Pre-malignant Disease in the Prostate

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Chapter 22
Pre-malignant Disease in the Prostate

Alastair D. Lamb, Anne Y. Warren, and David E. Neal

Abstract Carcinoma of the prostate (CaP) is the most common non-cutaneous cancer in men and the second most common cause of cancer related death. Mortality remains high despite improvements in diagnosis in the developed world. A better understanding of the mechanisms involved in the development of prostate cancer should allow targeted diagnosis, prevention and treatment, and may improve mortality. In this chapter, we outline the two principal pre-malignant histological types, prostate intraepithelial neoplasia (PIN) and atypical small acinar proliferation (ASAP) and the likelihood of progression to CaP if these diagnoses are made. We then assess current understanding of factors contributing to the initiation of pre-malignant disease and progression to CaP as they relate to stem cells, inflammation, diet and specific genetic mutations or aberrant pathways. Finally, we discuss the translational potential of these factors in early detection and prevention of CaP.

Introduction

Carcinoma of the prostate (CaP) is the most common non-cutaneous cancer in men and the second most common cause of cancer related death, killing approximately 10,000 men annually in the UK [1]. The majority of prostate cancer deaths occur in men aged 65 and over (Fig. 22.1); however, the disease is also found amongst younger men, with prevalence rates of up to 30% in 30–50 year olds reported on post-mortem analysis [2]. There is a 15-fold variation in prostate cancer mortality rates worldwide, and although North America ranks first in terms of incidence, mainly owing to high levels of PSA testing, it is eighth for mortality, with the highest mortality rates being recorded in the Caribbean (Fig. 22.2). Countries with higher levels of PSA testing detect a greater proportion of early stage disease, the consequent lead-time bias

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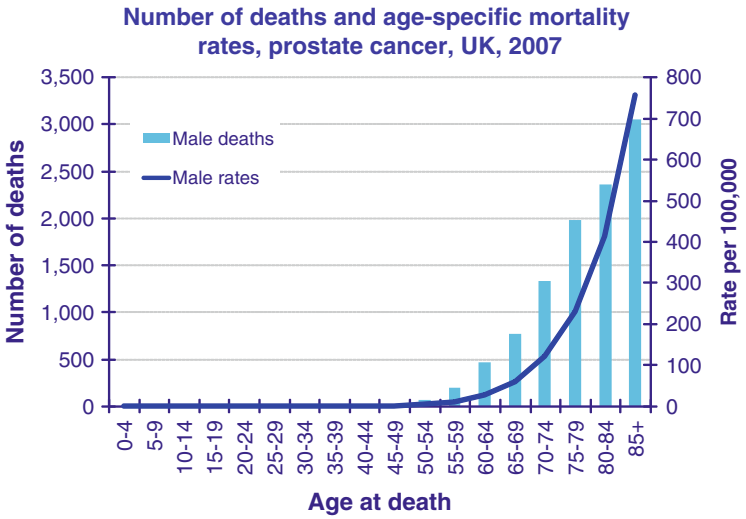

Fig. 22.1 UK age-specific mortality rates in 2007

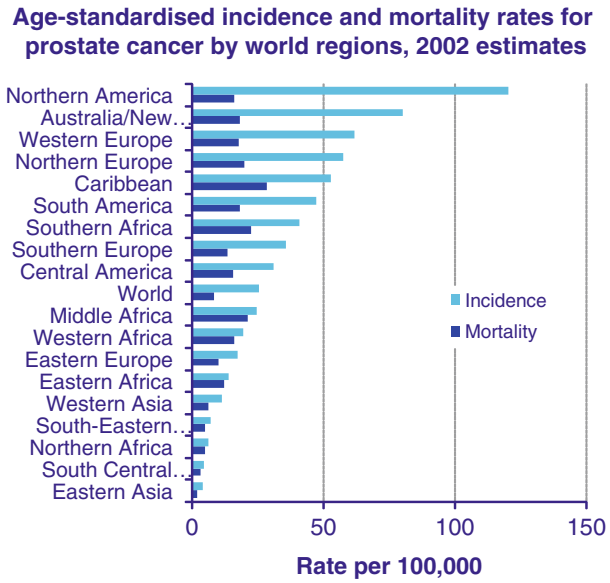

Fig. 22.2 World age-standardised incidence and mortality in 2002

giving higher survival rates compared to incidence [3]. Overall in the United Kingdom, prostate cancer mortality was fairly stable, but began to increase in the early 1980s. Mortality peaked in the early 1990s when the age-standardised death rate reached 30 per 100,000 in 1992. Since then there has been a slight fall in rates and in 2007, the age standardised rate was 25 per 100,000 (Fig. 22.3) [4]. Prostate cancer mortality remains high despite improvements in diagnosis in the developed world.

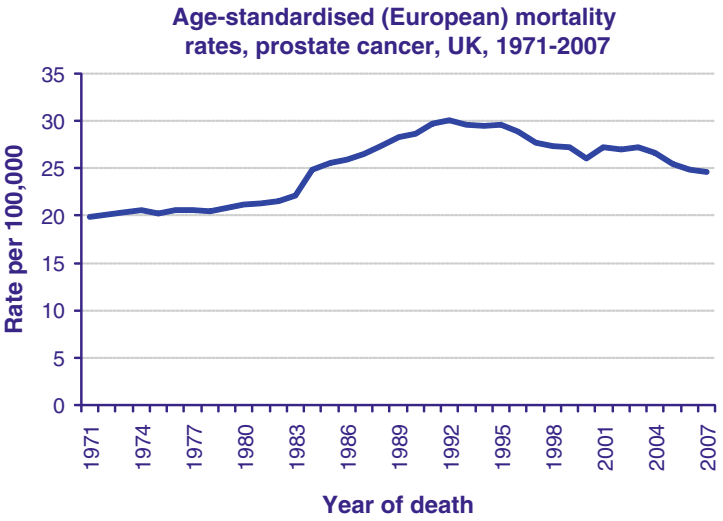

Fig. 22.3 UK mortality rates for three decades with age standardised to European averages

A better understanding of the mechanisms involved in the development of prostate cancer would allow targeted diagnosis, prevention and treatment, and may improve mortality.

Prostate cancer is a heterogeneous, often multifocal disease with numerous factors contributing to its initiation and progression. In this chapter, the two principal pre-malignant histological types will be outlined and current understanding of factors contributing to development of CaP will be assessed as they relate to stem cells, inflammation, diet and specific genetic mutations or aberrant pathways. Finally, the translational potential of these factors in early detection and prevention of CaP will be discussed.

Normal Prostate

The prostate is an accessory sexual organ with *exocrine* and *endocrine* functions, responsible for production and storage of 25–30% of constituents of the seminal fluid. Prostatic fluid is alkaline, which protects spermatozoa in the acidic environment of the vagina. The protein content of prostatic secretions is less than 1% and includes proteolytic enzymes, prostate acid phosphatase and prostate specific antigen as well as minerals such as zinc (free and protein bound) which has antimicrobial and semen coagulant effects [5, 6].

The prostate develops as part of the urogenital sinus (endodermal in origin) with contributions from the *Wolffian ducts* and from the surrounding mesenchyme. The prostate consists of branching ducts and *acini* which are often referred to as prostate glands collectively. The cells lining ducts and acini are identical with the exception of the larger peri-urethral ducts that have a urothelial lining. There are four main

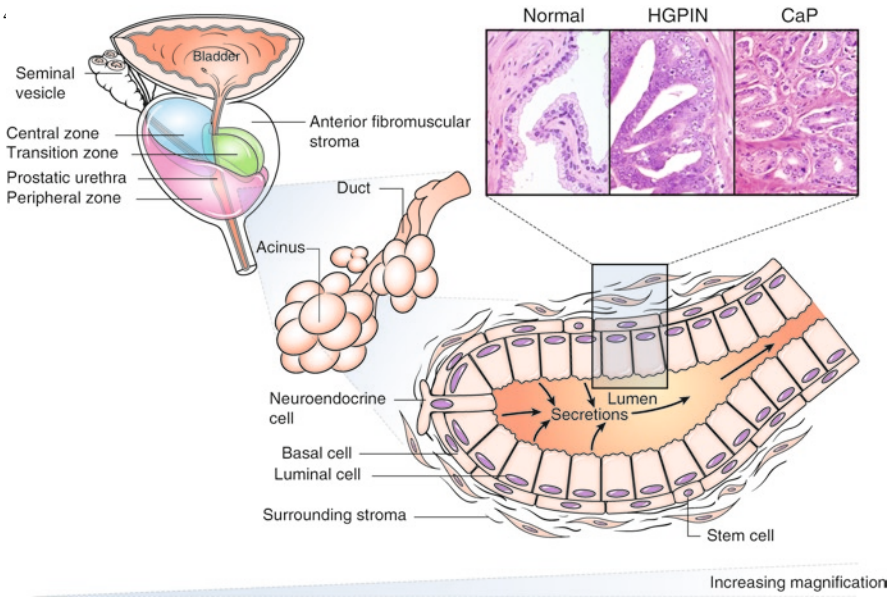

Fig. 22.4 Schematic of the prostate from organ to glands (ducts and acini). Different cell-types shown. Haematoxylin and Eosin sections show normal prostate, prostatic intraepithelial neoplasia (PIN) and Gleason score 6 carcinoma

anatomically distinct cell types (Fig. 22.4). First, prostate basal cells form a largely structural basal layer encircling each prostatic duct or acinus. Second, luminal (or glandular) cells form a columnar layer that make up the functional secretory surface of the glandular lumen. Third, rare neuroendocrine cells are interspersed between the basal and luminal cells. These are endocrine and sensory cells thought to share structural, functional and metabolic properties with neuronal cells found in the prostate. They secrete neuroendocrine peptides that support epithelial growth and viability [7]. Fourth, stromal cells surround the prostate glandular structures to guide and support growth and differentiation of the epithelium. Stromal cells include fibroblasts, myofibroblasts and smooth muscle cells and are derived from mesenchyme. Stem cells are generally thought to reside in the basal layer of the prostate contributing to all epithelial cell types of the prostate. The significance of prostate stem cells in development of prostate cancer will be discussed.

