

REVIEW

Prostate carcinogenesis and inflammation: emerging insights

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Prostate cancer remains a significant health concern for men throughout the world. Recently, there has developed an expanding multidisciplinary body of literature suggesting a link between chronic inflammation and prostate cancer. In support of this hypothesis, population studies have found an increased relative risk of prostate cancer in men with a prior history of certain sexually transmitted infections or prostatitis. Furthermore, genetic epidemiological data have implicated germline variants of several genes associated with the immunological aspects of inflammation in modulating prostate cancer risk. The molecular pathogenesis of prostate cancer has been characterized by somatic alterations of genes involved in defenses against inflammatory damage and in tissue recovery. A novel putative prostate cancer precursor lesion, proliferative inflammatory atrophy, which shares some molecular traits with prostate intraepithelial neoplasia and prostate cancer, has been characterized. Here, we review the evidence associating chronic inflammation and prostate cancer and consider a number of animal models of prostate inflammation that should allow the elucidation of the mechanisms by which prostatic inflammation could lead to the initiation and progression of prostate cancer. These emerging insights into chronic inflammation in the etiology of prostate carcinogenesis hold the promise of spawning new diagnostic and therapeutic modalities for men with prostate cancer.

Introduction

Prostate cancer continues to be a source of considerable morbidity and mortality for men around the world, accounting for an anticipated 30 000 deaths in the USA in 2004 alone (1). While prostate adenocarcinoma is often thought of as a disease of older men, several published autopsy series have demonstrated

Abbreviations: BPH, benign prostatic hyperplasia; COX-2, cyclooxygenase 2; EBV, Epstein-Barr virus; HHV, human herpes virus; HPV, human papilloma virus; HSV, herpes simplex virus; MSR, macrophage scavenger receptor; NOD, non-obese diabetic; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; PIA, proliferative inflammatory atrophy; PIN, prostate intraepithelial neoplasia; PSA, prostate-specific antigen; STIs, sexually transmitted infections.

that up to one-third of men between the ages of 30 and 40 harbor histological evidence of adenocarcinoma of the prostate (2). For men in their sixth decade of life in the USA the prevalence of the disease increases to ~60% (2). It has recently been hypothesized that a chronic process, such as inflammation, may be partly responsible for the apparent accrual of risk of prostate carcinogenesis as a man ages (3).

Recurrent or chronic inflammation has been implicated in the development of many human cancers, including those of the esophagus, stomach, liver, large intestine and urinary bladder (4). Nearly all of these malignancies have been associated with either a specific infectious agent and/or a defined environmental exposure. Inflammation, regardless of etiology, is thought to incite carcinogenesis by (i) causing cell and genome damage, (ii) promoting cellular replacement and (iii) creating a tissue microenvironment rich in cytokines and growth factors that can enhance cell replication, angiogenesis and tissue repair (5–7). Contemporary data from population and genetic epidemiology, molecular pathology and inflammatory toxicology seem to indicate that inflammatory-related processes are involved in the development of prostate cancer. Novel and established animal models of prostate inflammation should enable the systematic laboratory investigation of the hypotheses generated from this growing body of evidence. In this review we will discuss each of these aspects in detail and give evidence for the putative role of inflammation in prostate carcinogenesis.

Epidemiology

Prostatitis and prostate cancer

Several retrospective case-control studies have observed an association between clinical prostatitis and prostate cancer (8). All of these studies may be potentially influenced by biases, the two most notable of which are: patients with prostatitis are more likely to be followed by a urologist and thus may be more likely to be evaluated for prostate cancer (detection bias) and patients with prostate cancer may be more likely to remember or be willing to report a previous episode of prostatitis than men without prostate cancer (recall bias) (9). Epidemiological studies of prostatitis and prostate cancer may be further limited by a misclassification of a history of prostatitis, as an unknown proportion of prostatic inflammation is not associated with clinical signs and symptoms and, even when present, the clinical manifestations may be variable, leading to difficulties in diagnosis (10,11). Additionally, older men in particular may develop prostatitis with no apparent infectious component and some men may exhibit symptoms without the presence of inflammatory cells in the prostatic fluid (see below). Nevertheless, a recent meta-analysis found a small increase in the relative risk of prostate cancer in men with a prior medical history of clinical, or symptomatic, prostatitis (8).

Table I. Classification of prostatitis

NIH category ^a	Symptomatic	Acute versus chronic	Bacteria in EPS ^b	Leukocytes in EPS
I	Yes	Acute	Yes	Yes
II	Yes	Chronic	Yes	Yes
IIIa	Yes	Chronic	No	Yes
IIIb	Yes	Chronic	No	No
IV	No	Chronic	No	No

^aNational Institutes of Health classification of prostatitis.

