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DNA methylation as a dynamic regulator of development and disease processes: spotlight on the prostate

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Abstract

Prostate development, benign hyperplasia and cancer involve androgen and growth factor signaling as well as stromal–epithelial interactions. We review how DNA methylation influences these and related processes in other organ systems such as how proliferation is restricted to specific cell populations during defined temporal windows, how androgens elicit their actions and how cells establish, maintain and remodel DNA methylation in a time and cell specific fashion. We also discuss mechanisms by which hormones and endocrine disrupting chemicals reprogram DNA methylation in the prostate and elsewhere and examine evidence for a reawakening of developmental epigenetic pathways as drivers of prostate cancer and benign prostate hyperplasia.

Keywords

benign prostate hyperplasia; DNA methylation; epigenetics; lower urinary tract; prostate; prostate cancer

Overview of DNA methylation

Epigenetics is the study of chemical modifications to DNA and histone proteins that regulate gene expression without altering DNA sequence. A growing number of epigenetic marks have been identified including DNA methylation, histone tail modifications, non-coding RNAs and others. Here we focus on the epigenetic mark of DNA methylation which occurs by addition of a methyl group to the 5′ position of cytosine. DNA methylation most often occurs in the context of CpG dinucleotides, though non-CpG DNA methylation can occur [1,2]. DNA methylation is classically perceived as a stable and sometimes heritable mark. However, DNA methylation events can also be surprisingly dynamic and added or removed in a spatially and temporally defined context.

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DNA methylation is catalyzed by DNA methyltransferases (*Dnmts*), which include maintenance (*Dnmt1*) and *de novo* methyltransferases (*Dnmt3a*, *Dnmt3b*). An additional member, *Dnmt3L*, lacks catalytic activity but participates in *de novo* methylation by interacting with *Dnmt3a* and *Dnmt3b* and other transcription factors [3–5] and uses the histone landscape to recruit *de novo* DNA methyltransferases [3]. DNA methylation can also be lost, either through passive mechanisms whereby the methylation pattern is not maintained upon subsequent cell divisions, or through active mechanisms involving base modification, substitution, excision or repair (reviewed in [6]).

DNA methylation typically regulates gene expression by repressing transcription but in some cases DNA methylation can be transcriptionally activating. For example, it has recently been demonstrated that non-neuronal-derived serotonin increases *Shh* mRNA abundance in mouse mammary gland by increasing DNA methylation at one site of the *Shh* locus and decreasing DNA methylation at another [7]. DNA methylation also acts in concert with methyl-CpG binding proteins (MBDs) and chromatin modifiers to change the chromatin landscape. These events can be influenced by hormones, environment, drugs and other chemicals, leading to derangement of DNA methylation marks which can perturb development or trigger inappropriate growth later in life, potentially contributing to a host of diseases, including cancer.

Here we focus on prostate and describe developmental processes that implicate DNA methylation as a critical gene expression regulatory mechanism and describe how aberration of DNA methylation events influences prostate disease processes. We highlight evidence in other systems which may help bridge the knowledge gap in understanding how prostate cells establish, maintain and remodel DNA methylation in a time and cell specific fashion during prostate development and the onset and progression of prostate disease.

Overview of prostate development

The prostate arises from a subcompartment of the lower urinary tract known as the urogenital sinus (UGS). Prostate formation is dependent upon androgen action as well as reciprocal stromal–epithelial interactions. Androgen signaling via androgen receptor (AR) in UGS mesenchyme instructs and initiates prostate ductal precursors, called prostate buds, to form from UGS epithelium. In mouse, testicular androgen synthesis occurs around 13 days post coitus (dpc) and epithelial prostate buds emerge from UGS epithelium about three days later, creating a lag between the onset of androgens and bud formation [8]. After prostate buds initiate they elongate into UGS mesenchyme and undergo branching morphogenesis, which continues postnatally (Figure 1) to give rise to the adult prostate ductal network.

AR signaling in prostate mesenchyme is necessary for prostate epithelial morphogenesis, suggesting androgen-induced paracrine signaling factors guide prostate development. These factors have been termed andromedins. Several andromedins have been proposed but to date no single gene has been identified as the andromedin responsible for prostate development. Multiple gene families participate in prostate development, including *Fgf*, *Tgf*β, *Bmps*, *Shh* and others (reviewed in [9]). KGF/FGF7 and FGF10 were the first identified candidate andromedins [9]. *Wif1* was also identified as a candidate andromedin [10]. *Wif1* expression

is regulated by androgens and acts to promote androgen dependent bud formation, but is unable to stimulate prostate bud formation in the absence of androgens – a proposed characteristic of a true andromedin [10].

