development of prostatic intraepithelial neoplasia from which invasive carcinomas emerge that metastasize in more aggressive cases. Many genetic changes responsible for altered differentiation, sustained cell proliferation and invasive growth of prostate cancers have been elucidated in the last decade. Prominently among them figure chromosomal rearrangements generating novel fusion genes from which ETS transcription factors like ERG are overexpressed in an androgen-dependent manner.[1] Another common change in prostate cancer is inappropriate activation of PI3K/Akt signalling, which is most efficiently achieved by homozygous deletions of the *PTEN* gene that encodes a crucial inhibitor of the pathway.[2] Like oncogenic ETS transcription factors, PI3K signalling interacts with and modulates androgen action.[3–6] A major question in current prostate cancer research is how these and other genetic changes conspire to bring about the spectrum of prostate cancer phenotypes ranging from indolent, well-differentiated local nodules to highly aggressive and often poorly differentiated systemic cancers. Epigenetic abnormalities play a seminal role in shaping the varying phenotypes of prostate cancer.[7,8] Well-characterized abnormalities comprise DNA hypermethylation, DNA hypomethylation, overexpression of the histone methyltransferase enhancer of zeste homologue 2 (EZH2) and several distinctive changes in histone modification patterns. Typically, these epigenetic changes aggravate with tumour stage and Gleason grade and can be employed as prognostic biomarkers.[9,10] A prominent exception to this relation is constituted by consistent and distinctive DNA hypermethylation events associated with early development.[11,12] These events induce aberrant methylation in more than 80% of prostate cancers at the CpG islands of a dozen specific genes, which are normally free of DNA methylation. Additional hypermethylation events are observed in smaller subsets of the cases and many of these correlate with tumour progression. Accordingly, consistent hypermethylation events can be employed for detection of prostate cancers, whereas some additional hypermethylation events can serve as indicators of worse prognosis.[9,13] Hypermethylation of *GSTP1* and *PITX2*, respectively, exemplifies the two categories.[14,15] The hypermethylation of CpG islands at the transcriptional start region of genes is often associated with silencing. More than 25 genes have so far been verified to be silenced by hypermethylation in prostate cancer.[9,13] While many hypermethylation events have been investigated for their potential as biomarkers, their functional relevance to the development of malignancy is not well understood.[13] In prostate cancer cell lines, restoration of expression of several silenced genes, e.g., *PDLIM4* and *RASSF1A*, arrested proliferation or induced apoptosis.[16,17] The contribution of these genes to the phenotype of prostate cancers in patients remains unclear.

Despite the aberrantly increased methylation at certain sites in the genome, its overall content of methylcytosine decreases during

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progression of prostate cancer. This decrease reflects hypomethylation of sequences that are methylated in normal tissues, i.e., intronic and intergenic sequences and prominently, interspersed and centromeric repeat sequences. Therefore, hypomethylation is primarily associated with regional or global changes in chromatin organisation that facilitate epigenetic reprogramming and genomic instability across the genome.[18,19] Locally, hypomethylation may also lead to reactivation of some genes that are normally repressed by DNA methylation. Although the number of such genes is limited, they comprise important regulators of pluripotency, lineage determination and development.[18,20]

The global changes in epigenetic regulation are also reflected in altered patterns of histone modifications, some of which may also be prognostic.[10] However, neither the mechanisms underlying these changes nor their consequences are very clear at this point. A large number of studies have addressed changes in the Polycomb factor EZH2 in prostate cancer (reviewed in Refs. 21–23). The Polycomb group proteins are normally involved in lineage determination during embryonic development and tissue maintenance, especially by suppressing alternative differentiation choices. They aid in gene silencing or in postponing gene activation to later differentiation stages. This function is achieved—among others—by specific histone modifications. Thus, trimethylation of histone H3 at lysine 27 (H3K27me3) is catalysed by the EZH2 subunit of the PRC2 Polycomb complex. Overexpression of EZH2 in prostate cancer is believed to contribute to altered patterns of histone modifications and to silence tumour suppressor genes in a complementary or cooperative fashion with DNA hypermethylation.[24,25] Increases in EZH2 are brought about by various mechanisms, including induction by E2F transcription factors in proliferating cells,[26] gene amplification and loss of the regulatory microRNA *miR-101*.[27] Interestingly, EZH2 expression is elevated as a consequence of ETS gene fusions in prostate cancer and may in turn conspire with these transcription factors to block cell differentiation, increase cell proliferation, facilitate invasiveness and alter androgen responses.[6,28] Accordingly, EZH2 expression levels have been linked to cancer recurrence.[25,29–31]

EZH2 is by far not the only histone modifying enzyme upregulated in prostate cancer. In particular and somewhat curiously, certain histone demethylases of the JMJD2 family and KDM1/LSD1, which counteract the biochemical activity of EZH2, are likewise upregulated in prostate cancer.[32,33] They interact as cofactors with the androgen receptor and hypoxia-inducible factors and are implicated in the epigenetic changes during the development of castration resistance.[34–37]

GENOMIC IMPRINTING AND ITS REGULATION[a]

DNA methylation, histone modifications and Polycomb factors, in particular EZH2, are also instrumental in the prototypic epigenetic phenomenon of 'imprinting'. At least 62 genes in humans and twice as many in mice are known to be differentially expressed depending on their parental origin.[38] The regulation of many imprinted genes depends on DNA methylation marks that differ between the alleles inherited from mother or father, designated differentially methylated regions (DMR). Most DMRs are established during gametogenesis and inherited and propagated by the zygote, whereas others, designated somatic DMRs, are established upon fertilisation, still in a parent-of-origin-specific fashion. Most of these methylation marks persist throughout life and are only erased in the embryonic primordial germ cells to be re-established once more in the new generation. Depending on the gene, imprinting is maintained in all cell types or in a tissue-specific manner. In addition or alternatively to DNA methylation, other epigenetic mechanisms like histone modifications and inter- or intrachromosomal interactions such as looping are employed to ensure the monoallelic expression of imprinted genes.[39–42] With respect to histone modifications, a striking characteristic of imprinted genes is a seemingly bivalent pattern, with modifications typical of actively transcribed genes such as trimethylation of H3K4 on one allele and H3K27 trimethylation on the other one.[43–45]

Imprinted genes are often situated in clusters, allowing coordinate regulation by common control regions consequently designated as imprinting control centres (ICRs). ICRs often contain DMRs. Many imprinted gene clusters encompass genes encoding non-translatable regulatory RNAs, which are termed long non-coding (lnc-RNA) to distinguish them from shorter species like microRNAs. Seemingly isolated imprinted genes are often actually gene pairs, of which one encodes an lnc-RNA acting on its partner gene. The regulatory RNAs may function by recruiting chromatin modifying proteins, including EZH2, to silence selected genes in the cluster,[45–47] and by directing them into specific higher-order chromatin structures located in transcriptionally inactive subnuclear compartments.[46,48–50] In contrast, the expressed alleles of imprinted genes may be enabled to enter active subnuclear compartments to gain access to 'transcriptional factories'.[51]

The extraordinarily complex multi-level mode of regulation of imprinted genes probably aims to tightly control the levels of gene products. Indeed, similar mechanisms are employed in the analogous process of X-chromosome inactivation to adjust the dosage of X-chromosomal genes.[52,53] In addition, imprinting may limit the influences of foetal or maternal factors on certain genes in the developing embryo.[54] Although the biological rationale for imprinting is not firmly established, current thinking favours the 'battle of the sexes' theory.[55] This theory is based on the observation that many imprinted genes expressed from the maternal chromosome limit the acquisition of maternal resources by the foetus through the placenta, while paternally expressed genes function in the opposite direction to enhance foetal supply and growth. In another mirror fashion, maternally expressed genes regulate placental growth, while paternally expressed genes are crucial for foetal growth and development.[54,56]

In addition, imprinted genes regulate the specification of certain lineages such as the musculoskeletal system and the brain, but also postnatal metabolism and social behaviour.[56–58] In these functions, imprinted genes cooperate with each other as well as with many non-imprinted genes. In particular, a subnetwork containing the imprinted genes *IGF2*, *H19*, *PLAGL1*/(*ZAC1*), *CDKN1C*, *DLK1* and *PEG3* was characterized by remarkable co-expression across different organs and developmental stages and by functional association (**Figure 1**).[59–61] The genes of the network are thought to control energy homeostasis at the levels of signal-sending (hypothalamus, pituitary and pancreas) and signal-receiving (liver, fat, muscle, cartilage and bone) organs to regulate body size, energy storage and expenditure during embryonic and postnatal development.[56] The function of this imprinted gene network may thus consist in 'programming' the growing embryo for optimal resource acquisition during foetal and adult life, with adjustments to the current environment.[56] However, the increasing number of imprinted genes implicated in cancer and other diseases of adults underlines that their function is not restricted to the placenta and foetus.