^bExpressed prostatic secretions.

In an effort to better formalize the diagnostic criteria of prostatitis, the National Institutes of Health in 1999 issued a consensus statement (12). This report defined four distinct categories of prostatitis, three that are symptomatic and one that is clinically silent (see Table I). Category I prostatitis, or acute bacterial prostatitis, results from an acute bacterial infection, usually caused by *Escherichia coli* or other gram-negative species. Affected men can appear to be quite ill, with significant pelvic pain and signs of systemic infection (e.g. fever). Many men with category I prostatitis may be unable to urinate (i.e. urinary retention) as a result of prostatic swelling during the acute phase of infection. With appropriate antibiotic therapy category I prostatitis is usually a self-limited infection with no apparent long-term side-effects. Category II prostatitis, or chronic bacterial prostatitis, is the result of persistent bacterial infection despite antimicrobial treatment. Men with this type of infection complain of intermittent or constant pelvic pain but lack the manifestations of serious systemic infection. Diagnostic prostatic massage performed on these men to obtain expressed prostatic secretions often reveals prostatic fluid containing numerous leukocytes and bacteria. Prolonged antibiotic therapy with vigilance to the possibility of an abscess or of urinary obstruction is frequently needed to completely eradicate the infection. Category III prostatitis, also known as chronic non-bacterial prostatitis/chronic pelvic pain syndrome, is the most common form of prostatitis, accounting for >90% of cases (13). Men with this syndrome suffer from episodic to constant bouts of pain that can involve the pelvis, perineum or external genitalia. Analysis of expressed prostatic secretions from men suffering with this class of prostatitis, by definition, reveals no bacteria either by microscopy or laboratory culture. Prostate fluid from these patients may, however, show inflammatory cells (category IIIa) or can be devoid of leukocytes (category IIIb). Unfortunately, men with either type of class III prostatitis may experience symptoms for weeks to years. Current therapy, consisting of long-term antibiotics, non-steroidal anti-inflammatory drugs, α -adrenergic antagonists and 5- α reductase inhibitors, has had limited success in alleviating the debilitating pain experienced by those with this condition (14,15). Symptomatic prostatitis (categories I–III) has been reported to occur in ~9% of men between the ages of 40 and 79, with an estimated 1 in 11 chance that a man will be diagnosed with clinical prostatitis by the age of 79 (16). Category IV prostatitis, or asymptomatic prostatitis, is a histological diagnosis of prostate inflammation that is made on pathological examination of prostate tissue in a man with no symptoms of prostatitis. The vast majority of surgical prostate specimens (prostate biopsy, transurethral resection samples or radical prostatectomy specimens) contain some histological evidence of prostate inflammation, albeit to varying degrees (10,11,17).

Current data associating prostatitis with prostate cancer have focused only on categories I–III prostatitis. For instance, in a recent case–control study Roberts and colleagues observed a significant, albeit weak, association between a previous medical history of prostatitis (categories I–III) and prostate cancer (18). Since most epidemiological studies have relied upon patient report for information on prostatitis history, a relationship between category IV prostatitis and prostate cancer cannot be inferred from these reports (see below). It is possible that a distinguishing feature between symptomatic and asymptomatic prostatitis may be the anatomical location of prostatic inflammation (19). That is to say, inflammation in the periurethral area, or transition zone, may be more likely to manifest in urinary symptoms and, possibly, pain than inflammation in the outer areas of the gland, or the peripheral zone, where prostate cancer more commonly develops (20). Large-scale autopsy series that categorize areas of inflammation and cancer by intra-prostatic location and that correlate these findings with clinical history (i.e. urinary symptoms and history and frequency of symptomatic prostatitis) may shed some light on the association between the different types of chronic prostatitis and prostate cancer.

Sexually transmitted infections (STIs) and prostate cancer

As early as the 1950s Ravich and Ravich postulated that sexual transmission of a carcinogenic agent might explain observed differences in prostate cancer rates between men of varying religious backgrounds, owing to differences in circumcision practices among individuals with differing religious beliefs (21). Since then, several epidemiologic studies have been conducted to explore potential associations between STIs and prostate cancer (22). Initially these types of studies assessed STI exposure by self-report of sexual activity and STI history. Although a few of such studies were prospective, the majority featured case–control designs with retrospective assessment of STI exposure, thus allowing for a potential bias in recall between cases and controls (22). These studies may have been further affected by suboptimal patient interviewing methods, with inadequate blinding of interviewers to case–control status, thus introducing a potential bias (9). In a recent meta-analysis Dennis and Dawson reviewed these and other studies and calculated a relative risk of prostate cancer of 1.20 (95% confidence interval 1.11–1.30) for an average lifetime frequency of sexual activity of 3 times/week as compared with <1 time/week, 1.17 (1.05–1.30) for a history of 20 lifetime sexual partners, 1.19 (1.01–1.41) for ever visiting a prostitute, 1.44 (1.24–1.66) for a history of any STIs, 2.30 (1.34–3.94) for a history of syphilis (excluding the results from one cohort study) and 1.36 (1.15–1.61) for a history of gonorrhoea (22). Notably, the impact of antibiotics on once common STIs, such as gonorrhoea, has markedly reduced the frequency with which these genito-urinary infections may cause prostatitis.