Therefore, additional mechanisms likely drive prostate morphogenesis. One such mechanism may involve DNA methylation. Recently, DNA methylation has been shown to play a critical role in regulating expression of key genes involved in prostate morphogenesis, including the *Ar*. Existing evidence for DNA methylation roles in prostate development is described below.

Role of DNA methylation in prostate development

In situ hybridization has been used to map mRNA expression patterns for DNA methylation modifying genes in developing mouse prostate at 14.5 dpc - P5 [11, 12]. *Dnmts* are expressed throughout prostate development, but change in abundance and spatial distribution over the course of prenatal and postnatal prostate morphogenesis [11]. *Dnmts* predominate in prostate mesenchyme prior to prostate bud outgrowth (14 dpc) and localize to prostate bud epithelium during elongation (17 dpc) and branching morphogenesis (P5) [11]. The spatially defined *Dnmt* expression patterns in developing prostate guided the search for gene targets within UGS epithelium and mesenchyme that are regulated by DNA methylation to drive morphogenesis. These studies have highlighted specific roles of DNA methylation in regulating *Cdh1* and *Ar* gene expression during discrete windows of prostate development.

Regulation of E-cadherin expression in developing prostate

Dnmt localization to elongating prostatic buds at 17 dpc, a time when prostatic buds are elongating and are actively invading the adjacent stroma, raises the hypothesis that DNA methylation may control cell adhesion and prostatic bud outgrowth. Targeted studies at this developmental stage reveal a role for DNA methylation in regulating *Cdh1* mRNA expression [13]. *Cdh1* mRNA and protein are abundant in prostatic epithelium prior to bud formation (14 dpc), then decrease in basal epithelium of developing prostatic buds (17 dpc) [13]. DNA methylation of *Cdh1* increases during this time. Inhibition of DNA methylation *in vitro* increases CDH1 abundance in prostate buds and causes prostatic buds to be shorter, a phenotype reversed by disrupting homotypic interactions between CDH1 domains on adjacent epithelial cells [13]. Thus, DNA methylation contributes to prostate bud outgrowth at least in part by downregulating *Cdh1*, which presumably modifies cell adhesion and permits epithelial rearrangements needed for prostate ductal growth.

Regulation of androgen receptor expression in developing prostate

There is also evidence that DNA methylation is used to regulate androgen action at an early stage of prostate development. Prior to prostate bud outgrowth, during the period of prostate bud specification (14 dpc), *Dnmts* are present in developing prostate mesenchyme (Figure 1) [11]. Prostate mesenchyme is necessary for prostate development and is the site of androgen action [14,15]. This suggests that genes involved in AR signaling may be regulated by DNA methylation. It has recently been shown that *Ar* DNA methylation is lower in mesenchyme

at 17 dpc compared with 14 dpc [16]. Further, inhibiting DNA methylation during prostate specification *in vitro*, when *Dnmts* predominate in UGS mesenchyme, uniquely enhances prostate bud formation [16]. Inhibiting DNA methylation decreases *Ar* DNA methylation as assessed by methylated DNA immunoprecipitation (MeDIP) and pyrosequencing of bisulfite converted DNA, increases AR expression, UGS androgen sensitivity and prostate bud formation rate [16]. A proposed model of two DNA methylation roles in guiding prostate morphogenesis is shown in Figure 2. DNA methylation of the *Ar* during early prostate development constrains prostate bud formation and prevents precocious growth. Later in development, when *Dnmts* predominate in epithelium, DNA methylation of *Cdh1* facilitates prostate epithelial differentiation and outgrowth (Figure 2). There are still many unanswered questions in how androgens elicit their actions on prostate development and in how cells establish, maintain and remodel DNA methylation in a developmental time- and cell-specific fashion. For example, how is prostate DNA methylation established and removed during specific developmental stages? Are there other target genes regulated by DNA methylation during prostate organogenesis? Do androgens modulate DNA methylation? Can alterations in DNA methylation influence proliferative growth processes? In order to answer these questions in prostate, it is useful to examine known DNA methylation roles in other developmental processes. The following sections specifically focus on how DNA methylation is established, maintained and remodeled during embryogenesis. We also describe known roles of DNA methylation in development of nonprostate organs, how hormones influence DNA methylation and how endocrine disrupting chemicals and disease processes can alter DNA methylation and impact health.