Prostatic Intraepithelial Neoplasia

Prostatic intra-epithelial neoplasia (PIN) is defined as the presence of cytologically atypical cells within a generally normal ductal or acinar outline in the presence of a basal layer of cells. PIN is conventionally divided into low grade (LG) and high grade (HG). Morphologically, HGPIN is identified by enlarged, crowded, often multilayered nuclei with irregular spacing, prominent nucleoli and amphophilic

cytoplasm, features also found in high *Gleason* grade carcinoma (Fig. 22.4) [8]. The essential distinguishing feature between HGPIN and carcinoma is the presence of basal cells, which, though often patchy in HGPIN are always present [9]. LGPIN, by contrast, shows a lesser degree of atypia and lacks prominent nucleoli. Clinically, LGPIN is not reported as such because of poor reproducibility and uncertain diagnostic significance, instead being classified as benign prostatic tissue [10]. Genetic and molecular factors contributing towards the development of PIN will be discussed in subsequent sections.

There is strong histological evidence implicating HGPIN as a pre-neoplastic lesion. Morphologically, HGPIN is primarily found in the peripheral zone, in proximity to invasive CaP [11], is multifocal and similar in cytological appearance to CaP [6], and it generally precedes CaP by at least 10 years, consistent with a linear progression [2, 12]. At a cellular level, HGPIN displays similar chromosomal abnormalities to early invasive CaP [13] as well as similar markers of differentiation [14]. However, the question remains open as to whether PIN is truly a precursor lesion of CaP or whether the two conditions are simply commonly associated.

A diagnosis of PIN has important clinical implications. There is general consensus regarding the diagnosis of HGPIN and also regarding the general relationship of HGPIN to CaP [8, 15, 16]. The reported incidence of HGPIN on transrectal ultrasound (TRUS) biopsy varies considerably at 0.6–24% (mean 7.7%), largely dependent on variation in the population under study, with HGPIN incidence increasing with age [17]. There is less consensus regarding the actual risk of diagnosing CaP on repeat biopsy with figures ranging from 50% from studies in the 1990s compared to 20% in recent studies, which is little different from the risk after a "normal" biopsy [17, 18]. The explanation lies in the extent of HGPIN and timing of biopsy. HGPIN that is multifocal and present throughout the gland, as determined by involvement of multiple (two or more cores) biopsies, is associated with a 39–80% risk of CaP depending on the study [19, 20]. Similarly, delaying biopsy has been reported to increase the detection rate of CaP from 25% at 6 months to 44.6% at 1 year [21]. This has led to the advice that patients with HGPIN in more than one core should have repeat biopsies after a 1 year interval [22].

Atypical Small Acinar Proliferation

Atypical small acinar proliferation (ASAP) describes a group of small, often closely packed, acini that are regarded by the pathologist as being atypical, but lacking in sufficient morphological abnormalities to be regarded as unequivocally diagnostic of carcinoma. Although generally used to indicate a suspicion of malignancy, this term encompasses a range of pathological conditions including benign mimics of CaP such as atypical adenomatous hyperplasia (AAH) in the transition zone, reactive atypia and atrophy, high grade PIN and acini that are cytologically suspicious, but too few in number to allow a confident diagnosis of malignancy [8, 23]. ASAP is detectable in a mean of 5% of biopsies and is accepted as being

highly predictive for CaP on subsequent biopsies, with a 34–60% second biopsy detection rate [17, 24]. Advice at the time of writing is for repeat biopsy in the presence of ASAP within 3–6 months [22].

The Role of Stem Cells in the Initiation of Prostate Cancer

Stem cells or early progenitors in the prostate are thought to reside in the basal layer of the prostate. This is based on the observation that there is preferential survival of the basal layer of the prostate during androgen ablation with apoptosis of androgen receptor (AR)-expressing differentiated luminal cells [25]. It is supported by findings that mice null for the basal cell marker p63 are born without prostates (or mammary glands) [26, 27]. The existence and site of prostate stem cells has been most recently elucidated following studies incorporating murine renal-subcapsular grafting of a single adult mouse prostate stem cell defined by *Lin⁻Sca-1⁺CD133⁺CD44⁺CD117⁺* and located in the periurethral prostate. These cells are able to generate functioning secreting prostate tissue with long term self renewal capacity as demonstrated by serial transplantation in vivo [28]. This prostate tissue contains all three epithelial cell-types including neuroendocrine cells, dispelling previous notions that this rarer prostate cell may have a distinct cell of origin, common with surrounding neuroectodermal or stromal cells (Fig. 22.5) [7]. The peri-urethral region in the mouse prostate is rich in basal cells and characterized by a morphologically distinct band of smooth muscle rich in TGF-β, known to promote stem cell quiescence, consistent with this site being a prostate stem cell niche [29]. However, the site of prostate stem cells remains controversial, with another recent paper identifying cells in the luminal epithelium with full regenerative potential, defined by NKX3-1⁺CK18⁺AR⁺ [30], and these studies are yet to be replicated with human tissue. This debate has important implications for the cell of origin in prostate cancer.

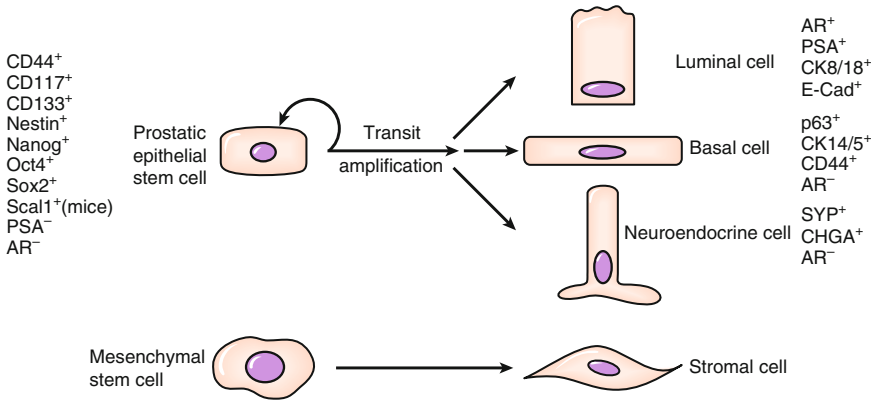

Fig. 22.5 Cell lineages of the prostate. The three epithelial cell types of the prostate are derived from a single epithelial stem cell. Stromal constituents are independently derived. Surface markers for different cell types as shown

The current dogma of stem cells as quiescent, slow-cycling cells, able to self-renew and pluripotent provides a strong case for these cells being the cell of origin in cancer. This was originally proposed in acute myeloid leukaemia [31], and subsequently many epithelial cancers including prostate [32–34]. Pathways that normally govern self-renewal or proliferation – such as PI3K [35], Wnt [36], SHh [37] and Notch [36] in haematopoietic, neural and epithelial systems – if dysregulated can contribute to tumorigenesis, for example in colon and mammary cancer, medulloblastoma, leukaemia and CaP. There are two main reasons for this. First, because stem cells have activated machinery for self-renewal, maintaining this activation may be easier than turning it on de novo. Second, if a number of mutations are required for neoplastic transformation to take place, then cells with longer lifespans have greater potential to accumulate these mutations and drive cancer [38]. The specific mutations involved in prostate cancer will be discussed later in this chapter.

Several properties of human prostate cancer suggest the disease may arise from a stem-like cancer initiating cell. The progression to androgen independent castrate resistant prostate cancer (CRPC) during androgen ablation therapy is consistent with prostate tumours containing populations of androgen-independent cells that survive and can expand in the absence of androgen. Given their androgen independent nature, prostate stem cells are a possible candidate. If the population of cells with such tumour regenerative potential is small this would fit with conventional wisdom regarding a precursor stem cell. However, it is also possible that a larger proportion of cells in a prostate tumour retains stem-like potential (a tumour is, after all, by definition, not fully differentiated), and when stressed by androgen ablation these cells alter their phenotype in a manner that is advantageous to their survival. One example of this is the propensity for castrate resistant prostate cancer to display a luminal to neuroendocrine shift [39].