More recently, epidemiological studies have begun to investigate associations between individual STIs and prostate cancer by serology, i.e. the detection of serum IgG antibodies against these agents (Table II). Five such studies have investigated human papilloma virus (HPV) serology and prostate cancer, one of which observed a statistically significantly higher risk of prostate cancer among HPV-16 seropositive and HPV-18 seropositive men, known high-risk serotypes for cervical cancer among women, but not among HPV-33 nor HPV-11 seropositive men (23,24). Two other studies observed a slightly but not statistically significantly higher HPV-16

Table II. Sexually transmitted infections and prostate cancer

Infection	Assay	Findings
Human papillomas virus 16	Serology	+/-
	<i>In situ</i> hybridization and PCR	+/-
Human papillomas virus 18	Serology	+
Human papillomas virus 33	Serology	None
Human papillomas virus 11	Serology	None
<i>Chlamydia trachomatis</i>	Serology	None
Syphilis	Serology	+/-
Herpes simplex virus 2	Serology	+/-
	Immunofluorescent staining, <i>in situ</i> hybridization	None
Human herpes virus 8 Cytomegalovirus	Serology	+/-
	Immunohistochemistry, PCR, <i>in situ</i> hybridization	+ ^a
Epstein-Barr virus	Immunohistochemistry, PCR	+ ^a

^aStudies lacked appropriate negative controls.

seropositivity among prostate cancer cases than among controls (25,26), while two additional studies found no association between HPV-16 seropositivity and prostate cancer risk when comparing prostate cancer cases with benign prostatic hyperplasia (BPH) or normal controls (27,28). It is important to note that while HPV is known to be an oncogenic virus, its influence on prostate carcinogenesis may be independent of inflammation.

To date, studies investigating *Chlamydia trachomatis* serology and prostate cancer have reported null results (24). Hayes and colleagues observed marginally statistically significantly higher odds of positive syphilis serology among prostate cancer cases than among controls (25). Two studies have investigated herpes simplex virus (HSV) 2 serology and prostate cancer, one of which detected significantly higher odds of HSV-2 serology among prostate cancer cases than among BPH controls (29), while the other observed no such association (30). Recently, Hoffman *et al.* conducted a case-control study of human herpes virus (HHV) 8 serology and prostate cancer, in which they observed significantly higher odds of HHV-8 seropositivity among prostate cancer cases than among controls in two study populations (31). A previous examination of HHV-8 seroprevalence among black men from South Africa, however, failed to reveal an association with prostate cancer (32). HHV-8 is of particular interest for prostate cancer because it encodes viral IL-6, a protein that shows homology to human IL-6. Viral IL-6 has been shown to promote growth of human cells *in vitro* and to activate the human IL-6 signaling cascade involved in inflammation (33).

Serological assessment has the advantage of assaying for an infection that may have resolved long before prostate cancer was detected. Put another way, the absence of histological evidence of an STI in prostate cancer specimens does not rule out a potential role of STI in prostate carcinogenesis. A possible disadvantage of serological testing for STIs is that a positive result does not necessarily mean that the prostate itself was infected by that particular organism, although this may be a reasonable assumption, in particular for agents that are frequently asymptomatic in men (e.g. *C.trachomatis*). Even so, actual prostate infection with an STI may not be required to induce an inflammatory response within the prostate. Infection anywhere within the genito-urinary tract may induce an inflammatory response in contiguous anatomic sites, including the prostate.