Defects in imprinting are responsible for a number of congenital paediatric diseases and syndromes. These often involve metabolic

[a]There is another usage of 'imprinting' that should not be confused with the phenomenon of 'genomic imprinting' discussed in this article. Accumulating evidence suggests that environmental effects on endocrine regulation *in utero* can have lasting influences throughout life, including the development of diseases like diabetes and prostate cancer. These effects are likely to be mediated by epigenetic mechanisms too. The phenomenon should better be designated as "developmental reprogramming" to avoid confusion.

disturbances, neurological deficiencies and either extensive or scanty growth together with specific morphological abnormalities. Perturbations in imprinted gene expression occur in Beckwith–Wiedemann syndrome (BWS), Silver–Russell syndrome, intrauterine growth restriction, transient neonatal diabetes mellitus and congenital Wilms tumours.[62–68] The lack of imprinting in such diseases originates from a failure in establishing epigenetic marks in germ cells or maintaining them in the zygote or early embryo.

Aberrant imprinting patterns may also represent a major factor in male infertility.[69–71] In the gametes of some infertile men, imprinted gene loci displayed abnormal methylation states.[70,72–74] Moreover, gametes of some infertile men contain reduced amounts of normal H3K4me or H3K27me bivalent marks at certain imprinted genes and at genes encoding essential transcription factors for early development.[75,76] These changes are associated with excessive methylation of imprinted gene promoters and silencing.[44,75,77–79]

While failure to establish imprinting is a cause of paediatric diseases and infertility, secondary loss of imprinting (LOI) contributes to diseases of adults. In particular, it is a common epigenetic disturbance in cancers, but is also quite often observed in preneoplastic and ageing tissues.[80–83] Strictly spoken, LOI denotes the loss of parent-of-origin monoallelic expression of genes such that the two alleles may become either both transcriptionally active or both silenced. Since monoallelic expression is typically maintained by differential DNA methylation and allelic histone modifications, LOI is also reflected by a convergence of these epigenetic marks on both alleles. Experimentally, monoallelic expression can be most easily ascertained through single nucleotide or short repeat polymorphisms in gene transcripts. Loss of differential DNA methylation and histone modifications are sometimes considered as surrogate indicators of LOI. In prostate cancer, the most studied imprinted genes are *IGF2* and *H19*.

INSULIN-LIKE GROWTH FACTOR 2 (IGF2)

IGF2 is the most widely studied imprinted gene in the context of tumourigenesis.[81,84] It belongs to a small imprinted gene cluster at 11p15.5 that also includes the *H19* gene. The cluster is organized around a differentially methylated ICR separating *IGF2* from *H19* and common enhancer regions. Normally, *IGF2* is expressed exclusively from the paternal allele and *H19* from the maternal allele. Paternal-specific *IGF2* expression is ensured by interactions between its promoter and distant enhancers. These interactions depend on the methylation status of a second DMR, *viz.* DMR0 upstream of the *IGF2* promoters, and on the epigenetic status of the major ICR. On the maternal chromosome both regions are unmethylated, whereas on the paternal chromosome both are methylated. Expression of the gene cluster is ultimately regulated by differential formation of three-dimensional chromatin loops that separate actively transcribed and silenced genes and bring together different regulatory regions on the two alleles on both chromosomes.[85–87] The correct looping is ensured by specific methylation patterns of the DMRs, and in particular by the insulator protein CTCF, which binds only unmethylated DNA in the central ICR.[88] The intensely studied regulation of *IGF2* and *H19* serves as a paradigm in the field.

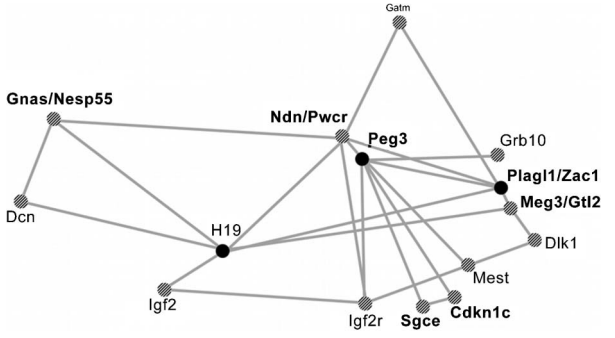

Figure 1. A network of imprinted genes. On the basis of 116 mouse microarray experiments, Varrault *et al.*[60] discovered significant co-expression of selected imprinted genes. The figure shows a simplified version of the original Pajek network representation of coregulated imprinted genes disregarding the exact degree of co-expression and omitting non-imprinted genes. The enlarged circles represent the central imprinted genes of the network (modified from Ref. 60). The genes indicated in bold are deregulated in prostate cancer according to our analysis (**Table 1**). Note that *IGF2R* is not imprinted in humans. IGF2, insulin-like growth factor 2.

The product of *IGF2* is a growth factor related to insulin that is essential for foetal development and growth. In adults, it acts as a cohormone together with follicle-stimulating hormone or luteinizing hormone. In general, IGF2 promotes cell growth, and also—like insulin—affects cell metabolism. Like its non-imprinted paralogue IGF1, IGF2 exerts its growth-promoting and anti-apoptotic effects mainly through the type 1 IGF receptor (IGFR1). Both factors act in a paracrine fashion on epithelial and stromal cells, but are more strongly expressed in stroma than in epithelium of normal prostate tissue.[89] Their expression is enhanced in malignant epithelial cells, especially in tumours with high Gleason scores.[90–94] IGFR1 activates PI3K/Akt signalling, a major determinant of prostate cancer development and progression.[95]

In prostate cancer, PI3K/Akt signalling is deregulated by different mechanisms, most severely by loss of the negative regulator *PTEN*, which reverses phosphorylation of phosphatidylinositol triphosphate by PI3K.[96,97] Particularly notable is downregulation of an inositol-triphosphate phosphatase, INPP4B, which restricts the substrate supply of PI3K.[98,99] In the absence of these negative regulators, activation of PI3K signalling by IGFs *via* IGFR1 is enhanced and sustained (**Figure 2**).

In prostate cancer, PI3K and androgen receptor (AR) signalling are linked by a mutual crosstalk with inhibitory and stimulating interactions.[5,37,100,101] On the one hand, PI3K signalling enhances AR transcriptional activity by stabilizing active AR homodimers and sensitizing AR to low levels of androgen/DHT.[95,102] On the other hand, the androgen receptor interacts with or influences the expression of several pathway components, especially feedback inhibitors like

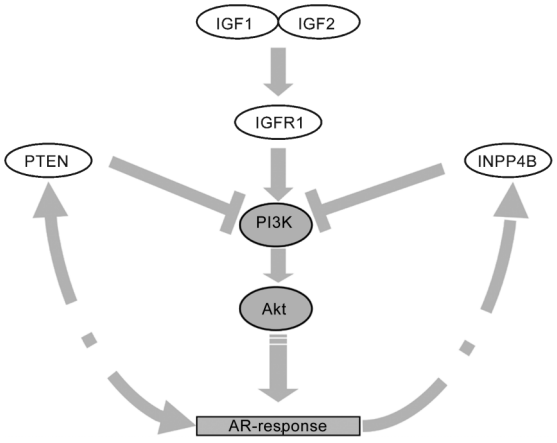

Figure 2. A sketch of the relationship between IGF signalling, the PI3K pathway and androgen responses in the prostate. IGF, insulin-like growth factor.