Sub-populations of cells within prostate cancer cell lines with increased proliferative capacity in vitro and increased tumour initiating and metastatic capacity in vivo have been shown to possess a $CD44^{+}CD133^{+}CD117^{+}$ profile similar to prostate stem cells [34, 40, 41], as well as expressing $\alpha 2\beta 1$integrin consistent with membrane adhesional properties encouraging metastasis [34], and demonstrate higher levels of Oct4, Sox2, Nanog, Nestin [41–43], all markers of "stemness" or pluripotency. They are predominantly AR^{-}. However, they are also $p63^{-}$ [24, 41], suggesting that cancer stem cells are probably an intermediate cell type; not luminal, but not truly basal either.

In summary, prostate cancers are heterogeneous and contain subpopulations of cells that have increased tumorigenic potential compared to surrounding cells. These cells are characterised by markers that are similar, if not identical to those thought to define prostate stem cells. These "cancer stem cells" are thought by some to be the sites of initiation and maintenance of prostate cancer.

There are several questions that still need to be answered. First, most work on prostate cancer stem cells has been performed on cells that are already malignant – if prostate cancer stem cells really are transformed normal prostate stem cells then what is or are the initiating event/s? Which factors dysregulate the self-renewal pathways in normal prostate cancer stem cells? Second, can it be assumed that prostate cancer

stem cells really are dysregulated normal prostate stem cells? Or, are they committed progenitors/transit amplifying cells which retain some self-renewal and multipotent properties? Or are they de-differentiated terminally differentiated (luminal) cells that re-acquire stem-like properties and phenotypic versatility when stressed (e.g. by androgen deprivation)? Third, if prostate cancer stem cells are the initiating event in prostate cancer, and the source of repopulation in recurrent tumours, is there a unifying pathway that can be targeted therapeutically either to destroy the stem cells with malignant potential, or to prevent expansion of their malignant progeny? There is no clear answer to any of these three questions at the time of writing.

The Role of Inflammation in the Initiation of Prostate Cancer

Chronic inflammation is implicated in the development of a diverse range of human cancers, with evidence causally linking it to several cancers of the gastrointestinal tract, bladder and lung [44]. In these cancers, inflammation often collaborates with environmental exposures such as dietary toxins to increase the risk further [45]. The molecular mechanisms that underlie the pathogenesis of inflammation-associated cancer are complex and involve both the adaptive and innate immune system [46–48]. The release of highly reactive compounds such as hydrogen peroxide, nitric oxide and superoxide by activated phagocytic cells of the innate immune system damages DNA in epithelial cells, leading to increased cell division to replace these damaged cells, these dividing cells then being further exposed to mutagenic agents. The release of cytokines by inflammatory cells promotes cell proliferation and stimulates angiogenesis further enhancing tumorigenesis. In addition, during chronic inflammation, T and B cell-mediated antibody activity of the adaptive immune response can cause excessive and prolonged activation of innate immune cells [47].

The prostate has been shown to harbour focal areas of epithelial atrophy, sometimes associated with inflammatory infiltrates. These are common in the ageing prostate and often encompass a large fraction of the peripheral zone [49]. Despite the atrophic architectural appearance, there is an increased fraction of epithelial cells in such lesions. One term that has been used to describe those which are also associated with an inflammatory infiltrate is proliferative inflammatory atrophy (PIA) [50]. These areas of PIA have been observed to transition to HGPIN and adenocarcinoma in morphological studies [51–54], although some debate persists regarding this continuum and the validity of the separation of PIA from other forms of atrophy [55, 56].

In most cases, the precise cause of prostatic inflammation is unclear. However, potential sources include (Fig. 22.6):

- Infectious agents (viral or bacterial)
- Reflux of urine into the prostatic ducts
- Hormonal agents (e.g. oestrogen)
- Dietary factors [57]

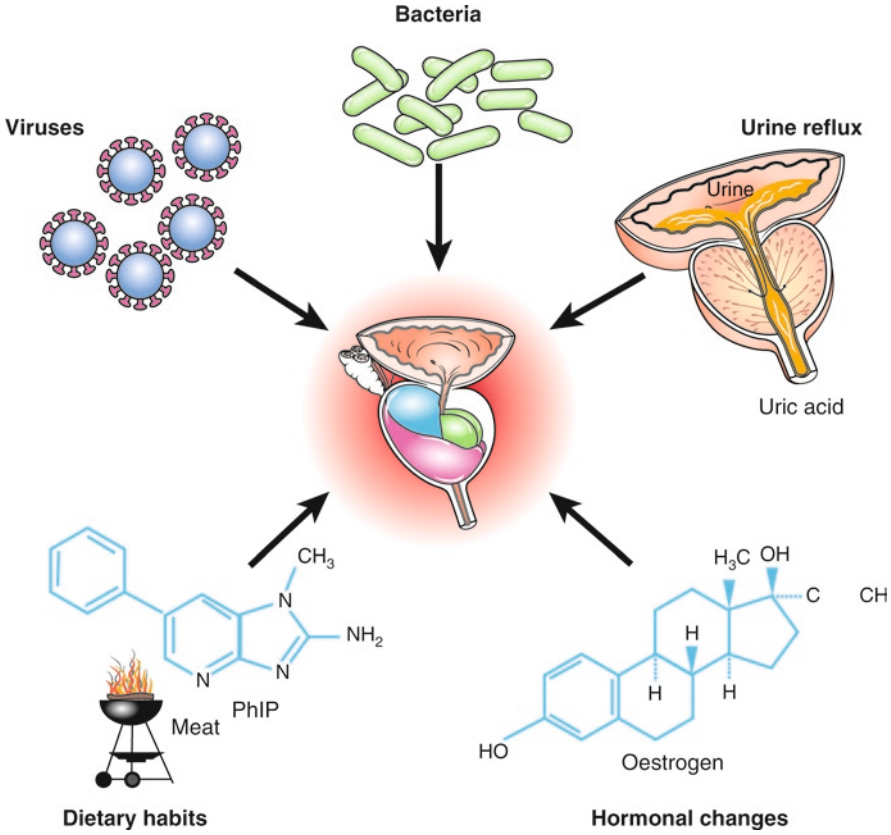

Fig. 22.6 Causes of inflammation in the prostate

Infectious agents. Sexually transmitted organisms that are known to infect the prostate include *Gonorrhoea* [58], *Chlamydia* [59], and *Trichomonas vaginalis* [60], while non-sexually transmitted bacteria include Gram-negative organisms such as *Escherichia coli* [61]. These can cause acute or chronic prostatitis, however severe acute inflammation and formation of prostatic abscesses is rare with antibiotic treatment, although TRUS biopsy of the prostate does increase this risk [62]. Nonetheless, asymptomatic infection and inflammation can still occur [58]. Viruses such as human papillomavirus (HPV) [63], cytomegalovirus (CMV) [64] and human herpes simplex virus (HSV) [65] have been detected in the prostate, although the extent to which they elicit an inflammatory response is largely unknown. It is possible that, in analogy to H. pylori in gastritis, there is a previously unidentified causative organism for PIA [57]. There is limited evidence linking specific infectious organisms with prostate cancer risk. This amounts to a lack of data rather than negative data per se, most likely due to the difficulties in assaying colonisation of the prostate. With prostatic massage being the only means of accurately

obtaining prostatic fluid cultures directly from the prostate it is difficult to accurately identify and quantify the extent of prostatic colonisation [66] and to correlate causative organisms with prostate cancer risk. There is also no evidence of an association between clinical prostatitis and prostate cancer, although this may be due to the complex nature and classification of clinical prostatitis which is not a true reflection of histological prostate inflammation [67]. The link is implicit. Infectious agents cause inflammation in the prostate, often chronic, and chronic inflammation causes PIA which is associated with prostate cancer risk.

One recent development has been the discovery of Xenotropic murine leukaemia-virus related virus (XMRV) in human prostate cancers. Gammaretroviruses have well-characterised oncogenic effects in animals but this is the first such virus known to infect humans. XMRV infection has been shown to be associated with a common polymorphism of the RNASEL gene [68]. XMRV transcripts and protein, when present, have been found to be predominantly expressed in malignant epithelial cells, especially more aggressive tumours [69]. This is, at present, still only an associative link.

Urine reflux. Chemical or traumatic irritation have been linked with inflammation in the prostate [70] for example, uric acid has been shown to directly engage inflammasomes, pro-inflammatory intracytoplasmic complexes in cells of the innate immune system, especially macrophages, resulting in recruitment of other inflammatory cells [71]. A rat model of partial urethral obstruction has shown increased Cox-2 levels in response to intraprostatic urinary reflux [72]. In addition the retrograde movement of spermatozoa into the prostate has been found in association with prostatic inflammation probably due to the adaptive immune response to these immune privileged cells [73].