Another avenue that has been pursued to investigate associations between STIs and prostate cancer is tissue analysis (Table II). It is important to note that STIs detected in prostate cancer tissues could have been acquired after the initiation of prostate cancer. Samanta and colleagues recently noted the presence of human cytomegalovirus, a very common HHV (34), nucleic acids and gene products in patients with prostate intraepithelial neoplasia (PIN) and prostate cancer (35). No information was cited in this report concerning prior medical history of either prostatitis or STI. In an analysis of Epstein-Barr virus (EBV), also a common HHV (36), and prostate cancer Grinstein *et al.* observed that 37% (7/19) of prostate cancer cases evaluated displayed EBV by immunohistochemistry and PCR (37). Prior EBV infectious history was not discussed in this report. Tissue analyses of human HSV-2 in benign and cancerous prostate tissue have yielded null results (38-40). Several studies have examined the presence of HPV in prostate cancer tissue with varying outcomes. Utilizing PCR and *in situ* hybridization techniques, some have noted the presence of HPV-16 in up to 20% of prostate cancers (41-43). In contrast, a case-control study by Strickler *et al.* that employed two HPV serum antibody assays as well as two different PCR primer sets in two distinct ethnic populations did not demonstrate an association between HPV-16 tissue positivity and prostate cancer (27). Other negative tissue studies with regards to HPV-16 have also been reported (44,45). Differences related to tissue handling and detection method/technique, as well as possible tissue contamination by agents in neighboring areas (e.g. urethra), most likely account for the discordant results thus far reported regarding HPV-16 and prostate cancer (44). For infections to account for anything but a small proportion of the risk of prostate cancer in the US population the prevalence of the infection must be relatively high. Thus, infections such as CMV, HPV and HSV may be more important risk factors for prostate cancer than the now less common STIs such as syphilis and gonorrhea. Further investigation is needed before definitive conclusions can be stated regarding the link between STIs and prostate cancer.

Genetics

Prostate cancer is associated with the greatest heritable risk of any human cancer (46-48). However, unlike the relatively common somatic genetic alterations observed in colon cancer, such as *p53* and *K-ras* mutations, the molecular pathogenesis of prostate cancer displays a great deal of heterogeneity both between individuals as well as within an affected organ (49-51). The diversity of currently identified somatic genetic abnormalities associated with prostate cancer suggests that there is not a single dominant molecular pathway required for prostate carcinogenesis (3,51). To date, numerous germline prostate cancer susceptibility genes as well as somatic genome alterations (i.e. mutations, deletions, rearrangements, amplifications and DNA methylation) have been identified (Table III) (52-54). For a discussion of the genetic variations and abnormalities presented in Table III but not discussed below the reader is directed to the recent review by Nelson *et al.* (3).

RNASEL

Changes in activity of the proteins encoded by genes involved in the innate immune response may result in an inadequate ability to fight infection, lead to infectious agent-mediated damage and, possibly, persistent infection and

Table III. Germline variations and somatic genome alterations in prostate cancer

Gene	Location	Alteration	Function
<i>RNASEL</i>	1q24–25	Germline; base substitutions/deletions	Encodes interferon-inducible endoribonuclease involved in RNA degradation
<i>ELAC2</i>	17p11	Germline; base insertions/substitutions	Unknown
<i>MSR1</i>	8p22	Germline; base substitutions	Encodes subunits of class A macrophage scavenger receptor
<i>AR</i>	Xq11–12	Germline; polymorphic trinucleotide repeats Somatic; amplification and mutations	Encodes androgen receptor
<i>CYP17</i>	10q24.3	Germline; base substitution in promoter	Encodes enzyme cytochrome P-450c17 α
<i>SRD5A2</i>	2p23	Germline; base substitutions	Encodes 5- α reductase type 2
<i>GSTP1</i>	11q13	Somatic; CpG island hypermethylation	Encodes carcinogen detoxification enzyme
<i>NKX3.1</i>	8p21	Somatic; allelic loss	Encodes prostate-specific homeobox gene
<i>PTEN</i>	10q23.31	Somatic; allelic loss, mutations, probable CpG island hypermethylation	Encodes phosphatase active against protein and lipid substrates
<i>CDKN1B</i>	12p12–13	Somatic; allelic loss	Encodes p27, cyclin-dependent kinase inhibitor