Mechanisms of establishing & erasing DNA methylation marks in embryogenesis that may be conserved in prostate development

Understanding how the DNA methylome is rapidly and extensively remodeled during embryonic development may inform future studies on whether aspects of this process occur during prostate development. A rapid wave of paternal genome demethylation occurs shortly after fertilization in preimplantation embryos. DNA methylation patterns are then re-established largely through *de novo* methylation to permit embryonic specification in the blastocyst [17,18]. Rapid genomic reprogramming also occurs in primordial germ cells (PGCs) prior to and once reaching the embryonic gonad around 10–13 dpc in mouse [19–21]. Unlike the wave of DNA demethylation which occurs in male pronucleus during fertilization, PGC loss of DNA methylation occurs in both male and female PGCs mainly by inactivation of maintenance DNA methyltransferase activity [20,22] and downregulation of *Dnmt3a*, *Dnmt3b* and *Dnmt3l* [23]. Once reprogrammed, sexspecific DNA methylation marks are established in a time- and stage-specific fashion [6,20,22,24–25].

The identification of active mechanisms for removing DNA methylation marks filled a longstanding gap in understanding epigenetic programming. It was first thought that passive loss of DNA methylation or presence of a demethylase enzyme capable of removing methyl groups was the only mechanism to mediate demethylation. While some evidence points to *Mbd2* as having independent demethylase activity [26] this is still controversial. In contrast, DNA demethylation has been shown to occur in a stepwise fashion, involving first

modification of methylated cytosines and then replacement by DNA repair pathways. DNA hydroxymethylation is a cytosine modification catalyzed by ten eleven translocation gene family members, which leads to base excision repair and demethylation [27–29]. Deoxycytidine deamination occurs via the activation-induced cytidine deaminase (*Aicda* or *Aid*) and *Apobec1*, *2* and *3* family members [30,31]. Deoxycytidine deamination generates DNA mismatches which trigger DNA base excision repair, activation of mismatch repair pathways [32–34] or activation of DNA glycosylases which remove damaged bases or T:G and U:G mismatches [35]. Active mechanisms of DNA demethylation provide for potentially rapid and context-specific addition and removal of DNA methylation marks during specific morphogenetic events.

Like the mRNAs of DNMT enzymes responsible for establishing DNA methylation, the mRNAs for *Tets*, *Aid*, *Apobecs*, DNA glycosylases and other DNA repair enzymes, capable of removing DNA methylation, are expressed in male mouse UGS during prostate development [11]. Many of the mRNAs encoding these enzymes are abundant in UGS mesenchyme prior to prostate bud formation before diminishing therein and localizing instead to prostate bud epithelium [11]. A temporal change in localization and expression of the chromatin modifier, *Ezh2* also occurs in developing prostate [36], suggesting that machinery for modifying histone structure, like DNA methylation machinery, is dynamically regulated during prostate development. These results raise the possibility that transient changes in DNA methylation and demethylation may contribute to morphogenetic changes associated with AR activation and onset of prostate development.

Roles of DNA methylation in organogenesis – conserved mechanisms & pathways important for prostate development

Developmental mechanisms, genes and pathways affected by DNA methylation in other developing organs inform future studies in developing prostate. How DNA methylation influences organogenesis is not as well understood as its developmental role in preimplantation embryos. This is largely because global DNA methylation is necessary for embryonic survival. Global inactivating mutations in mouse *Dnmt1*, *Dnmt3b* or *Dnmt3a +Dnmt3b* cause mid-gestational mortality (0.5–11.5 dpc) [37,38], the ultimate cause is still unknown. Early embryonic mortality and growth retardation of *Dnmt* mutant mice limits their utility in testing DNMT requirements in prostate development, which occurs late in gestation and relies on several prerequisites including successful cloacal septation, pathfinding of mesonephric and nephric ducts, cell differentiation and testicular testosterone synthesis [39].

The growing number of genetically modified mice that express cell type specific *cre* drivers facilitates an organ- and cell-type specific approach toward understanding the role of DNA methylation in development. Using currently available mouse strains, several studies have revealed context-dependent roles for DNA methylation. A role for *Dnmt1* and DNA methylation has been established in progenitor cell populations of developing pancreas, hematopoetic system and neurons, where conditional *Dnmt1* deletion impairs cell survival and differentiation, progenitor cell self renewal and lineage appropriate gene expression [40–42]. DNA methylation is not only important for progenitor cell survival and

differentiation, but also morphogenesis. Selective *Dnmt1* deletion in developing excitatory neurons and astroglia of cortex and hippocampus causes cortical degeneration and persistent learning and memory deficits [43]. Interestingly, a fraction of hypomethylated neurons survive postnatally but exhibit increased dendritic branching and impaired excitability [43]. Developmental mechanisms responsible for these phenotypes include changes to genes responsible for cell layer-specification, cell death and ion transport [43]. *Dnmt1* deletion in retinal pigment epithelium or neural retina disrupts retinal pigment epithelia morphology and polarization, photoreceptor development and neural retinal differentiation [44]. These events are accompanied by disrupted actin cytoskeleton morphology, decreased retinal adhesion and DNA methylation changes in Hedgehog, Notch and Wnt pathway genes [44].