Hormonal influence – oestrogen. Increased levels of oestrogens have long been seen to affect the growth and development of the prostate, which is known to express oestrogen receptor-α (ERα) primarily in the stroma and oestrogen receptor-β (ERβ) in the epithelium [74, 75]. Administration of oestrogens to neonatal rodents induces developmental defects, but also results in inflammation as well as hyperplasia, dysplasia or PIN [51]. It is therefore reasonable to associate oestrogens with chronic inflammation and prostate cancer risk in the adult prostate, although further work needs to be done.

In summary many mechanisms might lead to prostate epithelial inflammation. Continuous exposure to the stimulating agent can set up a sustained or chronic inflammatory response leading to PIA and potentially to cancer. Questions that still need to be answered include: first, does the breakdown of prostate epithelial cells (e.g. in response to chemical injury) release antigens that either initiate an autoimmune response or reduce tolerance to future injuries? Indeed, a T-cell immune response to PSA in patients with chronic prostatitis has already been reported [76]. Second, do the endogenous inflammatory cells present in normal prostate, such as T-lymphocytes contribute to PIA and carcinogenesis? Methods such as automated quantitative image analysis are crucial to answer this [77]. Third, are there specific

polymorphisms or mutations in inflammation-related genes that predispose an area of PIA to initiation of prostate cancer? Aspects of this last question will be answered later in this chapter.

The Role of Diet in the Initiation of Prostate Cancer

Epidemiological studies have revealed a link between prostate cancer incidence and mortality and the consumption of red meat and animal fats [78]. North-east Asian and Northern Atlantic populations have the lowest international prevalence of prostate cancer and yet they assume western risk profiles within two generations on migration [79]. This is generally thought to be due to dietary factors. Saturated and monounsaturated animal fat as well as linoleic acid have been associated in a number of case control and cohort studies with a higher risk of prostate cancer [80, 81]. By contrast, Japanese studies have demonstrated a negative association with soybean products, isoflavones, and long-chain polyunsaturated fatty acids such as eicosapentaenic acid (EPA) and docosahexanoic acid (DHA) [82]. In addition trace metals such as zinc supplements in the VITamins And Lifestyle (VITAL) cohort have been associated with a decreased risk of advanced prostate cancer [83].

Studies of the Mediterranean diet (Greece in particular) have revealed a protective effect for lycopenes, selenium, vitamin E, pulses and high plasma 1,25-dihydroxyvitamin D levels. Foods high in calcium such as milk, which is also rich in insulin-like growth factor-1 (*IGF-1*), increase the risk of prostate cancer [84].

Mechanisms by which dietary fat could affect CaP development include effects on insulin IGF-1 [85], steroid hormone metabolism, free radical damage and fatty acid metabolism pathways. Transgenic mice fed with low versus high fat diets have shown a significant delay in progression from mouse PIN to CaP and a reduction in AKT activation consistent with an IGF-mediated role (see explanatory box) [86]. This study used the *probasin* directed Hi-Myc model of mouse CaP; as yet, it has not been replicated in other models.

One mechanism by which meats might stimulate cancer development is through the formation of heterocyclic amines (HCAs), molecules produced by cooking red meat at high temperatures, and which can be metabolised to DNA damaging agents. Rat exposure to the most abundant HCA, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), results in intestinal carcinomas, mammary tumours and CaP [87, 88]. PhIP has also been shown to recruit epithelial macrophages and stromal mast cells and induce PIA before inducing PIN and CaP [89].

Recently, it has been shown that genistein, the major isoflavone of soy, and resveratrol, a polyphenolic phytoalexin found in red wine and grape-derived products, suppress the development of CaP in transgenic rat models when administered either alone or in combination. These nutritional polyphenols reduce cell proliferation and induce apoptosis by reducing levels of growth factors such as IGF-1 and steroid signalling proteins such as SRC-3 [90].

In summary, although conclusive evidence is limited, it is possible that a diet high in soybean products, fish, fruit and vegetables, and low in red meat, dairy products and calcium, similar to the Japanese or Greek diets may reduce the risk of prostate cancer [91]. However, well-designed epidemiological studies such as nested case-control studies with nutritional analyses of blood samples are needed to confirm these associations. In addition further laboratory studies in vivo models of prostate cancer are required to elucidate the mechanisms.

The Role of Specific Genetic Mutations and Pathways in the Initiation of Prostate Cancer (Table 22.1)

NKX3-1/8p. The NKX3-1 homeobox gene regulates prostate epithelial differentiation. One of the commonest events in prostate carcinogenesis (in ~80% of CaP) is loss of specific regions of chromosome 8p which encodes NKX3-1 [92]. Fluorescence in situ hybridisation (FISH) and allelic imbalance analysis studies concur that loss of 8p12-21 is an early event, while 8p22 is more common in advanced CaP [93]. Chromosome 8p deletions are also present in lung and colorectal tumours, but NKX3-1 expression is restricted to the prostate in adult tissues. NKX3-1 homozygous mutant mice develop PIN by 1 year of age [94]. In the intact adult mouse prostate, all luminal cells and 10% of basal cells express Nkx3-1. Expression is virtually abolished on castration and quickly restored after androgen re-administration. The small number of residual Nkx3-1 expressing cells has been further characterised with serial single cell transplantation and with lineage-marked Nkx3-1 knock-in mice to show that HGPIN and CaP develops from these cells in an inducible PTEN deletion mouse model of CaP. Nkx3-1 is therefore proposed as a marker of a cell of origin for CaP. As discussed earlier in this chapter, these cells also express the androgen receptor (AR) and keratin 18 (CK18) consistent with a luminal origin [30].

PTEN. Mutations involving phosphatase and tensin homolog deleted on chromosome 10 (PTEN) are common with loss of function mutations being reported in ~30% of primary cancers and ~60% of metastatic lesions [95]. PTEN dephosphorylates phophatidylinositol 3,4,5-triphosphate (PIP3) which is a product of phosphoinositide 3-kinase (Pi3K) activity. Increased levels of PIP3 in PTEN-deficient conditions alter the rate of protein translation, susceptibility to apoptosis and *anoikis*, entry in the cell cycle, differentiation and motility [35]. Key downstream effectors of PIP3 are PDK1, AKT and mTOR1&2, which play a fundamental role in supporting cancer cell metabolism, growth and survival [96]. Deletion of PTEN in the developing murine prostate leads to early onset and rapidly progressive neoplasia [97]. Cre-recombinase mediated PTEN excision in the murine prostate gland after puberty instead leads to a gradual onset of pre-malignant conditions within 16–20 weeks, following by progression to CaP at 1 year [98]. The delayed latency occurred despite evidence of prominent AKT/mTOR activation from the time of PTEN deletion. This indolent evolution of the disease after PTEN deletion in the mature mouse is reflective of disease initiation and progression in humans and is

Table 22.1 Common genetic and somatic changes in initiation of CaP

Gene	Location	Notes
Nkx3-1 [30, 117, 118]	8p12-22	Homeodomain transcription factor. Prostate specific – suppresses growth of epithelial cells/maintains prostate stem cells. One allele frequently deleted in primary tumours. Possible marker of CaP cell of origin
PTEN [98, 119]	10q23.31	Lipid phosphatase that suppresses cell proliferation and increases apoptosis. One allele lost in ~30% of primary tumours. Mutations in 60% of metastatic lesions. Constitutive PTEN deletion in mouse prostates leads to rapid CaP. Post-pubescent deletion leads to PIN and slow development of CaP at 1 year
AKT [99]	14q32.32	Pi3K pathway. Inhibited by PTEN. Activation of murine AKT induces uniform highly penetrant PIN. Only progresses to CaP if there is a secondary hit (e.g. p27)
Myc [107, 108, 120]	8q24	Transcription factor with multiple regulatory roles in epithelial proliferation, senescence, apoptosis and metabolism. Overexpression can directly transform cells. Amplified in ~70% of castrate resistant tumours
ERG ETV 1-4 [101, 103, 104] (ETS)	21q22.3 7p21.2 19q13.12 1q21-q23 17q21.31	Encode ETS transcription factors. Fusion transcripts with androgen-regulated TMPRSS2 present in ~50% of CaP at all disease stages. Particularly implicated in progression of PIN to CaP
p27 [99, 121] (CDKN1B)	12p12.3	Cell cycle regulator – regulates cyclin-CDK to inhibit cell cycle progression. Reduced levels observed in CaP progression. Ablation of p27 in mouse PIN induces CaP
Rb [122]	13q	Cell cycle regulator. Infrequently mutated in humans but homozygous mutant mice develop hyperplasia, dysplasia and CaP
Telomerase [123]	Chromosome termini	Maintains chromosomal stability. Shortened telomeres found in >90% of PIN and CaP
E-cadherin [124]	16q22.1	Cell adhesion. Prevents migration of epithelial cells. Reduced expression in PIN and CaP
c-Met [125, 126]	7q31	Tyrosine-kinase receptor. Overexpressed in PIN, CaP and metastasis. Levels repressed by normal AR signalling
FGFs [127, 128]	7:15q15-21.1 8:10q24 10:5p12-13	Growth factors. Regulators of prostate growth. FGF7 & 10 associated with progression in TRAMP mice. FGF8 enhances cell migration
p53 [118, 129]	17p13.1	Regulates apoptosis/senescence in response to DNA damage or telomere dysfunction. Mutations less common in primary CaP but occur in 50% of castrate resistant cancers
AR [130]	Xq11-12	Expressed in most primary CaP. Amplified or mutated in ~30% of castrate resistant tumours