consequent chronic inflammation. The *RNASEL* gene encodes a widely expressed latent endoribonuclease that is involved in interferon-inducible RNA degradation (55,56). Once activated by interferon, cells containing a functional *RNASEL* gene produce an enzyme that degrades single-stranded RNA, leading to apoptosis (57). This pathway is thought to be one method that cells utilize to combat viral infections. Mice bearing a homozygous deletion in the *RNaseL* gene display diminished anti-viral activity in response to interferon- α (58). Interestingly, *RNASEL* has been linked to the familial prostate cancer locus HPC1 on chromosome 1 (59). Carpten *et al.* reported that in one family four brothers with prostate cancer possessed a mutation resulting in a termination codon substitution at amino acid position 265 of *RNASEL* and that in another affected family four of six brothers with prostate cancer carried a base substitution at the *RNASEL* initiator methionine codon. The termination codon substitution at amino acid position 265 was detected by Rokman and colleagues in 1.8% of control men and in 4.3% of Finnish men with familial prostate cancer (60). Early studies of the *RNASEL* locus found an extremely low prevalence of this particular mutation in unaffected white males and no unaffected men with the previously described defect at the initiator codon (59). An analysis in the Ashkenazi Jewish population revealed a mutant *RNASEL* allele, consisting of a deletion at codon 157, in 6.9% of men with prostate cancer and in 2.9% of elderly men without prostate cancer (61). In addition, a less active *RNASEL* variant was observed to be associated with a higher risk of prostate cancer by Casey *et al.* (62). Notably, other studies thus far have failed to detect a significant relationship between *RNASEL* inactivating mutations and the risk of familial prostate cancer (63,64). Whereas the reasons for these discrepant results are unclear, and could include factors ranging from initial false positive results to lack of replication in subsequent studies due to differences in definition of control subjects or small sample sizes, it is tempting to speculate that genetic variants of *RNASEL* increase the risk for prostate cancer only in the presence of some environmental exposure (e.g. viral infection) that may vary from study population to population.

MSR1

Another component of the innate immune response is the macrophage scavenger receptor (MSR) 1, a macrophage plasma membrane spanning protein that is capable of binding

a variety of ligands, including bacterial lipopolysaccharide and lipoteichoic acid, as well as oxidized high-density lipoprotein and low-density lipoprotein in the serum (65). The *MSR1* gene is located on 8p22, an area of frequent allelic loss in prostate cancer (66). Mice deficient in *Msr* are highly susceptible to infection by *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and HSV1 (67). Several germline *MSR1* mutations have been observed in some families with hereditary prostate cancer (66). Of these, the nonsense mutation Arg293X has been detected in ~3% of men with non-hereditary prostate cancer compared with a prevalence of 0.4% in unaffected men (66). Areas within the prostate that show evidence of inflammation are often populated by macrophages that express MSR1 (3). Notably, there is one study that found no association between *MSR1* germline mutations and prostate cancer risk (68). In a case-control study sporadic prostate cancer cases had statistically significantly different allele and haplotype distributions for five polymorphisms compared with prostate-specific antigen (PSA)-screened controls (69); although the frequency of Arg293X was higher in cases than controls in a large Swedish study, no association with these five sequence variants was observed (70).

GSTP1

The π class glutathione S-transferase (*GSTP1*) gene encodes an enzyme that acts as a carcinogen detoxifier (71). *GSTP1* has been described as a 'caretaker' gene, as it actively protects the cell from oxidative genome damage mediated by carcinogens and electrophilic compounds (72,73). Cells devoid of *GSTP1* accumulate oxidized DNA bases in response to oxidative stress, a situation that may occur at sites of inflammation (unpublished data). Mice that are null for *Gstp1* at both alleles do not spontaneously develop tumors, although male mice beyond the age of 6 months can attain much greater body sizes and weights in comparison with wild-types (74). After exposure to a topical skin carcinogen (7,12-dimethylbenz-[a]anthracene), however, *Gstp1*^{-/-} mice display a strong tendency to develop skin papillomas at a frequency significantly higher than control animals (74). Hypermethylation of CpG island sequences in the promoter region of *GSTP1*, a somatic genome alteration, is an exceedingly common early epigenetic event in prostate cancer, occurring in >90% of cases (72,75). In normal prostate epithelium *GSTP1* expression is generally confined to the basal cell compartment. Benign luminal or columnar cells may be induced to express *GSTP1* in the face of environmental stress, a finding characteristic of the

histological lesion proliferative inflammatory atrophy (PIA) (76). Malignant prostate epithelium, however, almost universally does not express *GSTP1* due to CpG island hypermethylation in the promoter region of the *GSTP1* gene. (77). It has been hypothesized that chronic consumption of certain dietary carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), that are produced by charring protein-rich foods such as red meats, may increase a man's risk of developing prostate cancer (78,79). *In vitro* PhIP exposure causes the production of promutagenic PhIP-DNA adducts in LNCaP cells, a cell line that does not normally express *GSTP1* (78). Stable transfection of LNCaP cells with *GSTP1* in this model abrogates the development of these harmful adducts seen with PhIP treatment. Using a similar *in vitro* model DeWeese *et al.* have demonstrated that low dose radiation causes unmodified LNCaP cells to accumulate more oxidized DNA bases and to experience a rate of cell death that is significantly less than that of LNCaP cells overexpressing *GSTP1* (unpublished data). This peculiar finding of tolerance to oxidative genomic damage in the absence of functional *GSTP1* may be a clue to the function of somatic *GSTP1* inactivation in prostate carcinogenesis. Studies examining the association of *GSTP1* polymorphic variants have yielded null results (80).