DNA methylation also mediates key events in mouse hair follicle development and regeneration. Epidermal *Dnmt1* deletion causes uneven epidermal and hair fiber thickness, altered follicle size and an aging-related decline in hair fiber quantity [45]. Focal cell proliferation defects partially account for uneven epidermal thickness [45]. Molecular mechanisms responsible for changes in hair follicle size are unknown largely in part because few molecules are known to influence hair size. Proposed targets include *Bmp* and *Wnt* pathway members which can influence hair diameter and thickness [46,47]. Together these studies indicate that DNA methylation is not only important for cell survival, but can also influence structural morphogenesis and function.

Developing prostate relies upon several developmental mechanisms regulated by DNA methylation in retina, skin and hair follicle. Cytoskeleton rearrangement, cell adhesion and epithelial polarity are all essential for prostate epithelial development and differentiation [48]. Signaling pathways including Hedgehog, Notch, Bmp and Wnt have all been linked to prostate epithelial budding, branching and differentiation [49–51]. Whether DNA methylation regulates these pathways in prostate, as it does in retina and hair follicles, is an area of future study.

Interactions between DNA methylation & hormones

DNA methylation regulates estrogen receptor and AR expression in adult prostate and other tissues [52,53]. Estrogen receptor and AR signaling both influence prostate development (reviewed in [54]), but it is not yet known if and how DNA methylation acts in concert with these hormones during prostate morphogenesis.

There is growing evidence that hormone action can alter *Dnmt* expression, activity and DNA methylation levels. For example, changes in estrogen and progesterone levels during the female menstrual cycle alter endometrial *Dnmt* expression [55–57] influencing DNA methylation patterns and mRNA abundance of endometrial genes involved in transcription, apoptosis, proliferation, extracellular matrix and blood vessel morphogenesis [58]. Neonatal testosterone exposure can masculinize DNA methylation at sexually dimorphic CpG sites in female mouse brain [59].

Evidence links hormone-induced DNA methylation patterns to specific anatomical and physiological endpoints. Estrogen and androgens are responsible for mouse gender dependent differences in DNA methylation and expression of cardiomyocyte gene, *Csx2* in

cardiac ventricle, leading to sexually dimorphic cardiac structure and function [60]. Further, DNA methylation, ventricle structure and function are reversed upon gonadectomy [60]. Together these results reveal that hormones can influence expression of DNA methylation regulators, DNA methylation patterns, expression of target genes and physiological responses elicited by target genes. Further investigation into these interactions will likely shed light on both developmental and disease processes driven by steroid hormones. These results will also inform future studies to determine whether hormones influence DNA methylation in developing prostate. DNA methylation could be a means by which androgens or andromedins (genes responsible for mediating the actions of androgens) alter gene expression to drive prostate development.

DNA methylation & the fetal basis of adult disease in prostate

The fact that the epigenomic landscape is established during embryonic development also makes it a period of susceptibility to perturbations by environmental and endocrine disrupting chemicals. Chemically induced epigenomic reprogramming during embryonic development can lead to changes in gene expression and altered histology and physiology later in life. This is one aspect of a fetal basis of adult disease (reviewed in [61]). Perhaps the best examples come from the field of toxicology, where for decades, researchers have been studying how *in utero* exposure to environmental and endocrine disrupting chemicals influence health in adulthood. Two estrogenic chemicals receiving considerable research focus are diethylstilbesterol (DES) and bisphenol A (BPA). Some mouse and rat strains exposed *in utero* to DES and BPA exhibit altered developmental morphogenesis, reprogrammed DNA methylation patterns and altered adult histology/physiology of the prostate.

The developing prostate is extremely sensitive to endocrine disrupting chemicals. CD-1 mice exposed *in utero* to estradiol, DES or BPA exhibit changes in the number of prostate buds formed and fetal UGS morphology [62]. Mice exposed during fetal and neonatal development to high doses of DES (200 μg/kg) generally develop fewer prostatic buds, while mice exposed to low doses of BPA (10 μg/kg) and DES (0.1 μg/kg) form more prostatic buds and exhibit increased UGS AR expression [62–64]. In Sprague–Dawley rats, *in utero* BPA exposure increases incidence of carcinogen-induced hyperplastic and precancerous lesions in adult prostate [65]. Molecular mechanisms driving abnormal prostate development and altered histology in adulthood are still under investigation. Evidence exists to support the hypothesis that BPA-induced changes in DNA methylation alter gene expression to disrupt homeostatic regulation of prostatic growth later in life. Male rats exposed *in utero* to BPA exhibit increases in prostatic *Dnmt3a* and *3b* expression at day 10 and 90 which diminishes by day 200 [66]. This *in utero* BPA exposure induces hypo- or hyper-methylation in a gene specific context in prostate. Some of these marks persist throughout life, while others are not observed until after sexual maturity or stimulation with pro-oncogenic factors in adulthood [66]. These studies provide evidence that early life exposure to endocrine disruptors, and potentially endogenous hormones, do not necessarily elicit immediately detectable changes in DNA methylation or gene expression. In some cases, these responses are not detectable until later in life-puberty or in response to a ‘second hit’ stimulus such as changes in hormone levels. Together the results of these studies have