consistent with PTEN deletion being one of the key genetic modifications accumulated in the development of prostate cancer. Inactivation of PTEN leads to deregulated PI3K signalling and subsequent AKT activation. Transgenic overexpression of AKT1 in luminal cells of the ventral murine prostate has been shown to induce PIN with increased expression of senescence markers which are proposed to limit progression to CaP. Only on genetic ablation of $p27^{Kip1}$ does progression occur. Similarly, in humans, senescence markers such as p27 are seen to be elevated in PIN that does not progress to CaP as opposed to cancer-associated PIN and CaP [99]. This is consistent with the need for a double hit: lesions initiated by PTEN deletion and upregulation of AKT also need inactivation of cell cycle limiters such as p27 in order for progression to CaP to take place.

TMPRSS2-ETS. Gene fusions involving members of the ETS family of transcription factors occur frequently in human CaP. The TMPRSS2-ERG fusion gene is generated by an interstitial deletion on chromosome 21 or by reciprocal translocation and is the most common rearrangement in CaP, being found in ~50% of localised tumours [100]. Cell line experiments suggest that the androgen-responsive promoter elements of TMPRSS2 mediate overexpression of ERG (or other ETS family members) [101]. Microdissection and fluorescence in situ hybridisation (FISH) studies have shown that, when present, the fusion is detected in essentially all the malignant cells within a focus of tumour, as well as in adjacent PIN. However, it is less commonly found in premalignant PIN when there is no tumour present [102]. Mice with probasin driven overexpression of ERG alone do not develop premalignant changes [103]. However, when TMPRSS2-ERG fusion mice are crossed with PTEN deletion/AKT activation, all mice develop PIN at 6 months, and CaP by 10–12 months [104]. This suggests that PI3K pathway changes are necessary for disease initiation and development of PIN, while ETS fusion rearrangements play a role in disease progression from PIN to CaP.

Myc/8q24. Chromosome 8q24 is an established risk locus for many common epithelial cancers, including prostate, colon, breast and bladder. It was originally discovered by fine mapping of a prostate cancer linkage peak from a family based study [105]. Most of the *single nucleotide polymorphisms* (SNPs) at 8q24 are contained within an approximately 500 kb sequence devoid of well-characterised genes. However, c-Myc, a well known oncogene in these cancers is nearby [106]. Copy number gain and amplification of 8q24 and increased c-Myc activity has been shown as an early event in some prostate cancers and in a high proportion of resistant tumours [107,108]. c-Myc has been shown to have varying oncogenic/tumour suppressor effects, from direct malignant transformation of benign cells in culture and induction of a pro-resistance phenotype [109] to increases in cell proliferation rates and recruitment of quiescent stem cells into rapidly dividing intermediate progenitors [91], to induction of apoptosis and senescence via effects on p53 and p21 [110, 111]. Overexpression of c-Myc in mouse prostates under the control of probasin (Lo-Myc) and probasin/AR promoters (Hi-Myc) leads to development of murine PIN and rapid development of invasive CaP within 6–12 months. These mice were also found to have a distinct loss of Nkx3-1 expression at the transition

from PIN to CaP but Nkx3-1 is not directly regulated by c-Myc [108]. With c-Myc having such varied effects it remains crucial to delineate its direct targets in prostate carcinogenesis and to clarify its interaction with the other pathways mentioned here. Cooperativity between c-Myc and PTEN deletion has been recently shown in development of HGPIN and CaP. PTEN deletion alone in mouse prostates was shown to activate p53, thought to have a "protective" senescent effect to limit the extent of malignant transformation. However, concomitant c-Myc activation suppressed the protective effect, probably by regulation of p21, to magnify the proliferative and tumorigenic effect [109].

SNPs/GWAS. Genome-wide association studies (GWAS) have emerged as a powerful approach to identify common disease alleles without prior knowledge of position or function. Genotype frequencies are compared between cases and controls at large numbers of single nucleotide polymorphisms (SNPs), chosen to report on most known common variants in the genome [112]. This technique has identified several interesting loci associated with CaP. Most recently, analysis of around 500,000 SNPs in 2,000 cases and controls, followed up with analysis of smaller numbers of SNPs in greater numbers of cases (4,000 cases in the second stage; 16,000 in the third) have revealed prostate cancer susceptibility loci in 14 separate chromosomal regions [112, 113], as well as eight loci in the 8q24 region [114] (Table 22.2) and it is expected that more will soon follow. These studies confirm that CaP is genetically complex and

Table 22.2 Single nucleotide polymorphisms (SNPs) associated with prostate cancer

Chromosome	SNP Marker	Potentially causative genes
3 [111]	rs2660753	*CHMP2B, POU1F1*
6 [111]	rs9364554	*SLC22A3, SLC22A2, LPAL2, LPA*
7 [111]	rs6465657	*LMTK2, BHLHB8*
10 [111]	rs7920517, rs10993994	*MSMB*
11 [111]	rs7931342	
19 [111]	rs2659056, rs266849, rs2735839	*KLK2, KLK3(PSA)*
X [111]	rs594561	*X NUDT10, NUDT11, GSPT2, MAGED1/4B/4, CTD-2267G17.3, XAGE2/1C/1D/5/3, SSX8/7/2/2B, SPANXN5, TMEM29B/29*
8q24 [113]	rs12543663, rs10086908, rs1016343, rs13252298, rs6983561, rs620861, rs6983267, rs10090154	*c-Myc*
2p21 [112]	rs1465618	*THADA*
2q31 [112]	rs12621278	*ITGA6*
4q32 [112]	rs17021918	*PDLIM5*
4q24 [112]	rs7679673	*TET2*
8p21 [112]	rs2928679, rs1512268	*NKX3-1*
11p15 [112]	rs7127900	*IGF2, IGF2AS, INS, TH*
22q13 [112]	rs5759167	*TTLL1, BIK, MCAT, PACIN2*

Potentially causative genes coded by or regions of regulation associated with these SNPs as shown

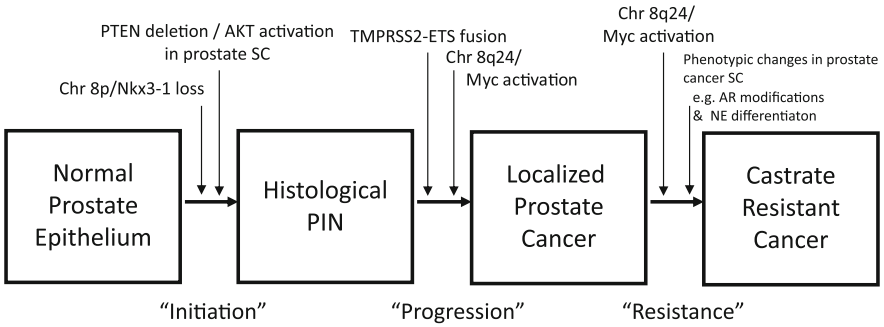

Fig. 22.7 Summary of current knowledge of the key steps involved in the initiation and progression of prostate cancer, as well as in development of resistant disease (not covered in this chapter). *SC* stem cell; *AR* androgen receptor; *NE* neuroendocrine

help to clarify the genetic architecture of CaP. Few of these loci are located directly within exon coding sequences (with the exception of MSMB and LMTK2) suggesting that diverse regulatory pathways are likely to be involved.

In summary, there are multiple specific genetic and regulatory pathway changes that occur in the development of CaP, some of which have been outlined here (Fig. 22.7). There is no doubt that there is parallel and chronological heterogeneity with a variety of factors interacting alongside each other in initiation of PIN and at subsequent progression to CaP and also to more resistant disease. A more complete understanding of these changes and their interactions will provide biomarkers for disease risk and deliver targets for therapeutic manipulation.

Implications for Early Detection and Prevention of Prostate Cancer

Specificity in therapeutic targeting of cancer stem cells will always be difficult. We are in a unique position with prostate cancer in that the patient population who develop the disease are rarely in need of their prostate. Therefore, a treatment targeted specifically to prostate stem cells, even if not specific to prostate cancer stem cells, is a viable option. However, few of the markers for prostate cancer stem cells discussed in this chapter is specific to the prostate. There is one exception: NKX3-1 is specific to the prostate after puberty and is a putative marker for the cell of origin for prostate cancer. If the cells that accumulate the genetic changes responsible for initiation and progression of prostate cancer could be destroyed then prostate cancer would not develop/progress. No compounds capable of this have yet been developed.