PTEN and INPP4B. *Via* PI3K signalling, IGFs regulate the androgen response. Accordingly, the IGFR1 inhibitory antibody A12 blocked AR transactivation induced by IGFR1 in the absence of androgens.[103]

Interactions between IGF signalling and the androgen response occur at some further points. To name just a few, the pathway affects the activity of AR cofactors, e.g., *via* stabilisation of β-catenin by inhibition of its negative regulator GSK3. Furthermore, IGFR1 signalling stimulates AR cofactors like GRIP and IDE.[104,105] The AR coactivators SRC1 and TIF2 are substrates for MAP kinases activated by IGFs and EGF tyrosine kinase receptors.[106] Consequently, endogenous IGF1 or constitutive PI3K/Akt signalling is required for androgen-induced prostate-specific antigen expression.[95] IGF signalling relieves Foxo-corepressor mediated inhibition of AR ligand-mediated transactivation.[107]

Together, enhancement of PI3K/Akt signalling by increased IGFR1 activity and loss of negative and feedback regulators like PTEN promote AR transcriptional activity even at the low androgen concentrations achieved by androgen deprivation therapy. Ultimately, these mechanisms permit androgen-independent activation of the AR, termed illustratively 'outlaw' AR activation.[108] Apart from their effect on androgen signalling, IGFs through Akt create a potent survival signal by inactivating the pro-apoptotic tumour suppressor proteins FOXO1A and BAD, and activating the anti-apoptotic MDM2.[95,107,109] This part of the signalling pathway may compensate for the lack of androgen pro-survival signals in castration-resistant prostate cancers. Therefore, pharmacological targeting of the IGFR1/PI3K/AKT signalling pathway by direct interference at the receptor or by decreasing circulating IGF1 levels is currently investigated in clinical trials.[110]

The deregulation of *IGF2* in the cancerous prostate is likely caused by epigenetic mechanisms that resemble those observed in other cancers.[80,84,111] IGF2 overexpression, often caused by loss of imprinting, is frequent across the range of paediatric tumours seen in BWS, in congenital and sporadic cases.[112] LOI at the *IGF2*/*H19* locus has been found in various cancers, including bladder and breast cancers, and in colorectal carcinoma.[81,84] Moreover, *IGF2* LOI has a very promising diagnostic potential having been associated with an increased risk for developing several adult-type cancers.[80,83] For instance, LOI of *IGF2* in blood cells has been proposed as a biomarker for colorectal cancer risk.[113] A similar association has not yet been reported in prostate cancer patients.

A common mechanism leading to biallelic *IGF2* expression is gain of methylation at the maternal ICR, which allows the interaction of the *IGF2* promoter with the enhancers on both chromosomes. This mechanism has been observed in BWS patients.[112] Alternatively, loss of methylation of the paternal DMR0 is associated with increased *IGF2* expression. This mechanism is not completely understood, since the unmethylated maternal allele ought to function as a silencer, suppressing *IGF2* expression independent of *H19* imprinting. Instead, loss of methylation at the DMR0 is associated with increased *IGF2* expression in breast, colorectal and Wilms tumours, perhaps because silencing of the maternal allele depends on additional repressor proteins that are no longer available in somatic cells.[87,112] In some cases, a switch to a different promoter may reactivate the normally silent maternal *IGF2* allele independent of ICR methylation status.[114]

Importantly, in the prostate, as in some other organs, *IGF2* LOI is also observed in the ageing tissue and especially in normal tissues adjacent to the cancer. Due to this 'field effect' the highest expression of IGF2 and H19 in the prostate is actually found in benign tissues adjacent to cancer in comparison to fully normal tissues and cancer tissues. Two studies reported significant associations between overexpression and epigenetic aberrations at the locus in cancer adjacent tissues.[82,115] One study found >80% methylation of the ICR in benign prostatic hyperplasia samples, while 9/30 prostate carcinoma samples showed ~40% methylation. Furthermore, the ICR was associated with the repressive H3K9me2 modification in benign prostatic hyperplasia but not in cancer tissues.[115] In addition to ICR hypermethylation, the second study[82] reported an association between increased IGF2 expression in tumour-adjacent tissue and decreased methylation of the DMR0, but no such changes in cancer tissues. Thus, DNA methylation aberrations and altered chromatin structures are likely to cause widespread *IGF2* LOI in ageing prostates. This idea is supported by an investigation of cultured primary prostate epithelial cells demonstrating a conversion of the *IGF2* imprinting status from monoallelic to biallelic, with a 10-fold induction of *IGF2* expression, when these cells became senescent. Mechanistically, *IGF2* LOI was traced to a diminished CTCF expression in the senescent cells.[80,116]

H19

As compared to *IGF2*, its fellow gene *H19* has hardly been studied in prostate cancer. While under negative regulation by p53, *H19* is induced by c-myc, E2F1, FOXA and ZAC1.[117] It is also upregulated by hypoxia, where it promotes cell survival.[117] All these factors are also relevant in prostate cancer. *H19* might potentiate IGF2 levels by inhibiting its suppressor IGFBP4 and has been implicated in epithelial–mesenchymal transition in a breast cancer metastasis model.[118] Knockdown of *H19* during hypoxia led to decreased expression of several genes which include *AKT1*, the prostate cancer biomarker *AMACR* and the imprinted genes *CDKN1C* and *INPP5A*.[119] In transgenic mice, *H19* activated the expression of imprinted genes including *Dlk1*, *Rtl1*, *Gnas*, *Peg3*, *Slc38a4*, *Igf2R* and *Cdkn1c*.[59] Broader disturbances of imprinted genes are also observed following *H19* deregulation in human congenital diseases.[120,121]

The molecular mechanism by which the *H19* RNA exerts its effects is not entirely clear.[119] By a better established mechanism, during germ cell development, the *H19* ICR interacts upon CTCF-binding with other imprinted genes *in cis* and *in trans* generating chromatin structures poised for transcription, establishing an 'imprinted interactome', and regulating the replication timing of further interacting imprinted genes.[85] *H19* was shown to be regulated by steroid hormones, including androgens, and prolactin in androgen-sensitive LNCaP, but not in androgen-insensitive DU145 prostate cancer cells. If the imprinted interactome persists in adult prostatic cells, *H19* and other imprinted genes might be influenced by androgen responses in prostate cancer.[122]

Taken together, these findings infer that relaxed *IGF*/*H19* imprinting may occur early in prostate carcinogenesis where environmental factors or ageing may influence its epigenetic state. One could speculate that the resulting overexpression of *IGF2* and *H19* may promote the enlargement of an epigenetically unstable but not neoplastic progenitor cell pool and skew the androgen response, thereby changing the microenvironment and facilitating cancer formation.[123]

A SYSTEMATIC SURVEY OF IMPRINTED GENE EXPRESSION IN PROSTATE CANCER

In addition to *IGF2*, activation or inactivation of further imprinted genes may influence prostate cancer development and progression. One wonders, in particular, to which extent imprinted genes may manage to uphold their exquisitely regulated epigenetic patterns in the face of the severe and progressive disturbances of epigenetic regulation in prostate cancer. There are therefore many open questions.

How many imprinted genes are deregulated in prostate cancer? Might their deregulation result straightforwardly from an 'epigenetic chaos' in these carcinomas? How might imprinted genes contribute to the phenotype of prostate cancer?

For a systematic approach to these questions, we have used the Oncomine database (http://www.oncomine.org) in order to search for changes in the expression of imprinted genes in the results of 16 microarray studies comparing benign *vs.* cancerous tissues from prostatectomies. At the time of the search, the website 'Geneimprint' (http://www.geneimprint.com/) listed 62 genes proven to be imprinted in humans. Of these, 52 had been investigated in at least two independent microarray studies. Of them only 12 genes showed consistent changes which at least approached statistical significance across multiple studies (**Table 1**).

Most genes identified by this approach belong to the network of coordinately regulated imprinted genes that regulate growth and differentiation in the mouse embryo (**Figure 1**). Interestingly, *IGF2* or *H19* were not among them, in keeping with the idea that deregulation of these genes may occur across the entire organ. Instead, *CDKN1C* followed by *MEG3* presented the most significant changes. Other significant expression changes concerned the 7q and 15q clusters and a pair of imprinted genes at 6q24, of which *PLAGL1* encodes the ZAC1 transcription factor and *HYMAI* a non-coding RNA. With the exception of *PPP1R9A* and *GNAS*, all genes were expressed at reduced levels in the cancers compared to benign tissues. Of note, only the 7q and 10q regions and to a lesser extent 6q undergo significant copy number changes in prostate cancer.[96] For *SNRPN* at 15q an almost equal number of studies described significant up- and downregulation. This may be due to the extremely complex transcription pattern of the gene with multiple splice variants confounding microarray analyses. It encodes a splicing factor acting mainly in the brain.[124,125] There is no information on SNRPN function in the prostate or in cancer.