important implications in assessing potential mechanisms of hormone action and health risks associated with endocrine disrupting chemical exposures in that timing of assessment is potentially as important as doses tested and endpoints measured.

Collectively, in prostate there are links between endocrine disruptor exposure and altered development, changes in DNA methylation and abnormal organ function and histology associated with disease later in life. These observations can help to inform future studies to understand how DES and BPA act to impair development of prostate and other organs. One potential mechanism is that *in utero* exposure to endocrine disrupting chemicals could influence prostate morphogenesis and homeostasis throughout life by altering DNA methylation during the embryonic period when DNA methylation guides prostate ductal development. This mechanism may extend to other environmental chemicals. For example, embryonic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) also alters the number of prostate buds formed and the anatomical morphology of the UGS in fetal mice [8]. TCDD has been shown to increase DNA methyltransferase enzyme activity and alter DNA methylation in preimplantation mouse blastocysts [67]. Whether TCDD induces changes in DNA methylation to alter prostate bud formation is yet to be determined.

Prostate cancer & benign prostate hyperplasia (BPH): a reawakening of developmental signals

A full discussion of epigenomics as it relates to prostate cancer and BPH is beyond the scope of this review, interested readers are directed toward several reviews on the topic [68–70]. There are numerous genes regulated by DNA methylation in prostate cancer [52,68–71]. In the following section we specifically focus on genes known to be regulated by DNA methylation during prostate development (*Ar* and *Cdh1*) which may be inappropriately reactivated in prostate cancer and BPH.

Prostate cancer

It has been proposed that a reawakening of developmental signals drives inappropriate growth in prostate cancer and BPH [72]. Since DNA methylation is critical for controlling expression of key prostate development genes (*Cdh1* and *Ar*), it is possible that aberrant DNA methylation patterns of these genes and others reawakens prostate growth mechanisms to drive onset and progression of BPH and prostate cancer. This notion is supported by multiple studies. Recently, a chromatin remodeling gene (*Hmga2*) was identified as being selectively activated during prostate development [73]. When *Hmga2* was over-expressed in adult prostate stromal cells, combined with prostate epithelium and grown as xenografts, the grafts formed high-grade prostate intraepithelial neoplasia [73]. Prostate intraepithelial neoplasia precursor lesions precede cancer formation in predisposed mice and in humans [74]. This study provides evidence that inappropriate expression of epigenetic modifiers in prostate stroma may be sufficient to alter epithelial proliferative activity. DNMT protein abundance and activity are inappropriately elevated in human prostate cancer tissue and cell lines versus nonmalignant controls [75,76]. DNMT protein expression also increases in prostate intraepithelial neoplasia and well-differentiated tumors in transgenic

adenocarcinoma of mouse prostate mice that are genetically predisposed to prostate cancer [77].

Androgen receptor

DNA methylation of *Ar* as assessed by methylated DNA immunoprecipitation and pyrosequencing of bisulfite converted DNA occurs during prostate development to control onset and rate of androgen dependent prostate bud formation, confining initiation of prostate development to a specific embryonic development window [16]. There is evidence that this developmental role of *Ar* DNA methylation could also be hijacked to drive prostate cancer. DNA methylation silences *AR* gene expression in primary human prostate cancer tissues [78,79] as well as advanced (androgen-insensitive) prostate cancer cell lines [52] as assessed by methylation specific PCR and bisulfite sequencing. Whether these changes are also observed with other highly quantitative approaches such as pyrosequencing of bisulfite converted DNA in human prostate cancer tissues is not clear. Chemical inhibition of *Ar* DNA methylation can restore *AR* expression and androgen responsiveness [80]. Administration of DNA methylation inhibitor 5′-aza-2′deoxycitidine to transgenic adenocarcinoma of mouse prostate mice impairs progression to androgen independent prostate cancer and when combined with castration increases survival [81]. These findings raise new hypotheses about the role of DNA methylation in progression of prostate cancer to an aggressive form that loses its growth dependence on androgens and can be lethal.