NKX3.1

NKX3.1, located at 8p21, encodes a prostate-specific homeobox gene that is essential for normal prostate development (81,82). *NKX3.1* has been noted to bind DNA and repress *PSA* gene transcription (83). Heterozygous or homozygous deletion of *Nkx3.1* in mice leads to prostate dysplasia and lesions resembling PIN (84). In men, loss of heterozygosity at polymorphic 8p21 sequences has been noted in as many as 63% of PIN lesions and 90% of prostate cancers (85,86). Despite allelic loss in this region, somatic mutations of *NKX3.1* have yet to be identified in a single case of prostate cancer, hence calling into question the role of *NKX3.1* inactivation in prostate cancer development (87). Nevertheless, loss of *NKX3.1* expression in prostate cancer tissue has been reported to be associated with disease progression (88). In their report Bowen *et al.* noted diminished *NKX3.1* expression in as many as 20% of PIN lesions, 6% of low stage prostate cancers, 22% of high stage prostate cancers, 34% of androgen-independent prostate cancers and 78% of prostate cancer metastases. As *NKX3.1* plays an important role in prostate development, one might speculate that chronic inflammatory injury might potentiate abnormal prostate gland regeneration in the setting of absent or diminished *NKX3.1* activity. The relationship between somatic *NKX3.1* genomic alterations and reduced *NKX3.1* expression in the context of prostate carcinogenesis is, however, yet to be determined.

Molecular pathology

Proliferative inflammatory atrophy (PIA)

Focal prostatic epithelial atrophy has long been known to be a relatively common finding in the periphery of the prostate of aging men (89,90). Recently, novel observations regarding this pathological entity have brought renewed attention to the potential clinical significance of these lesions (76). Many of these areas of epithelial atrophy are associated with acute or chronic inflammation, contain proliferative epithelial cells and occur diffusely in the anatomic peripheral zone of the

prostate where prostate cancers predominantly develop (91-93). Furthermore, such areas may be contiguous with high grade PIN lesions or small volume cancers and may be commonly seen in the prostates of older men (94). Because of the high proliferative index of the epithelial cells in these lesions as well as the frequent association with inflammation, the term PIA has been used to describe these foci of inflammation (76). PIA represents a diverse spectrum of morphological patterns of prostate atrophies, including simple atrophy and post-atrophic dysplasia, and is currently the subject of an international classification project.

Substantial evidence of the putative role of PIA as a prostate cancer precursor lesion has been reported. For instance, focal areas of epithelial atrophy accompanied by inflammation have been proposed to be involved in the development of prostate cancer in a rodent model (95,96). Also, it has been noted that gain of the centromeric region of chromosome 8, as determined by fluorescence *in situ* hybridization, can be seen in human PIA, PIN and prostate cancer (92,97). In addition, *p53* mutations have been detected in ~5% of post-atrophic hyperplasia lesions, a variant of PIA, a rate similar to that seen in high grade PIN (98). Moreover, Nakayama *et al.* observed that ~6% of PIA lesions show evidence of *GSTP1* promoter CpG island hypermethylation (94). Thus, PIA lesions appear to share at least some of the genetic characteristics of prostate cancer. One may hypothesize then that areas of PIA result from epithelial damage due to local ischemia, infection and/or toxin exposure (endogenous or exogenous), followed by epithelial regeneration and inflammation. Indeed, the inflammatory response itself can lead to oxidative damage of epithelial cells (5). Whatever the cause, luminal columnar cells in areas of PIA display high levels of *GSTP1*, *GSTA1* and cyclooxygenase 2 (COX-2) expression, signifying cellular stress (76,99,100).

The evidence linking PIA to prostate cancer is not beyond question. Not all PIA lesions are associated with cancer and concordantly not all cancers occur within or adjacent to regions of PIA (101,102). Our current working model describes PIA as a response to microenvironmental stress experienced by normal prostate epithelial cells (Figure 1). Individual regions of PIA that are unable to adequately defend themselves against oxidative genome damage may subsequently progress to PIN or prostate cancer. In this model it is hypothesized that many, although not all, high grade PIN lesions may develop by first proceeding through a period of atrophy. In this scenario some atrophy lesions associated with inflammation would progress to cancer by first going through a step of high grade PIN. Other times, we speculate that atrophy can directly proceed to carcinoma, without a prior phase of high grade PIN. Another subset of cancers appear to develop from PIN lesions without associated atrophy, while still others may manifest without any evidence of nearby precursor lesions. Finally, some low grade transition zone carcinomas have been proposed to arise from adenosis (atypical adenomatous hyperplasia) (103-105).