Below, we will summarize the current knowledge on the function and regulation of the imprinted genes listed in **Table 1** (except *SNRPN*), with an accent on their potential influence on prostate cancer progression mechanisms. Although not formally identified by our bio-informatic analysis, *DLK1* and *DCN* will be treated, since they are part of the imprinted gene network and some evidence already links them to prostate cancer.

PLAGL1/ZAC1 AND HYMAI

The chromosomal region 6q24 lost in various cancers, including a fraction of prostate carcinomas, is thought to contain a tumour suppressor gene. The most likely candidate is pleiomorphic adenoma gene-like 1 (*PLAGL1*), also known as *ZAC1*, which encodes a C2H2 seven-zinc-finger transcription factor and nuclear receptor coactivator. Two isoforms are generated *via* usage of alternative promoters. Transcription of the shorter isoform starts from a DMR downstream of the start of the longer isoform, which also includes the first exon of the non-coding RNA gene *HYMAI*. Both genes are ubiquitously paternally expressed. Defects in imprinting of the twin locus are implicated in transient neonatal diabetes mellitus, a rare disease characterized by intrauterine growth retardation, dehydration, and failure to thrive due to a lack of normal insulin secretion.[68,126]

During embryonic development ZAC1 participates in molecular switches controlling progenitor cell fate decisions, proliferation and differentiation of bone, muscle and adipose tissue, secretory organs, including those of the gonadotropic axis (hypothalamus, pituitary and gonads) and the endocrine pancreas.[127] Its function during development depends on its ability as a transcriptional activator. Monomeric or dimeric ZAC1 binds to GC-rich palindromic DNA elements[128,129] in the regulatory regions of the imprinted protein-coding genes *IGF2*, *CDKN1C*, *DLK1* and *MEST*, the imprinted ncRNA genes *H19*, *KCNQ1OT1* and *MEG3*; as well as in the non-imprinted genes *PPARG*, *CK14* and *PACAP1*.[129–131] Zac1 was suggested to represent a key regulator of the imprinted gene network in **Figure 1**.[60]

Apart from its function as a DNA-binding transcriptional activator, ZAC1 acts as a powerful coactivator for the hormone-dependent

Table 1 Changes of imprinted gene expression in prostate benign *vs.* cancerous tissues

Gene symbol	*Gene product*	*Chromosomal localisation*	*Expression*	*Differential expression in PCa*	*Number of studies with*		*Total number of studies*
					Upregulating	*Downregulating*	
HYMAI	Hydatidiform mole associated and imprinted (non-protein coding)	6q24.2	Paternal	↓	0	3	4
PLAGL/ZAC1	Zinc finger protein PLAGL1	6q24–q25	Paternal	↓	1	12	16
SGCE	Epsilon-sarcoglycan	7q21–q22	Paternal	↓	1	11	15
PEG10	Paternally expressed gene 10 protein	7q21	Paternal	↓	1	6	14
PPP1R9A	Protein phosphatase 1, regulatory (inhibitor) subunit 9A	7q21.3	Maternal	↓	5	0	6
INPP5F	Inositol polyphosphate-5-phosphatase F	10q26.11	Paternal	↓	0	10	15
CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57,Kip2)	11p15.5	Maternal	↓	0	12	12
MEG3/GTL2	maternally expressed 3 (non-protein coding)	14q32.2	Maternal	↓	4	13	15
NDN/PWCR	Necdin	15q11.2–q12	Paternal	↓	0	10	14
SNRPN	Small nuclear ribonucleoprotein-associated protein N	15q11.2	Paternal	↓ ↑	6	7	16
PEG3	Paternally expressed gene 3 protein	19q13.4	Paternal	↓	2	10	16
GNAS/NESP55	Guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1	20q13.32	Paternal	↓	11	6	15

Abbreviation: PCa, prostate cancer.

Imprinted genes found to be frequently differentially expressed in prostate benign *vs.* cancerous tissues, as found by *in silico* analysis of changes in 16 microarray studies available in Oncomine. The arrows indicate over- or underexpression. Note: in cases when some studies measured no difference in expression between benign and cancerous tissues, the total number of studies is bigger than the sum of studies with up- and downregulation. The sum of studies reporting up- or downregulation can be bigger than the total number of studies due to differences between identifiers in complex genes such as *GNAS*.

activity of nuclear receptors, including the androgen, estrogen, glucocorticoid, and thyroid hormone receptors. In this role, it functions as a scaffolding protein recruiting chromatin activators (p160 as well as histone acetyltransferases CBP, p300 and PCAF), but also corepressors (HDAC1 and mSin3a) to nuclear receptor target genes.[132–134] Specifically, when interacting with the AR ZAC1 binds other coactivators (GRIP1 and SRC1) to enhance AR transcriptional activity in a hormone-dependent manner. The physiological role of ZAC1 may be the prevention of AR activation by other coactivators in the absence of hormone. Thus, ZAC1 synergized with GRIP1 in activating AR at lower androgen concentrations but suppressed the ability of GRIP1 to coactivate AR in a hormone-independent fashion.[133] In effect, loss of ZAC1 may therefore promote castration-resistance in prostate cancer. However, this has not been investigated explicitly in human cancer tissues.

ZAC1 may exert antitumour effects by enhancing the transcriptional activity of p53 in the induction of certain antiproliferative proteins such as $p21^{Cip1}$ or apoptosis protease-activating factor-1.[134–136] Moreover, ZAC1-can inhibit proliferation by directly activating the *PPARG* gene, eliciting differentiation and growth arrest.[130]

Loss of *ZAC1* expression has accordingly been observed in numerous tumour types, including breast and ovarian tumours, melanomas, astrocytomas, renal cell carcinomas and pituitary adenomas.[137–142] *ZAC1* overexpression inhibited tumour cell growth through induction of apoptosis or cell cycle arrest.[131,143] *PLAGL1/ZAC1* could therefore be relevant as a tumour suppressor in prostate cancer beyond its function in androgen action.

SGCE, PEG10 AND THE 7Q21 IMPRINTED GENE CLUSTER

Chromosome 7q is among the most frequently altered chromosomes in prostate cancer and subject to both gains and losses.[96] These changes also involve the 7q21 region that contains an imprinted gene cluster. Several imprinted genes are clustered around two head-to-head oriented paternally expressed genes—paternally-expressed gene 10 (*PEG10*) and sarcoglycan epsilon (*SGCE*). *PEG10* is derived from an ancient retrotransposon integration, which has been conserved in many mammalian genomes. A maternally methylated region in the first exon comprises an ICR which is marked by allele-specific histone modifications, i.e., H3K9me3 and H4K20me3 on the inactive maternal allele *vs.* H3K4me2 and acetylated H3K9 on the active paternal allele.[144] Of note, *PEG10* provides a striking example of how retrotransposon insertions contribute to evolution.[145] Due to the sharing of a common promoter, *PEG10* and *SGCE* are often coexpressed, but in some cases their expression diverges.[146]

The 7q21 cluster further contains the maternally expressed gene tissue factor pathway inhibitor 2 (*TFPI2*). It is considered as a tumour suppressor candidate, because it is silenced by genetic and epigenetic aberrations in several types of cancer, including prostate cancer.[147–150] TFPI2 and its non-imprinted homolog TFPI inhibit the coagulation-initiating protease tissue factor. Besides its well-established role in control of coagulation, it is a component of prostatic secretions contributing to stroma remodelling and angiogenesis. In a previous study, we investigated expression and methylation of *TFPI2* and two further reciprocally imprinted genes in the locus—the maternally expressed paraoxonase 2 (*PON2*) and the paternally expressed *SGCE*.[151] Whereas prostate cancer cell lines varied quite strongly in the expression of these genes, no significant differences were observed between prostate cancer and adjacent normal tissues, with the exception of *PON2*. Nevertheless, we observed a substantial variation of *TFPI2* expression between different prostate cancer tissues. Moreover, the *TFPI2* promoter was found to be partially methylated in several samples with low gene expression. Repressive chromatin modifications were more enriched in the weakly expressing LNCaP cell line with low *TFPI2* expression in comparison to the PC3 cell line with high expression. Thus, *TFPI2* might be prone to hypermethylation in prostate cancer. In contrast, the epigenetic status of the ICR did not correlate with *TFPI2* expression in the prostate cancer cell lines. We concluded that epigenetic disturbances in the 7q21 cluster affect imprinted genes in a non-coordinated manner, suggesting an unstable epigenetic state prone to selection for specific expression changes.[151]