By contrast, treatment of inflammation is well established. The results of multicentre randomised controlled trials are awaited, for example studying the effect of non-steroidal anti-inflammatories (NSAIDS) in prevention of CaP.

However, a recent study of neoadjuvant celecoxib in clinically localised prostate cancer reported no difference in primary or secondary outcomes [115]. The search continues for an effective way to manipulate CaP risk by anti-inflammatory treatment: a randomised trial assessing the effect of treatment at the pre-malignant stage is needed. The discovery of viruses associated with CaP such as XMRV also raises the possibility of using vaccines or anti-retroviral drugs to treat CaP-associated infections and lower the risk the disease. Given its association with higher risk disease, XMRV might also serve as a useful marker to identify patients that would most benefit from early treatment [69].

Biomarkers of disease risk and likelihood of recurrence and progression will be an important means of targeting appropriate treatment. Biomarker information can be readily obtained from serum or urine samples, attractive to patients because of their non-invasive nature. In addition, more detailed analysis of prostate biopsy material with particular attention to field tumourigensis of 'near normal' tissue [115], will allow more information to be obtained from currently available material. Functional understanding is not a prerequisite for a biomarker to be useful in providing diagnostic and prognostic information when candidates have been rigorously validated by correlation of tissue expression and clinical outcome. For example, microseminomaprotein-beta (MSMB) expression is consistently high in normal prostate tissue and PIN, but lost in CaP [116]. This could be useful in assisting TRUS biopsy diagnostics and in directing the need for repeat biopsy. However, given the heterogeneity of prostate cancer development, a more complete knowledge of the key genetic players and their functional interactions will be required and this will most likely lead to a validated panel of diagnostic and prognostic markers for use in the clinical setting.

The identification of prostate cancer susceptibility genes by GWAS has a variety of clinical implications. The location of SNPs within regions that directly code for MSMB and LMTK2 suggest that these proteins might have a role in prostate cancer screening or provide potential therapeutic targets [111]. There are also implications for risk counselling although the relative risks conferred by each loci is modest (odds ratio of 2 at best) suggesting that "at-risk" SNP identification will be most useful at a population rather than individual level.

Conclusion

Prostate cancer is a heterogeneous disease with multiple factors contributing to the development of pre-malignant lesions and progression to adenocarcinoma. The role of cancer stem cells, inflammation, diet and certain specific genetic changes have been discussed in this chapter. With the identification of specific dietary factors contributing to the development of CaP the outlook is positive for lifestyle modification making a significant difference to the impact felt by this disease. It may be that targeting particular high risk populations identified through SNP profiling could lead to early preventative interventions. Prostate cancer research is a

rapidly developing field with a healthy commitment of personnel and resources and new genetic players are being identified all the time. The jigsaw of heterogeneity is beginning to be pieced together and, it is hoped, will soon provide us with exciting new additions to the currently available diagnostic and therapeutic options.

Comment Boxes

Exocrine: Exocrine glands secrete their products (excluding hormones and other chemical messengers) into ducts (duct glands) which lead directly into the external environment. They are the counterparts to endocrine glands, which secrete their products (hormones) directly into the bloodstream (ductless glands) or paracrine glands that release hormones that affect only target cells nearby the release site. Examples include the sweat and salivary glands (exocrine), stomach, liver, pancreas, and prostate (mixed).

Wolffian duct: The Wolffian duct is a paired organ found in mammals during embryogenesis. It was named by Caspar Friedrich Wolff in 1759. It connects the primitive kidney to the cloaca and serves as a clustering site for embryonic cells of the reproductive tracts. The Wolffian duct goes on to form the epididymis, vas deferens and seminal vesicles.

Acinus: Acinus refers to the cluster of cells that make up the termination of an exocrine gland such as the prostate. Acinus is Latin for berry.

Gleason: The Gleason staging system is based upon the microscopic architectural appearance (size, spacing and irregularity) of the prostate glands. The pathologist assigns a grade (from 1 to 5) to the most common tumour pattern and a second grade to the next most common. The combined score is the Gleason score. In practice the lowest score commonly allocated is 6 and the highest score is 10.

$Lin^{-}Sca\text{-}1^{+}CD133^{+}CD44^{+}CD117^{+}$: Cells can be sorted according to the presence of cell surface proteins which act as specific markers for different groups of cells. These particular cell surface markers have been proposed as the unique signature of stem cells in the mouse prostate.

IGF-1: Insulin-like growth factor (IGF-1) is a protein hormone similar to insulin in molecular structure. It has an important role in child growth and continues to have anabolic effects in adulthood. It is stimulated by growth hormone. 98% is protein bound by one of 6 binding proteins (IGF-BP). It binds to IGF-1 receptor (IGF1R), a tyrosine kinase receptor, to initiate intracellular signalling and is one of the most potent activators of Pi3K/AKT signalling and has been implicated in carcinogenesis.

Probasin (PB): Probasin (PB) is a prostate-specific nuclear and secreted protein found in differentiated luminal epithelial cells. The probasin promoter is used in

mouse and rat models to direct transgene expression specifically to prostate epithelial cells. This commonly-used technology may not allow for tumour initiating events that occur in undifferentiated prostate stem cells.

Anoikis: Apoptosis triggered by detachment of anchorage-dependent cells from surrounding extracellular matrix. Metastatic tumour cells resist anoikis to allow survival and attachment at distant sites. Anoikis is a Greek derivative meaning "the state of being without a home".

SNPs: A single-nucleotide polymorphism (SNP, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual). For example, two sequenced DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide.

References

1. Office for National Statistics (UK) (2007) Mortality statistics: cause, 2005 Newport: Office for National Statistics
2. Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD (1993) The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. J Urol 150(2 Pt 1):379–385
3. Hsing AW, Tsao L, Devesa SS (2000) International trends and patterns of prostate cancer incidence and mortality. Int J Cancer 85(1):60–67
4. Cancer Research UK cancerstats (2009) http://info.cancerresearchuk.org/cancerstats/types/prostate/mortality/index.htm
5. Patel M, Chhasatia M, Parmar P (2010) Antibacterial and DNA interaction studies of zinc(II) complexes with quinolone family member, ciprofloxacin. Eur J Med Chem 45(2):439–446
6. Yoshida K, Kawano N, Yoshiike M, Yoshida M, Iwamoto T, Morisawa M (2008) Physiological roles of semenogelin I and zinc in sperm motility and semen coagulation on ejaculation in humans. Mol Hum Reprod 14(3):151–156
7. Shariff AH, Ather MH (2006) Neuroendocrine differentiation in prostate cancer. Urology 68(1):2–8
8. Parkinson MC (1995) Pre-neoplastic lesions of the prostate. Histopathology 27(4):301–311
9. Hedrick L, Epstein JI (1989) Use of keratin 903 as an adjunct in the diagnosis of prostate carcinoma. Am J Surg Pathol 13(5):389–396
10. Epstein JI, Grignon DJ, Humphrey PA, McNeal JE, Sesterhenn IA, Troncoso P et al (1995) Interobserver reproducibility in the diagnosis of prostatic intraepithelial neoplasia. Am J Surg Pathol 19(8):873–886
11. Bostwick DG, Brawer MK (1987) Prostatic intra-epithelial neoplasia and early invasion in prostate cancer. Cancer 59(4):788–794
12. Bostwick DG, Amin MB, Dundore P, Marsh W, Schultz DS (1993) Architectural patterns of high-grade prostatic intraepithelial neoplasia. Hum Pathol 24(3):298–310
13. Sakr WA, Macoska JA, Benson P, Grignon DJ, Wolman SR, Pontes JE et al (1994) Allelic loss in locally metastatic, multisampled prostate cancer. Cancer Res 54(12):3273–3277
14. Nagle RB, Brawer MK, Kittelson J, Clark V (1991) Phenotypic relationships of prostatic intraepithelial neoplasia to invasive prostatic carcinoma. Am J Pathol 138(1):119–128
15. Bostwick DG, Montironi R, Sesterhenn IA (2000) Diagnosis of prostatic intraepithelial neoplasia: prostate working group/consensus report. Scand J Urol Nephrol Suppl (205):3-10
16. Bostwick DG, Qian J (2004) High-grade prostatic intraepithelial neoplasia. Mod Pathol 17(3):360–379