Inflammatory toxicology

Approximately one-quarter of all malignancies are thought to arise in part due to chronic inflammation (4,6). The initiating event often involves an infectious agent or environmental toxin, although this may not always be the case. Once activated, how does the inflammatory response potentiate carcinogenesis? Inflammation is a complex phenomenon,

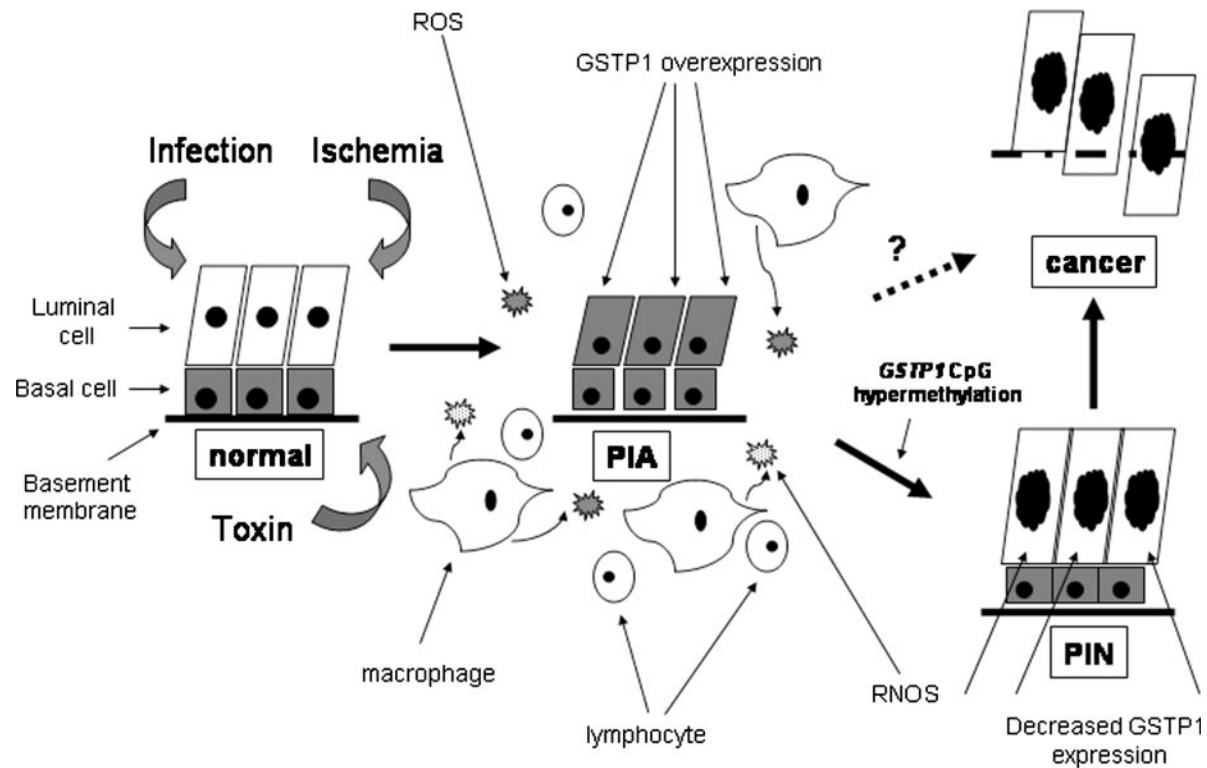


Fig. 1. Proposed paradigm of proliferative inflammatory atrophy (PIA). Our hypothesis is that chronic insult to normal prostate epithelial cells, due to infection, ischemia or a toxin (endogenous or exogenous), can result in the influx of inflammatory cells (lymphocytes and macrophages), increased expression of carcinogen-detoxification enzymes such as *GSTP1* (represented in gray) in luminal cells and induce histological changes that resemble atrophy (i.e. shrunken appearing cells with compact nuclei). Normally, basal epithelial cells express *GSTP1* (represented by gray shading) whereas luminal cells do not (represented by no shading). Inflammatory cells may produce reactive oxygen and nitrogen species (ROS and RNOS, respectively) that can cause further epithelial cell damage. Selective PIA lesions that sustain *GSTP1* hypermethylation (clear cells), resulting in decreased expression of *GSTP1*, may progress to prostatic intraepithelial neoplasia (PIN). PIA lesions have been found to occur in continuity with prostate cancer, suggesting a possible direct relationship between these two entities.