Our *in silico* analysis (**Table 1**) reveals that the expression of three imprinted genes from the 7q21 locus differs significantly between normal and cancer prostate tissues. Peculiarly, while the adjacent paternally expressed *PEG10* and *SGCE* genes were downregulated in the majority of prostate cancer studies, *PPP1R91A* was upregulated. Since the epigenetic state of the ICR seemed unchanged in our former analysis,[151] these expression changes might derive rather from gains and losses at 7q21, as observed in other cancer types. This explanation would also account for the lack of difference in *SGCE* expression in our sample series, which contains a relatively low fraction of cases with 7q alterations compared to other studies.[152]

PEG10 promotes embryonic growth and is overexpressed in several malignancies including B-cell ALL and CLL, hepatocarcinoma and Wilms tumour.[153–155] In hepatocellular carcinoma (HCC), the 7q21 region was reported to be frequently amplified, which correlated with overexpression of both *PEG10* and *SGCE*.[156,157] The oncogenic function of PEG10 might be mediated by several mechanisms. In HCC cells PEG10 inhibited SIAH1-mediated apoptosis.[154] In androgen-dependent HCC cells, *PEG10* expression was elevated upon treatment with androgen and necessary for induction of *hTERT*.[158] Ablation of *PEG10* by siRNA blocked the ability of DHT to enhance cell growth and apoptotic resistance in HCC cells.[158] Another inducer of *PEG10* is c-Myc.[159] Furthermore, PEG10 can interfere with TGF-β signalling through interaction with two of its receptors—the activin receptor-like kinases ALK1 and ALK5. Moreover, the balance between these receptors is known to be crucial in determining proliferation and migration *vs.* quiescence of vascular endothelial cells during angiogenesis.[160] Conceivably, this effect might also contribute to metastasis of prostate cancer cells.

The *SGCE* gene encodes the protein sarcoglycan epsilon which is part of a dystrophin—sarcoglycan complex in muscle and other tissues, thought to functionally link the cytoskeleton to the extracellular matrix.[161] *SGCE* aberrations are implicated in intrauterine and postnatal growth retardation. The inherited disease *Myoclonic dystonia* characterized by irregular arrhythmic movements and behavioural disorders is caused by *SGCE* mutations.[162] As in prostate cancer, *SGCE* is often downregulated in colorectal cancers, especially in cases with microsatellite instability and its loss may favour a less invasive phenotype.[163]

PPP1R9A encodes the protein phosphatase 1 regulatory (inhibitory) subunit 9a, also called neurabin I. It is an F-actin binding protein targeting protein phosphatase 1 to the actin cytoskeleton to limit the activity of the growth regulatory kinases PKA and p70S6K.[164] Therefore, neurabin I was proposed to regulate adhesion-dependent signalling and function as a tumour suppressor.[165]

INPP5F

The *INPP5F* gene was recently shown to contain an imprinted transcript variant,[166] coding for a phosphatidylinositol phosphatase which limits the substrate for PI3K and phospholipase C that are activated by

tyrosine kinase receptors. To some extent, therefore, the phosphatidylinositol phosphatases function in a similar way to PTEN. For instance, INPP5F limits cardiomyocyte cell size and cardiac hypertrophy by inhibiting PI3K signalling during stress responses. PTEN loss likewise favours cardiac hypertrophy.[167] A homolog of *INPP5F*, *INPP4B*, was recently found to be downregulated in prostate cancers. *INPP4B* is induced by AR in prostate cancer cells and diminishes phosphorylation of Akt.[99] It is not known whether any of the *INPP5F* transcripts is regulated in a similar manner. However, phosphatidylinositol phosphatases are expected to dampen signalling from growth factor receptors like IGFR1 and EGFR in general. In particular, they may act as feedback inhibitors in the interaction of PI3K/Akt and AR signalling (**Figure 2**). Their potential as suppressors of development of prostate cancer and especially castration resistance is evident.

CDKN1C

The maternally expressed gene *CDKN1C* is located in a second imprinted gene cluster at 11p15.5 together with *KCNQ*, encoding an ion channel protein, and other genes. *CDKN1C* codes for the protein $p57^{KIP2}$, a cyclin-dependent kinase inhibitor. It binds to various CDK-cyclin heterodimers and at low concentrations promotes their assembly, but at high concentrations abrogates the activity of CDKs 1, 2, 3, 4 and 6 leading to cell cycle arrest.[168]

Expression of $p57^{KIP2}$ is high during embryogenesis, typically peaking at key stages of differentiation for each organ, after which it declines. In many developing tissues, particularly strong protein expression can be observed at the interface between mesenchymal and epithelial layers, perhaps corresponding to the sites where tissue-specific stem cells are suspected to reside in stem cell niches. These observations suggest a function of $p57^{KIP2}$ in stem cell determination. Less speculatively, the protein is involved in regulating cell cycle exit during differentiation of many tissues.[168,169] In this function, $p57^{KIP2}$ cooperates with RB family proteins to inactivate E2F proteins and induce G_1 arrest and eventually a G_0 state associated with terminal differentiation. Chromatin immunoprecipitation revealed $p57^{KIP2}$ to be associated with E2F1-regulated promoters of genes crucial for DNA synthesis, where the protein inhibits RNA polymerase CTD phosphorylation and activity.[170]

Due to its growth-regulatory functions, CDKN1C has been studied extensively in various human cancers and is generally considered a tumour suppressor.[171] Somewhat surprisingly, data on its role in prostate cancer are relatively limited. In cultured primary human normal prostate epithelial cells, $p57^{KIP2}$ has been implicated in the acquisition of a senescent phenotype. Loss of the protein may be required for immortalisation of prostate cells.[172] Accordingly, *CDKN1C* was shown to be a target of cancer-specific CpG hypermethylation in 56% of primary prostate cancer tissue samples. Promoter hypermethylation was also found in prostate cancer cell lines, where treatment with the DNA methylation inhibitor 5-aza-deoxycytidine and the histone deacetylase inhibitor trichostatin A significantly upregulated *CDKN1C* mRNA levels.[173] In contrast to other cancers, such as urothelial carcinoma,[174] allelic losses of *CDKN1C* at 11p15.5 are rare in prostate cancer. Very likely, $p57^{KIP2}$ provides an important barrier to tumourigenesis in the prostate.

Aberrant methylation and histone modifications seem to dominate as mechanisms of *CDKN1C* inactivation in many malignancies. In breast cancer, EZH2 directly suppresses *CDKN1C* through H3K27 trimethylation.[175] Likewise, restoration of the chromatin remodelling factor SMARCB1, which is frequently inactivated in cancer, led to *CDKN1C* upregulation *via* promoter histone H3 and H4 acetylation. Factors like SMARCB1 are crucial for pRB-mediated repression of E2F factors and their target genes.[176] Another transcription factor regulating *CDKN1C* gene expression is ZAC1 (see below). Together, such findings suggest that multiple regulatory mechanisms converge at the *CDKN1C* gene to ensure adequate levels of $p57^{KIP2}$ and that these may be disrupted by multiple mechanisms in different cancers.

In paediatric cancers associated with BWS, *CDKN1C* suppression involves loss of methylation at the differentially methylated ICR of the 11p15.5 imprinted gene cluster.[65] This region contains the promoter of the ncRNA *KCNQ1OT1* (formerly named *LIT-1*) which is methylated on the maternal allele. The ncRNA expressed from the paternal allele antagonizes the expression of *CDKN1C* on the same allele (i.e., *in cis*) by recruiting Polycomb complexes and histone modifying enzymes to the *CDKN1C* promoter and silencing its transcription. Upon LOI with loss of maternal methylation of the ICR, *KCNQ1OT1* RNA is biallelically expressed, leading to silencing of *CDKN1C* on both chromosomes. In the paediatric tumours, a concomitant overactivation of the more distant *IGF2* locus is also often observed. At least partial hypomethylation of the *KCNQ1OT1* DMR has also been observed in cancers of adults including hepatocellular carcinoma and bladder cancer.[174,177] It is not known yet whether it also occurs in prostate cancer.