17. Epstein JI, Herawi M (2006) Prostate needle biopsies containing prostatic intraepithelial neoplasia or atypical foci suspicious for carcinoma: implications for patient care. J Urol 175(3 Pt 1):820–834
18. Gallo F, Chiono L, Gastaldi E, Venturino E, Giberti C (2008) Prognostic significance of high-grade prostatic intraepithelial neoplasia (HGPIN): risk of prostatic cancer on repeat biopsies. Urology 72(3):628–632
19. Netto GJ, Epstein JI (2006) Widespread high-grade prostatic intraepithelial neoplasia on prostatic needle biopsy: a significant likelihood of subsequently diagnosed adenocarcinoma. Am J Surg Pathol 30(9):1184–1188
20. Schoenfield L, Jones JS, Zippe CD, Reuther AM, Klein E, Zhou M et al (2007) The incidence of high-grade prostatic intraepithelial neoplasia and atypical glands suspicious for carcinoma on first-time saturation needle biopsy, and the subsequent risk of cancer. BJU Int 99(4):770–774
21. Roscigno M, Scattoni V, Freschi M, Raber M, Colombo R, Bertini R et al (2004) Monofocal and plurifocal high-grade prostatic intraepithelial neoplasia on extended prostate biopsies: factors predicting cancer detection on extended repeat biopsy. Urology 63(6):1105–1110
22. Oderda M, Gontero P (2009) High-grade prostatic intraepithelial neoplasia and atypical small acinar proliferation: is repeat biopsy still necessary? BJU Int 104(11):1554–1556
23. Bostwick DG, Srigley J, Grignon D, Maksem J, Humphrey P, van der Kwast TH et al (1993) Atypical adenomatous hyperplasia of the prostate: morphologic criteria for its distinction from well-differentiated carcinoma. Hum Pathol 24(8):819–832
24. Scattoni V, Roscigno M, Freschi M, Briganti A, Fantini GV, Bertini R et al (2005) Predictors of prostate cancer after initial diagnosis of atypical small acinar proliferation at 10 to 12 core biopsies. Urology 66(5):1043–1047
25. English HF, Santen RJ, Isaacs JT (1987) Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. Prostate 11(3):229–242
26. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT et al (1999) p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 398(6729):714–718
27. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L et al (2000) p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 157(6):1769–1775
28. Leong KG, Wang BE, Johnson L, Gao WQ (2008) Generation of a prostate from a single adult stem cell. Nature 456(7223):804–808
29. Salm SN, Burger PE, Coetzee S, Goto K, Moscatelli D, Wilson EL (2005) TGF-{beta} maintains dormancy of prostatic stem cells in the proximal region of ducts. J Cell Biol 170(1):81–90
30. Wang X, Kruithof-de Julio M, Economides KD, Walker D, Yu H, Halili MV et al (2009) A luminal epithelial stem cell that is a cell of origin for prostate cancer. Nature 461(7263):495–500
31. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3(7):730–737
32. Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB (2003) Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422(6929):313–317
33. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D et al (2006) Purification and unique properties of mammary epithelial stem cells. Nature 439(7079):993–997
34. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ (2005) Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 65(23):10946–10951
35. Hawkins PT, Anderson KE, Davidson K, Stephens LR (2006) Signalling through Class I PI3Ks in mammalian cells. Biochem Soc Trans 34(Pt 5):647–662
36. Yardy GW, Bicknell DC, Wilding JL, Bartlett S, Liu Y, Winney B et al (2009) Mutations in the AXIN1 gene in advanced prostate cancer. Eur Urol 56(3):486–494
37. Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A et al (2004) Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 431(7009):707–712
38. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414(6859):105–111
39. Vias M, Ramos-Montoya A, Mills IG (2008) Terminal and progenitor lineage-survival oncogenes as cancer markers. Trends Mol Med 14(11):486–494

40. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT (2004) CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci 117(Pt 16):3539–3545
41. Gu G, Yuan J, Wills M, Kasper S (2007) Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. Cancer Res 67(10):4807–4815
42. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S et al (2006) Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 25(12):1696–1708
43. Sotomayor P, Godoy A, Smith GJ, Huss WJ (2009) Oct4A is expressed by a subpopulation of prostate neuroendocrine cells. Prostate 69(4):401–410
44. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420(6917):860–867
45. Groopman JD, Kensler TW (2005) Role of metabolism and viruses in aflatoxin-induced liver cancer. Toxicol Appl Pharmacol 206(2):131–137
46. Condeelis J, Pollard JW (2006) Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124(2):263–266
47. de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6(1):24–37
48. Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y et al (2009) Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature 457(7225):102–106
49. McNeal JE (1988) Normal histology of the prostate. Am J Surg Pathol 12(8):619–633
50. De Marzo AM, Marchi VL, Epstein JI, Nelson WG (1999) Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am J Pathol 155(6):1985–1992
51. Putzi MJ, De Marzo AM (2000) Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. Urology 56(5):828–832
52. Wang W, Bergh A, Damber JE (2009) Morphological transition of proliferative inflammatory atrophy to high-grade intraepithelial neoplasia and cancer in human prostate. Prostate 69(13):1378–1386
53. Elkahwaji JE, Hauke RJ, Brawner CM (2009) Chronic bacterial inflammation induces prostatic intraepithelial neoplasia in mouse prostate. Br J Cancer 101(10):1740–1748
54. Tomas D, Kruslin B, Rogatsch H, Schafer G, Belicza M, Mikuz G (2007) Different types of atrophy in the prostate with and without adenocarcinoma. Eur Urol 51(1):98–103; discussion 03–04
55. Billis A (1998) Prostatic atrophy: an autopsy study of a histologic mimic of adenocarcinoma. Mod Pathol 11(1):47–54
56. Bostwick DC, Cheng L (eds) (2008) Urologic surgical pathology. Chapter 8: Non-neoplastic diseases of the prostate, 2nd ed. Mosby Elsevier, Philadelphia, PA
57. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG et al (2007) Inflammation in prostate carcinogenesis. Nat Rev Cancer 7(4):256–269
58. Handsfield HH, Lipman TO, Harnisch JP, Tronca E, Holmes KK (1974) Asymptomatic gonorrhea in men. Diagnosis, natural course, prevalence and significance. N Engl J Med 290(3):117–123
59. Poletti F, Medici MC, Alinovi A, Menozzi MG, Sacchini P, Stagni G et al (1985) Isolation of *Chlamydia trachomatis* from the prostatic cells in patients affected by nonacute abacterial prostatitis. J Urol 134(4):691–693
60. Stark JR, Judson G, Alderete JF, Mundodi V, Kucknoor AS, Giovannucci EL et al (2009) Prospective study of *Trichomonas vaginalis* infection and prostate cancer incidence and mortality: Physicians' Health Study. J Natl Cancer Inst 101(20):1406–1411
61. Drach GW (1975) Prostatitis: man's hidden infection. Urol Clin North Am 2(3):499–520
62. Ozden E, Bostanci Y, Yakupoglu KY, Akdeniz E, Yilmaz AF, Tulek N et al (2009) Incidence of acute prostatitis caused by extended-spectrum beta-lactamase-producing *Escherichia coli* after transrectal prostate biopsy. Urology 74(1):119–123
63. Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP (2002) Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate 53(4):263–276
64. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS (2003) High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol 170(3):998–1002

65. Doble A, Harris JR, Taylor-Robinson D (1991) Prostatodynia and herpes simplex virus infection. Urology 38(3):247–248
66. Batstone GR, Doble A (2003) Chronic prostatitis. Curr Opin Urol 13(1):23–29
67. Sutcliffe S, Giovannucci E, De Marzo AM, Leitzmann MF, Willett WC, Platz EA (2006) Gonorrhea, syphilis, clinical prostatitis, and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 15(11):2160–2166
68. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA et al (2006) Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2(3):e25
69. Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR (2009) XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci U S A 106(38):16351–16356
70. Kirby RS, Lowe D, Bultitude MI, Shuttleworth KE (1982) Intra-prostatic urinary reflux: an aetiological factor in abacterial prostatitis. Br J Urol 54(6):729–731
71. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440(7081):237–241
72. Liu W, Li SW, Zheng XM, Hu LQ, Luo Y (2008) Intraprostatic urinary reflux associated prostatitis caused by partial urethral obstruction in the rat model. Zhonghua Nan Ke Xue 14(1):11–14
73. Chen X, Zhao J, Salim S, Garcia FU (2006) Intraprostatic spermatozoa: zonal distribution and association with atrophy. Hum Pathol 37(3):345–351
74. Huang L, Pu Y, Alam S, Birch L, Prins GS (2004) Estrogenic regulation of signaling pathways and homeobox genes during rat prostate development. J Androl 25(3):330–337
75. Huang L, Pu Y, Alam S, Birch L, Prins GS (2005) The role of Fgf10 signaling in branching morphogenesis and gene expression of the rat prostate gland: lobe-specific suppression by neonatal estrogens. Dev Biol 278(2):396–414
76. Ponniah S, Arah I, Alexander RB (2000) PSA is a candidate self-antigen in autoimmune chronic prostatitis/chronic pelvic pain syndrome. Prostate 44(1):49–54
77. Lamb AD, Qadan M, Roberts S, Timlin H, Campbell FM, Gregor K, et al (2009) CD4+ and CD8+ T-lymphocyte scores cannot reliably predict progression in patients with benign prostatic hyperplasia (BPH). BJU Int *in print–DOI awaited*
78. Michaud DS, Augustsson K, Rimm EB, Stampfer MJ, Willet WC, Giovannucci E (2001) A prospective study on intake of animal products and risk of prostate cancer. Cancer Causes Control 12(6):557–567
79. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM (1991) Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 63(6):963–966
80. Whittemore AS, Kolonel LN, Wu AH, John EM, Gallagher RP, Howe GR et al (1995) Prostate cancer in relation to diet, physical activity, and body size in blacks, whites, and Asians in the United States and Canada. J Natl Cancer Inst 87(9):652–661
81. Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC (1993) A prospective cohort study of vasectomy and prostate cancer in US men. JAMA 269(7):873–877
82. Mori M, Masumori N, Fukuta F, Nagata Y, Sonoda T, Sakauchi F et al (2009) Traditional Japanese diet and prostate cancer. Mol Nutr Food Res 53(2):191–200
83. Gonzalez A, Peters U, Lampe JW, White E (2009) Zinc intake from supplements and diet and prostate cancer. Nutr Cancer 61(2):206–215
84. Itsiopoulos C, Hodge A, Kaimakamis M (2009) Can the Mediterranean diet prevent prostate cancer? Mol Nutr Food Res 53(2):227–239
85. Yu H, Rohan T (2000) Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 92(18):1472–1489
86. Kobayashi N, Barnard RJ, Said J, Hong-Gonzalez J, Corman DM, Ku M et al (2008) Effect of low-fat diet on development of prostate cancer and Akt phosphorylation in the Hi-Myc transgenic mouse model. Cancer Res 68(8):3066–3073