E-cadherin

DNA methylation of cell adhesion molecule, *Cdh1*, allows for prostate epithelial outgrowth during prostate development [13]. Epigenetic regulation of *Cdh1* also influences epithelial cell migration and behavior in prostate cancer. *CDH1* is inappropriately silenced by DNA methylation in prostate cancer [82]. Silencing of *CDH1* is associated with tumor invasion and metastasis and is predictive of poor patient outcome [83]. Treatment with DNA methylation inhibitor restores *Cdh1* expression in prostate cancer cell lines and decreases invasion potential [84]. Together these results suggest that DNA methylation is not only an important aspect of development but inappropriate DNA methylation activity is associated with prostate disease and progression.

Benign prostate hyperplasia

Benign growth of the prostate associates with lower urinary tract symptoms (LUTS) which include irritative symptoms like increased frequency, urgency, pain and nocturia as well as voiding or obstructive symptoms like weak stream, hesitancy, dribbling, incomplete emptying and overflow incontinence. LUTS decrease quality of life and represent a significant healthcare burden to the aging population [85]. Approximately 70% of men over the age of 70 experience BPH and/or LUTS [86]. Prostate volume positively associates with risk of developing bothersome LUTS, however men with small prostates can experience LUTS and men with large prostates can be relatively asymptomatic, making diagnosis and treatment complex [87]. While these diseases have a complex etiology, few studies have focused on the epigenome as a contributing factor.

It was first hypothesized by the pathologist John McNeal that the proliferative growth pattern in BPH nodules resembled developing prostate ducts undergoing branching morphogenesis [88]. He hypothesized that inappropriate reawakening of prostate development signaling pathways could contribute to BPH pathogenesis [89]. Evidence now exists to support the hypothesis that epigenetic changes co-occur with histological prostate hyperplasia. Global 5-methylcytosine levels are reduced [90] and localized DNA hypermethylation is observed in tumor suppressor genes [91] in benign hyperplasic prostate compared with histologically normal prostate tissue.

Inflammation is one of the major factors commonly observed in prostates from patients with BPH/LUTS. Men with chronic prostatic inflammation are 6.8 times more likely to have histological BPH than individuals without [92]. Men with higher prostate inflammation also report more bothersome LUTS [93]. The cause of inflammation is not well understood and since not all incidences are accompanied by histological or culturable presence of bacteria [94], multiple inflammatory mediators are likely involved. Evidence now exists that epigenetic abnormalities in the immune system may contribute to BPH associated with LUTS (symptomatic BPH). The innate antiviral immune response is activated in prostate tissue from patients with BPH and LUTS versus those with histologic BPH but few LUTS (asymptomatic) [95]. The cytosine deaminase, APOBEC3G, is upregulated in prostates from patients undergoing surgery for symptomatic BPH compared with patients with asymptomatic BPH or histologically normal prostate tissue [95]. Concurrently, demethylation and increased expression of LINE-1 retrotransposable elements occurs in BPH tissues from patients with symptomatic BPH compared with histologically normal tissues [95]. LINE-1 demethylation activates the immune response in autoimmune disorders [96]. Together these results raise the hypothesis that *Apobec* mediated demethylation of LINE-1 may occur in prostate to drive inflammation contributing to BPH and symptomatic LUTS.

Changes in the hormonal mileau have also been hypothesized as a driving factor in onset and progression of BPH and LUTS. As men age, testosterone levels decline while estrogen levels remain the same or even rise [97]. Severe bladder outlet obstruction and increased prostate wet weight is observed in rodent models mimicking this hormonal change [98]. Interplay between the epigenome and hormone action is also evident in BPH. Testosterone is converted to the more potent dihydrotestosterone by SRD5A2 within prostate, driving proliferative growth in development, BPH and prostate cancer. 5 alpha reductase inhibitors like finasteride are commonly used in men to alleviate symptoms of BPH with varying success. Currently, it is not understood why some men respond to these therapies better than others. Improved understanding of *SRD5A2* regulation may shed light on factors responsible for patient drug responsiveness. *SRD5A2* has CpG regions and DNA methylation has been shown to regulate its expression in prostate [99]. Interestingly, men exhibit varying levels of SRD5A2 expression in prostate [99]. Why some adult men express low levels of prostatic SRD5A2 is not known [99]. The possibility that men with highly methylated *SRD5A2* may be more resistant to 5 alpha reductase inhibitor therapy has been raised [100]. What regulates SRD5A2 DNA methylation and why it is only seen in some men experiencing BPH is unknown but a promising field of study for designing personalized therapies.