MEG3/GTL2, DLK1 AND THE 14Q32.2 IMPRINTED GENE CLUSTER

The imprinted gene cluster on human chromosome 14q32.2 comprises protein coding genes like *DLK1, RTL1* and *DIO3*, non-coding RNA genes (*MEG3*/*GTL2*, *RIAN* and *anti-RTL1*) as well as genes for micro- and small nucleolar RNAs.[178] These genes have important roles in embryogenesis and post-natal behaviour. In particular, they have been implicated in regulation of stem-cell properties of embryonic stem cells as well as cancer stem cells,[179,180] especially the reciprocally imprinted gene pair consisting of the paternally expressed *DLK1* gene and the maternally expressed non-coding RNA gene *MEG3*.[181,182]

The epigenetic regulation of the 14q32.2 cluster is similar to that of the *IGF2*/*H19* cluster on chromosome 11p15.5, where the promoter of an ncRNA (*H19*) contains a DMR whose methylation status influences the activity of a protein-coding gene (*IGF2*).[183] In the *DLK1*/*MEG3* cluster, the expression of *MEG3* is dependent on the activity of the DMR in its promoter. In mice, the epigenetic patterning and transcriptional activity of the *Meg3*/*Gtl2* promoter DMR together with a neighbouring intergenic DMR has been demonstrated to regulate the expression of paternally (*Dlk1*, *Rtl1* and *Dio3*) and maternally expressed genes (*Mirg*, *Rian*, *anti-Rtl1*, *miR-127* and *miR-410*) alike.[184] Targeted deletion of the unmethylated maternally inherited *Meg3*/*Gtl2* DMR or the intergenic DMR resulted in loss of imprinting across the entire cluster.[184,185] Accordingly, hypermethylation of the *MEG3* DMR resulted in abnormal inactivation of *DLK1* in human renal cell carcinoma.[186] The *MEG3* ncRNA regulates expression of the cluster *in cis* probably in a similar way as the lnc-RNA *XIST* mediates X-chromosomal silencing, i.e., through recruitment of heterochromatin to RNA-coated regions. Furthermore, the *MEG3* gene hosts miRNAs that might also participate in silencing of transcripts from the imprinted locus.

Apart from its regulatory functions in the 14q32.2 gene cluster, *MEG3* RNA may control cell proliferation by suppressing *MDM2* and enhancing $p16^{INK4A}$ expression, thereby promoting activity of p53 and RB1 respectively.[187] The *MEG3*-encoded miRNAs likewise have an established role in tumourigenesis. Furthermore, *MEG3* functions bear on the Notch pathway.

Notch signalling is crucial for the development and homeostasis of many tissues including the neural system, skeletal muscle, pituitary, pancreas, gut and the prostate.[188,189] Typically, the Notch pathway regulates the size of the progenitor cell pool and its proliferative and differentiation capacity. Active Notch signalling results in translocation of the C-terminal part of the receptor protein to the nucleus to induce several target genes like *HES1* and *HEY1* capable of repressing lineage-specific genes and controlling choices between alternative differentiation pathways.

The best studied function of the paternally expressed DLK1 protein also concerns Notch signalling. As originally discovered in *Drosophila*, Dlk1 is a non-canonical Notch ligand capable of heterodimerizing with the Notch1 receptor proteins without eliciting their activation. Thereby, it limits Notch1 activation by canonical Jagged or delta ligands in a competitive manner.[190]

In the mature human prostate, *DLK1* was reported to be expressed in some cells of the basal epithelial layer and in rarer neuroendocrine cells. It has been speculated that these *DLK1*-positive basal cells may represent a common prostate epithelial cell which has the potential to give rise to neuroendocrine, basal and luminal epithelial cells.[191] NOTCH1 and its ligand JAGGED1 are predominantly expressed in the luminal cell layer, but since no NOTCH1 nuclear immunoreactivity can be observed, it is unclear whether Notch signalling is active. In cells at intermediate differentiation stages, DLK1 is lower and NOTCH1 expression rises.[191] This suggests that the balance between NOTCH1 and DLK1 in prostate cells may be associated with epithelial differentiation.

The Notch target gene *HEY1* is especially interesting in the context of prostate cancer, as it acts as an AR corepressor. Ceder *et al.*[191] therefore proposed a dual role of Notch signalling in the human prostate. On the one hand, it may allow early luminal commitment by inhibition of neuroendocrine differentiation, by downregulating *DLK1* and by upregulating *HES1*. On the other hand, Notch signalling may interfere with AR-regulated transcription through HEY1 to inhibit luminal differentiation.[192] The authors suggest that Notch activity becomes downregulated as cells terminally differentiate.

Notch signalling contributes to the development of cancers in several tissues,[193] but its role in prostate cancer is unclear. According to one study, prostate tumour foci exhibited fewer cleaved NOTCH1 as compared with surrounding benign tissue and lower HEY1.[194] In other studies, NOTCH1 and JAGGED1 were reported to be overexpressed in metastatic cancers.[195–197] It has therefore been suggested that not expression of NOTCH1 protein as such but its activation status may be essential for the onset and maintenance of prostate cancer.

The proposed downregulation of *HEY1* in prostate cancers[194] may relieve repression of the androgen receptor allowing increased cancer cell growth in the presence of androgen as well as under castration conditions.[198] In contrast, the other Notch-induced transcriptional repressor HES1 might favour cancer development through binding and repression of the *PTEN* promoter.[199] In this fashion Notch activity could enhance PI3K/Akt signalling. Therefore, the effects of active Notch signalling in prostate cancer, if it occurs, might be strongly context-dependent. In any case, the expression level and distribution of DLK1, which is expected to depend on *MEG3*, would be crucial.

NDN

Necdin is the product of the paternally expressed *NDN* gene from the melanoma antigen family that is lost in Prader–Willi syndrome, a hereditary imprinting disease characterized by mental retardation, hypogonadism and obesity.[200,201] Numerous studies suggest major functions of necdin in regulating cell cycle and differentiation. It contributes to myogenic and neuronal differentiation and survival, while inhibiting adipogenic programs. In order to promote myogenic differentiation, necdin acts as a transcriptional repressor silencing adipogenic differentiation genes like *PPARG* and *DLK1*.[202] Similar to the pRB1 protein, necdin interacts with cell cycle factors like E2F1 and E2F4, resulting in suppression of proliferation and terminal differentiation.[203,204] A particular intriguing facet of necdin repressor activity is its interaction with hnRNP-U in a specific nuclear compartment, where it might be involved in maintaining a higher order chromatin structure, suppressing the transcriptional activity of colocalized cell cycle regulators.[205] The pro-survival function of necdin is exerted at least in part through attenuation of p53. The protein interacts with SIRT1 and p53 to potentiate p53 deacetylation by SIRT1.[206] In summary, necdin, on the one hand, has a potential to function as a tumour suppressor by inhibiting the cell cycle, but on the other hand, it might promote cell survival during stress by its attenuating effect on p53.[207] The function of necdin in the prostate and in human cancers is poorly studied.

PEG3

Paternally expressed gene 3 (*PEG3*), also known as zinc finger imprinted domain 2 (*ZIM2*), is situated on chromosome 19q13.4 and is paternally expressed. A CpG island within the gene is methylated on the maternal chromosome and participates in its silencing *in cis*. This DMR contains tandem binding sites for the Polycomb-interacting protein YY1, which binds to the unmethylated paternal allele. This constellation suggests an important role of DNA methylation and Polycomb-dependent histone modifications in the regulation of *PEG3* imprinting and expression.[208] In glioma, ovarian and other gynaecological cancers, *PEG3* was found to be silenced by DNA hypermethylation of the DMR, which could be relieved by demethylating drugs, especially in combination with a histone deacetylase inhibitor.[209–213] Interestingly, the methylation of the intragenic *PEG3* DMR correlated also with the expression of the neighbouring *ITUP1* gene oriented head-to-head to *PEG3*.[214]

The *PEG3* gene product is a Krüppel-type zinc finger protein. High levels of PEG3 are detected during embryo development and in the placenta. In the adult, it remains mainly expressed in the ovary, testis, muscle and brain, characterizing in particular adult stem and progenitor cell populations.[215] Like other imprinted genes, *PEG3* affects metabolic set-up. It regulates hypothalamic circuits that program energy homeostasis, and thereby growth and development of the entire body, evident from the phenotype of knockout mice.[216] Various studies in mice and men show its role in neuronal development, parental and sexual behaviour, as well as in muscle development and homeostasis.