87. Knize MG, Felton JS (2005) Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat. Nutr Rev 63(5):158–165
88. Sugimura T, Wakabayashi K, Nakagama H, Nagao M (2004) Heterocyclic amines: mutagens/carcinogens produced during cooking of meat and fish. Cancer Sci 95(4):290–299
89. Nakai Y, Nelson WG, De Marzo AM (2007) The dietary charred meat carcinogen 2-amino-1-methyl-6-phenylimidazo[4, 5-b]pyridine acts as both a tumor initiator and promoter in the rat ventral prostate. Cancer Res 67(3):1378–1384
90. Harper CE, Cook LM, Patel BB, Wang J, Eltoum IA, Arabshahi A et al (2009) Genistein and resveratrol, alone and in combination, suppress prostate cancer in SV-40 tag rats. Prostate 69(15):1668–1682
91. Ma RW, Chapman K (2009) A systematic review of the effect of diet in prostate cancer prevention and treatment. J Hum Nutr Diet 22(3):187–199; quiz 200–202
92. Chang M, Tsuchiya K, Batchelor RH, Rabinovitch PS, Kulander BG, Haggitt RC et al (1994) Deletion mapping of chromosome 8p in colorectal carcinoma and dysplasia arising in ulcerative colitis, prostatic carcinoma, and malignant fibrous histiocytomas. Am J Pathol 144(1):1–6
93. Abate-Shen C, Shen MM (2000) Molecular genetics of prostate cancer. Genes Dev 14(19):2410–2434
94. Kim MJ, Bhatia-Gaur R, Banach-Petrosky WA, Desai N, Wang Y, Hayward SW et al (2002) Nkx3.1 mutant mice recapitulate early stages of prostate carcinogenesis. Cancer Res 62(11):2999–3004
95. Tomlins SA, Rubin MA, Chinnaiyan AM (2006) Integrative biology of prostate cancer progression. Annu Rev Pathol 1:243–271
96. Faivre S, Kroemer G, Raymond E (2006) Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 5(8):671–688
97. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J et al (2003) Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 4(3):209–221
98. Luchman HA, Benediktsson H, Villemaire ML, Peterson AC, Jirik FR (2008) The pace of prostatic intraepithelial neoplasia development is determined by the timing of Pten tumor suppressor gene excision. PLoS One 3(12):e3940
99. Majumder PK, Grisanzio C, O'Connell F, Barry M, Brito JM, Xu Q et al (2008) A prostatic intraepithelial neoplasia-dependent p27 Kip1 checkpoint induces senescence and inhibits cell proliferation and cancer progression. Cancer Cell 14(2):146–155
100. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM (2008) Recurrent gene fusions in prostate cancer. Nat Rev Cancer 8(7):497–511
101. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW et al (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310(5748):644–648
102. Perner S, Mosquera JM, Demichelis F, Hofer MD, Paris PL, Simko J et al (2007) TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol 31(6):882–888
103. Carver BS, Tran J, Chen Z, Carracedo-Perez A, Alimonti A, Nardella C et al (2009) ETS rearrangements and prostate cancer initiation. Nature 457(7231):E1; discussion E2–E3
104. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH et al (2009) Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet 41(5):524–526
105. Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA et al (2006) A common variant associated with prostate cancer in European and African populations. Nat Genet 38(6):652–658
106. Jia L, Landan G, Pomerantz M, Jaschek R, Herman P, Reich D et al (2009) Functional enhancers at the gene-poor 8q24 cancer-linked locus. PLoS Genet 5(8):e1000597
107. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao X, Carver BS et al (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18(1):11–22

108. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R et al (2003) Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell 4(3):223–238
109. Bernard D, Pourtier-Manzanedo A, Gil J, Beach DH (2003) Myc confers androgen-independent prostate cancer cell growth. J Clin Invest 112(11):1724–1731
110. Kim J, Eltoum IE, Roh M, Wang J, Abdulkadir SA (2009) Interactions between cells with distinct mutations in c-MYC and Pten in prostate cancer. PLoS Genet 5(7):e1000542
111. Watt FM, Frye M, Benitah SA (2008) MYC in mammalian epidermis: how can an oncogene stimulate differentiation? Nat Rev Cancer 8(3):234–242
112. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK et al (2008) Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40(3):316–321
113. Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G et al (2009) Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nat Genet 41(10):1116–1121
114. Al Olama AA, Kote-Jarai Z, Giles GG, Guy M, Morrison J, Severi G et al (2009) Multiple loci on 8q24 associated with prostate cancer susceptibility. Nat Genet 41(10):1058–1060
115. Antonarakis ES, Heath EI, Walczak JR, Nelson WG, Fedor H, De Marzo AM et al (2009) Phase II, randomized, placebo-controlled trial of neoadjuvant celecoxib in men with clinically localized prostate cancer: evaluation of drug-specific biomarkers. J Clin Oncol 27(30):4986–4993
116. Nonn L, Ananthanarayanan V, Gann PH (2009) Evidence for field cancerization of the prostate. Prostate 69(13):1470–1479
117. Whitaker HC, Warren AY, Eeles R, Kote-Jarai Z, Neal DE (2010) The potential value of microseminoprotein-beta as a prostate cancer biomarker and therapeutic target. Prostate 70(3):333–340
118. Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, Kim M, Desai N, Young P et al (1999) Roles for Nkx3.1 in prostate development and cancer. Genes Dev 13(8):966–977
119. Lei Q, Jiao J, Xin L, Chang CJ, Wang S, Gao J et al (2006) NKX3.1 stabilizes p53, inhibits AKT activation, and blocks prostate cancer initiation caused by PTEN loss. Cancer Cell 9(5):367–378
120. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI et al (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275(5308):1943–1947
121. Gil J, Kerai P, Lleonart M, Bernard D, Cigudosa JC, Peters G et al (2005) Immortalization of primary human prostate epithelial cells by c-Myc. Cancer Res 65(6):2179–2185
122. Yang RM, Naitoh J, Murphy M, Wang HJ, Phillipson J, deKernion JB et al (1998) Low p27 expression predicts poor disease-free survival in patients with prostate cancer. J Urol 159(3):941–945
123. Li C, Larsson C, Futreal A, Lancaster J, Phelan C, Aspenblad U et al (1998) Identification of two distinct deleted regions on chromosome 13 in prostate cancer. Oncogene 16(4):481–487
124. Zhang W, Kapusta LR, Slingerland JM, Klotz LH (1998) Telomerase activity in prostate cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium. Cancer Res 58(4):619–621
125. Umbas R, Isaacs WB, Bringuier PP, Schaafsma HE, Karthaus HF, Oosterhof GO et al (1994) Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Res 54(14):3929–3933
126. Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LW (1995) c-met proto-oncogene expression in benign and malignant human prostate tissues. J Urol 154(1):293–298

127. Verras M, Lee J, Xue H, Li TH, Wang Y, Sun Z (2007) The androgen receptor negatively regulates the expression of c-Met: implications for a novel mechanism of prostate cancer progression. Cancer Res 67(3):967–975
128. Djakiew D (2000) Dysregulated expression of growth factors and their receptors in the development of prostate cancer. Prostate 42(2):150–160
129. Darby S, Sahadevan K, Khan MM, Robson CN, Leung HY, Gnanapragasam VJ (2006) Loss of Sef (similar expression to FGF) expression is associated with high grade and metastatic prostate cancer. Oncogene 25(29):4122–4127
130. Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC (1993) p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res 53(14):3369–3373
131. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C et al (1995) In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9(4):401–406