Age, one of the best known risk factors for BPH/LUTS, is also a predictor of DNA methylation status [101–103]. Epigenetic drift, or age associated changes in the DNA methylation landscape, occurs in prostate as it does in other organs [101]. Typically, a global loss of DNA methylation is associated with aging [104]. However, gene specific changes in DNA methylation (including hypo- and hypermethylation) as well as prostate tissue region specific changes in DNA methylation have also been observed [101]. Whether changes in DNA methylation contribute to BPH/LUTS onset or progression and whether interventions to enhance DNA methylation will be therapeutically beneficial has not been examined.

Conclusion

Figure 3 encompasses a summary of events in prostate and other organs which are capable of establishing, maintaining or removing DNA methylation marks, which in turn have downstream actions on embryogenesis, organogenesis and disease in prostate and other organs.

Future perspective

Given the complexity in studying roles of DNA methylation, new tools and models will likely emerge. This will refine the intricate study of DNA methylation in carrying out actions of hormones, remodeling tissue structure and function in response to aging or endocrine disrupting chemicals. While significant progress has been made, it is still often difficult to manipulate the epigenome in a cell and DNA locus selective fashion. Global inhibitors of DNA methylation are available but have off target and widespread effects. Specific DNMT inhibitors are also becoming more widely used but they too cannot be targeted to a specific organ, specific genetic locus or specific time. Deletion of *Dnmts* is currently possible and continued expansion of available *cre* drivers will facilitate investigating the role of *Dnmts* in specific cell populations at critical periods of time and in specific cell populations. Development of new techniques to study DNA methylation in a gene, cell or developmental stage specific context will exponentially expand the field of epigenetics.

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• of interest;

•• of considerable interest

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Executive summary

Role of DNA methylation in prostate development

- Androgens regulate prostate development but how they elicit their actions is not completely understood.
- DNA methylation acts during specific developmental stages to guide prostate morphogenesis.
- DNA methylation of e-cadherin contributes to prostate epithelial outgrowth.
- DNA methylation of androgen receptor guides prostate bud patterning and rate of formation.
- Understanding how DNA methylation is remodeled in other developmental context may shed light on how DNA methylation acts in prostate.

Mechanisms of establishing & erasing DNA methylation marks in embryogenesis that may be conserved in prostate development

- DNA methylation is rapidly and extensively remodeled during embryonic development.
- DNA demethylation occurs through base modifications, base excision repair, mismatch repair and DNA damage repair pathways.
- Genes involved in these processes are expressed in developing prostate.

Roles of DNA methylation in organogenesis – conserved mechanisms & pathways important for prostate development

- Transient changes in DNA methylation can regulate developmental pathways guiding organ morphogenesis in a stage and cell type specific fashion.
- Mouse models employing conditional deletion of *Dnmts* have increased our ability to probe the roles of DNA methylation during organogenesis.
- Conditional deletion of DNMT1 has revealed developmental pathways which rely on appropriate DNA methylation, including cell polarity, adhesion and signaling pathway members like *Notch, Bmp* and *Wnt*.
- These developmental mechanisms all contribute to prostate development, whether DNA methylation regulates them in developing prostate is an area of future study.

Interactions between DNA methylation & hormones

- DNA methylation acts to regulate hormone receptor expression in prostate and elsewhere.
- Hormones are capable of influencing DNA methylation machinery and altering gene specific DNA methylation leading to changes in tissue structure and function.

- Whether hormones influence DNA methylation in developing prostate is unknown.

DNA methylation & the fetal basis of adult disease in prostate

- Endocrine disrupting chemicals such as diethylstibesterol and bishenol A are capable of altering prostate development.
- Developmental exposure to these chemicals in prostate and other organs is capable of modifying DNA methylation, which has been linked to altered histology/physiology later in life.
- This raises the hypothesis that these chemicals may act by altering DNA methylation in developing prostate.

Prostate cancer & benign prostate hyperplasia: a reawakening of developmental signals

- Reawakening of developmental signaling pathways has been proposed as a mediator of prostate disease onset and progression.
- Inappropriate activation of DNA methylation pathway genes as well as changes in DNA methylation are observed in prostate cancer and benign prostate hyperplasia.

Future perspective

- New techniques and animal models capable of manipulating DNA methylation at specific cell types and specific stages will enhance our understanding of the complex and intricate regulation of DNA methylation in development and disease.