Although growth promoting in the embryo, in the adult *PEG3* is suspected to have a tumour suppressor function that may be exerted by interactions with several pathways regulating cell proliferation and apoptosis. In cortical neurons *Peg3* was upregulated after DNA damage in a p53-dependent manner, mediating between p53 and Bax in the induction of cell death.[217] Overexpression of PEG3 and SIAH1 induced apoptosis independently of p53.[218] PEG3 also promoted TNF/TNFR-dependent apoptosis by interaction with TRAF2.[219]

PEG3 moreover limits the activity of the canonical WNT pathway, in particular by decreasing β-catenin levels. Inhibition of PEG3 expression led to enhanced β-catenin expression, inhibited p53-dependent apoptosis and promoted proliferation in human glioma stem cells.[220]

This function of PEG3 might be particularly pertinent to prostate cancer, as deregulation of the WNT/β-catenin pathway is thought to contribute to prostate cancer progression, prominently by influencing AR signalling.[221–226]

Through its involvement in major important pro-apoptotic pathways and its participation in the control and fine tuning of WNT signalling which impacts on androgen responses, PEG3 may serve to maintain the homeostasis in the normal prostate. Therefore, the strong tendency towards downregulated *PEG3* expression in prostate cancer indicated by our *in silico* analysis might indicate a major function in suppressing cancer development. However, so far no direct evidence for its functional significance in prostate cancer has been reported. Moreover, compared to the tissues with the highest levels in the ovary, placenta, adrenal and pituitary glands PEG3 levels in human prostate tissues are relatively low. An early study reported downregulation of *PEG3* mRNA by androgen treatment in LNCaP cells and expression could be detected neither in the androgen-responsive MDA-PCA-2 prostate cancer cell lines nor the poorly differentiated androgen-independent cell lines PC-3 and DU145. While the authors concluded that both androgens and loss of differentiation may affect *PEG3* expression in the prostate,[227] this remains to be ascertained in tissue samples and any consequences need to be elucidated further.

GNAS

The extremely complex G*NAS* locus is situated at chromosome 20q13.11 and codes for a variety of alternative transcripts with parental-of-origin-dependent expression. Best characterized are the protein-coding *GNAS* ($G_{s\alpha}$) and its variants *GNASXL* ($XL\text{-}G_{s\alpha}$) and *NESP55*, and the non-coding *NESP55as*, and *Exon 1A*. Although most of the transcripts share exons 2-13 of the $G_{s\alpha}$ transcript, their function, especially of the non-coding transcripts, may be to antagonize $G_{s\alpha}$ ($XL_{s\alpha}$) and to ensure its tissue-specific imprinted expression.[228–230] Three DMRs control the monoallelic expression of the transcripts in the *GNAS* locus. Differential histone modifications, too, are involved in regulating its imprinting.[231] In addition, remote cis-acting elements have been suggested to impact on imprinted expression. For a detailed review of *GNAS* locus regulation refer to ref.[232].

$G_{s\alpha}$ is a guanine nucleotide binding heterotrimeric G-protein stimulatory subunit that activates adenylate cyclase in response to ligand binding to G-protein coupled receptors. It functions in signal transduction from hormone and growth factor receptors to induce cyclic AMP, protein kinase A activity and activation of cAMP-responsive genes through the transcription factor CREBBP. This signalling directs metabolic processes like glycogenolysis and lipolysis, but also secretion or cell survival. In particular, $G_{s\alpha}$/cAMP signalling is involved in the stimulation of many endocrine glands by trophic hormones.[230,233,234] Very likely, the complex regulation of the *GNAS* gene reflects the requirement to achieve a precise setting of energy metabolism.

This conclusion is underlined by the highly tissue-specific imprinting pattern of *GNAS*. Its expression is biallelic in most tissues, but silenced on the paternal allele in specific hormone-secretory or hormone-responsive tissues like the kidney, anterior pituitary, thyroid and ovary. In these tissues, cAMP acts as a growth factor for endocrine cells, and $G_{s\alpha}$ overactivation is often oncogenic.[235] Accordingly, activating mutations of *GNAS*, which alter key residues required for its GTPase activity, are found in kidney, thyroid, pituitary, adenocortical and colorectal tumours, bone fibrous dysplasia and in patients with McCune-Albright syndrome.[236] In these diseases, the activating mutations cause overactivity of $G_{s\alpha}$, $XL_{s\alpha}$ and adenylate cyclase, resulting in autonomous synthesis of cAMP and constitutive activation of PKA and its downstream pathways.[235] *GNAS* gene amplification and overexpression have been found and may be relevant in ovarian and colorectal cancers.[237,238]

Relaxed imprinting of *GNAS* locus and biallelic $G_{s\alpha}$ expression may constitute further mechanisms for oncogenic activation in secretory organs like the pituitary, thyroid and ovary, as observed, e.g., in pituitary tumours.[239] Mutations in exon 1 inactivate $G_{s\alpha}$ but do not affect $XL_{s\alpha}$ and *NESP55* and may cause haploinsufficiency in tissues that express $G_{s\alpha}$ biallelically, and complete insufficiency in tissues with monoallelic expression. Maternally inherited mutations of this kind cause resistance to PTH, TSH and gonadotropins in their target organs (kidney, thyroid and ovaries) in addition to the bone growth defects of Albright hereditary osteodystrophy patients. Paternal transmission of the mutations, in contrast, does not cause hormonal resistance.[233]

Polymorphisms constitute a further factor to be considered in GNAS physiological and oncogenic functions. Homozygosity for the *GNAS* T393C polymorphism, which correlated with increased $G_{s\alpha}$ levels, was linked to a more favourable clinical course in bladder and colorectal cancer, as compared to patients with TC or CC genotypes.[240,241] Similarly, in clear cell renal cell carcinoma and gastric cancer,[242] homozygous CC patients were found to be at highest risk for progression.[242,243] In prostate cancer, no effect of this polymorphism could be found.[244]

In view of the involvement of *GNAS* in endocrine regulation and many human cancers, it is surprising that the gene has hardly been studied yet in prostate cancer. The results of our bioinformatic analysis, with 11 studies reporting significant upregulation, clearly call for a study of GNAS expression, mutation and function in prostate cancer, even though this would have to detailed, given the complex regulation of the locus.

DCN

The product of the *DCN* gene, decorin (also known as PG-40), is a proteoglycan secreted by mesenchymal cells into the extracellular matrix, where it regulates growth factor signalling and receptor tyrosine kinase activity. In this fashion, it influences signalling by IGFs, EGF-like growth factors and TGF-β.

Altered expression of decorin has been linked to ageing and carcinogenesis in various organs. *DCN* was reported to be hypomethyated and overexpressed in the stroma of human colon carcinoma.[245] In the Tiensin Albino 2 mouse, which develops spontaneous breast cancer, *Dcn* along with *Igf2*, *Mest*, *Ndn* and *Peg3* is differentially expressed between normal mammary gland and cancer tissues. The decrease in decorin is supposed to contribute to carcinogenesis in the mammary gland *via* increased EGFR signalling.[246] In human breast tissues, decorin accumulation was observed in areas of increased mammographic density and malignant-appearing microcalcifications, and was associated with in situ carcinomas.[247] Decorin together with other small leucine-rich proteoglycans was proposed to be involved in the regulation of inflammatory and fibrotic renal disorders.[248] Taken together, these studies suggest a major function of decorin in the remodelling and regeneration of inflamed and ageing tissues that might be highly pertinent to benign and malignant tumours in the prostate.

Indeed, decorin blocked AR nuclear translocation and decreased prostate-specific antigen levels in the androgen-dependent LNCaP cell line. Importantly, decorin inhibited the growth of both androgen-dependent and androgen–independent prostate tumour cells by suppressing signalling by EGFR, PI3K-Akt and the androgen receptor.[249] In mouse prostates, decorin inhibited PI3K and Akt phosphorylation even after *PTEN* knockout. Downregulation of decorin has been

reported during the transition from high-grade prostatic intraepithelial neoplasia to invasive carcinoma.[250] Together, these properties suggest an ability of decorin to function as a tumour suppressor in the human prostate by influencing the crucial interacting PI3K/Akt and androgen response pathways.

CONCLUSIONS

In this final section, we will summarize the implications of our data mining and literature survey and draw some tentative conclusions as working hypotheses for further investigations.

The first conclusion from our analysis is evident. Despite the extensive epigenetic changes associated with the initiation and progression of prostate cancer, most imprinted genes remain stably regulated. In particular, there is surprisingly little evidence for LOI, apart from the *IGF2*/*H19* locus. Notably, the changes altering the imprinted state at this locus are most likely early events in prostate carcinogenesis that precede deregulation of global DNA methylation patterns and histone modifications. In particular, IGF2 deregulation appears to be involved in the field effect evident in cancerous prostates that manifests in changes in gene expression and DNA methylation.[251] There is so far little evidence that changes in the actual imprinting mechanism are responsible for altered expression of other imprinted genes in prostate cancer. At the 7q21 cluster and to a lesser extent at the 6q *PLAGL1*/*HYMAI* locus, chromosomal gains and losses may contribute to deregulation. At *TFPI2* and *CDKN1C*, promoter hypermethylation has been observed,[151,173] but the state of the corresponding ICR is rather unchanged (in the former case) or has not been investigated yet (in the latter case).

The second conclusion is that the changes in the expression of imprinted genes in prostate are strongly selected for by their function. A large fraction of those that are deregulated have functions related to PI3K/Akt signalling and androgen response, which interact in the development and progression of prostate cancer to CRPC. Additionally, there is preliminary evidence that other pathways, including WNT and Notch signalling, could be influenced by altered expression of individual imprinted genes (**Figure 3**). This relationship is not incidental, since the regulation of foetal growth and the establishment of set points for endocrine regulation are key functions of imprinted genes during development. In that context, they influence some of the same pathways that are relevant for prostate carcinogenesis and development. Regulation of the involved imprinted genes may be as critical in prostate carcinogenesis.

Thirdly, the imprinted genes controlling foetal growth in this fashion are linked with each other in a tight network (**Figure 1**). Thus, they do not only exert common functions, but are also subject to common regulatory mechanisms. In particular, some of the gene products of this network serve as hubs by regulating many other genes and the overall activity of the network. ZAC1 and MEG3 are prime candidates for such a hub function and *H19* in some instances may play a similar role. This means that deregulation of hub genes in prostate cancer may spread through the network (**Figure 1**) and lead to coordinated changes as during embryonic development. Notably, such coordinated changes can also occur during regenerative processes in adult organs.[155,252,253]

In embryonic development and during tissue regeneration, the function of the network resides in controlling the extent of cell growth and proliferation as well as the expansion of progenitor cell compartments. In brief, the network is first activated to aid in the growth phase of tissues and ensures its transition to a stable and final size, whereupon the network genes are downregulated. In analogy, one might speculate that during prostate cancer development, activation of the network may occur at an early stage of carcinogenesis, but would be

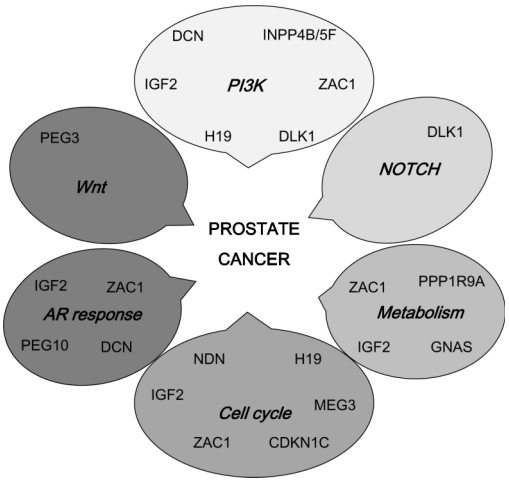

Figure 3. An overview of signalling pathways and cellular regulatory systems influenced by and acting upon imprinted genes with altered expression in prostate cancer.

selected against once it begins to limit the tumour progression. This argument is supported by the observation that *CDKN1C*, which encodes one of the strongest antiproliferative proteins in the network, is most consistently downregulated in prostate cancer.

Of note, apart from coordinated regulation in the imprinted gene network, overexpression or downregulation of imprinted genes may occur in normal and neoplastic tissues by mechanisms unrelated to imprinting, e.g., by tissue-specific transcription factors or as a consequence of responses to hypoxia, stress or infection. To give a few examples, expression of *CDKN1C* is highly tissue-specific, although the factors involved are not well characterized.[168] Overexpression of *IGF2* in some cancers is associated with a switch to a different promoter.[254] *H19* is upregulated by hypoxic stress dependent on the p53 status.[117] *PLAGL1* is induced upon TLR3 activation during virus infection.[255] Certain chemical mutagens change the expression level of the imprinted genes *SNRPN*, *PEG3*, *NDN* and *ZAC1*.[256]

Finally, several imprinted genes subject to altered expression in prostate cancer have been postulated to regulate stem cell and precursor population. Obviously, since expression analyses by microarrays or quantitative PCR measure mRNA levels across tissue samples, neither our *in silico* bio-informatic analysis nor the published data from the literature can reveal the expression status of such imprinted genes in subpopulations of epithelial cells in the normal prostate or in prostate cancer. Clearly, their expression and function needs to be considered in the context of cellular hierarchies. Unfortunately, the issue of cancer stem cells in normal and cancerous prostate is highly controversial (see Ref. 257 for a balanced account). However, a few points might be considered.

In the adult, imprinted genes are expressed at low levels and are thought to be mostly restricted to progenitor cell populations. Upregulation of imprinted genes with growth-promoting functions during organ regeneration might therefore be accounted for by the expansion of progenitor cells.[258] Furthermore, a similar overexpression of imprinted genes is reported during immortalisation of cells and to contribute to their clonal expansion.[259] Deregulation of imprinted genes in progenitor cells could therefore affect not only their number and their response to the microenvironment, but also their ability to give rise to correctly differentiated progeny. Such considerations underlie the theory of the epigenetically disrupted progenitor cells

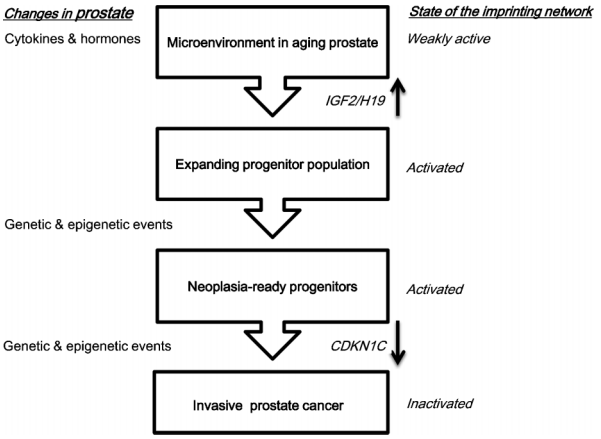

Figure 4. A hypothesis on the role of imprinted genes in prostate cancer initiation and progression. IGF2, insulin-like growth factor 2.

from which tumours may arise.[258] According to this theory, epigenetic events perturb the regulation of precursor cells, creating a population of 'neoplasia-ready cells' from which cancer originates. These cells would be present as well in morphologically normal tissue and explain the broader distribution of epigenetic changes in many neoplastic organs.[258] In prostate carcinogenesis, changes at the *IGF2/H19* locus may reflect this situation. Interestingly, it has been postulated that microenvironmental influences like cytokines and growth factors secreted during infection or altered hormone homeostasis provide the milieu that causes LOI in tissue-resident stem cells.[260] Accordingly, increased levels of IGF2 have been postulated to cause cancer by increasing the progenitor cell population in tumours.[261]

In summary, thus, we may propose the following model to account for the specificity of changes in imprinted gene expression in prostate cancer (**Figure 4**). Changes in *IGF2* imprinting might be caused by micro-environmental changes in the ageing prostate and consequently contribute to the emergence of a tumourigenic precursor population. Genetic changes such as *ERG* translocations and certain chromosomal losses and gains may contribute to the establishment of that population[8] or may follow. Certainly, they are required for the emergence of invasive cancers. These genetic changes along with epigenetic alterations elicit changes in the growth factor/PI3K/androgen response axis that are crucial for tumour growth and progression. In response to the expansion of the precursor cell pool or the activation of growth-stimulating signals, the imprinted gene network controlling tissue growth becomes activated and becomes subject to selection against its effects that limit proliferation and drive differentiation. This selection appears to result in the specific inactivation of individual genes that act as effectors or hubs within the network, eliciting broader changes in expression.

COMPETING FINANCIAL INTERESTS

The authors declare no competing commercial interests in relation to the submitted work.

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