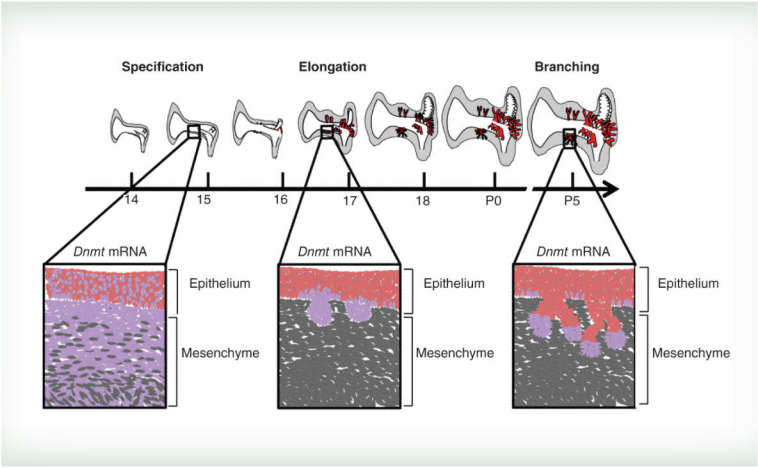

Figure 1. Mouse prostate development and localization of *Dnmt1* expression over time

In mouse the prostate develops from the urogenital sinus (UGS). Prostate specification begins at 14 days post coitus after which time epithelial prostate buds (red) begin to emerge and elongate (16–18 dpc) into surrounding mesenchyme (grey). Prostate buds then undergo branching morphogenesis, which continues postnatally. Insets represent magnified images of prostate epithelial and mesenchymal morphology over the course of bud formation. *Dnmt1* mRNA expression patterns are shown in purple displaying widespread expression in mesenchyme prior to bud formation before diminishing in mesenchyme and localizing to developing prostate buds and bud tips.

For color images please see online at: www.futuremedicine.com/doi/full/10.2217/EPI.15.8.

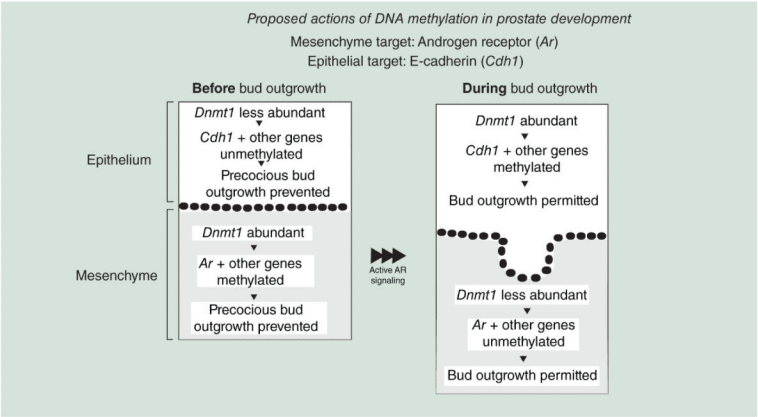

Figure 2. Proposed mechanisms of action for DNA methylation during prostate development

Early in prostate development, when DNA methyltransferase expression predominates in mesenchyme, DNA methylation of *Ar* acts to constrain prostate bud formation and prevents precocious growth. Later in development, when *Dnmts* predominates in epithelium, DNA methylation of *Cdh1* facilitates prostate epithelial differentiation and outgrowth.

Ar: Androgen receptor; Cdhl: E-Cadherin.

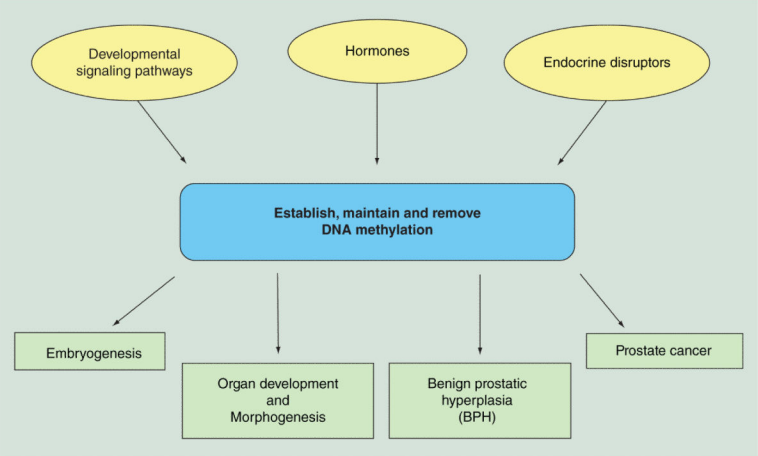

Figure 3. Dynamic DNA methylation events in development and disease

Summary of events in prostate and other organs which are capable of establishing, maintaining or removing DNA methylation marks, which in turn have downstream actions on embryogenesis, organogenesis and disease.