consisting of humoral (cytokines and chemokines) and cellular components (leukocytes, lymphocytes and granulocytes). The purpose of the inflammatory response is thought to be the creation of a tissue microenvironment that promotes the recognition and repair of cellular damage as well as the eradication of foreign particles, infected cells and irreparably damaged cells (4). The primary mediators of the non-specific host immune defense system are free radicals, predominantly oxygen and nitrogen species (106). Hydroxyl radical (OH^\bullet), peroxynitrite (ONOO^-) and nitric oxide (NO^\bullet) are the reactive oxygen species (ROS) and reactive nitrogen oxide (RNOS) species most commonly linked to the deleterious oxidative effects of inflammation (107). These reactive species can alter protein structure and function, cause lipid peroxidation and induce somatic gene changes (108). Free radicals have been shown to cause post-translational modifications of several key proteins, including those involved in DNA repair, apoptosis, cell signaling and essential enzymatic pathways (109–111). Experimental non-prostatic models of chronic inflammation have revealed that NO^\bullet is able to cause structural changes to p53 that can affect its function (112). It is not difficult, therefore, to expect substances like free radicals to promote carcinogenesis. Lipid peroxidation creates the reactive aldehyde species malondialdehyde and can trigger the prostaglandin synthesis pathway via activating COX-2 (4,113). Several studies have observed increased expression of COX-2 in PIA and prostate cancer lesions in comparison with benign tissue, at both the mRNA and protein levels

(100,114). In addition, COX-2 reactions themselves can produce ROS that can cause further cellular and genomic damage (5,114). Contrastingly, decreased expression of COX-2 via CpG island promoter hypermethylation has also been noted in cases of prostate cancer (115).

Since the 1950s, when Phillips first demonstrated that hydrogen peroxide can cause chromosome fragmentation, the impact of ROS on genomic integrity has been aggressively studied as a potential cause of cancer (116). ROS and RNOS can produce oxidized DNA bases (i.e. 8-oxo-deoxyguanine), initiate mutations, form DNA adducts and produce DNA strand breaks (117). The *OGG1* gene codes for a protein that is involved in repair of the oxidized DNA base 8-oxo-deoxyguanine (111). Interestingly, polymorphisms at this locus have been associated with an increased risk of prostate cancer (118). Also, *GSTP1* inactivation, a near ubiquitous finding in prostate cancer, may expose affected prostate epithelial cells to sustained electrophilic and oxidative stress during episodes of inflammation, thus increasing genome damage and compounding the likelihood of cellular transformation (119). Free radical-related genetic damage amassed over time may provide a fertile environment for tumorigenesis. Recently, data on NO^\bullet have emerged that demonstrate that this radical species can set off pathways that, paradoxically, can either initiate carcinogenesis or protect the cell from developing malignant characteristics, depending on the biological and genetic environment (120–122). While the role of free radical injury and inflammation has been extensively studied in the

development of other human cancers, such as the colon and stomach, relatively little is currently known about these factors with relation to prostate carcinogenesis. Clearly, the transforming role of ROS and RNOS is complex and deserves further study with regards to the initiation and progression of prostate cancer.

Animal models of prostatic inflammation

Laboratory models are essential for studying the impact of prostatic inflammation on carcinogenesis. While a number of animal models of prostate cancer exist, accompanying inflammatory changes in these models have been sparsely commented upon (123,124). Fortunately, several rodent models of prostate inflammation are available, each with their own unique features (Table IV).

Spontaneous prostatitis

The majority of Wistar, Lewis and Copenhagen rats develop spontaneous non-acute prostatitis in the lateral lobes as early as 13 weeks of age (125,126). This process is age-dependent and is characterized by infiltration of the stroma with lymphocytes and macrophages (127). Bacterial infection may accompany the finding of prostatitis in this spontaneous model, possibly incurred through bacterial accumulation due to acinar obstruction caused by sloughed cellular residue (128). Interestingly, Sharma *et al.* were able to abrogate spontaneous inflammation by treating rats with a diet rich in soy protein for 11 weeks (129). A novel hypothesis on the etiology of spontaneous prostatitis in rats was recently proposed by Keith *et al.* (127). Their results suggest that mast cells may play an important role in creating a prostatic environment that is conducive to spontaneous inflammation.

Hormone-associated prostatitis

Treatment of adult Wistar male rats with 17 β -estradiol for 30 days has been shown to increase the intensity of

spontaneously occurring lateral lobe prostate inflammation (128). This effect was shown to be significantly attenuated when testosterone was administered simultaneously with 17 β -estradiol to adult Wistar rats, but was not altered by concomitant dihydrotestosterone treatment. Curiously, castration of adult rats seemed to have the same effect as adult rat testosterone administration in these experiments (128). In addition, Naslund *et al.* were able to induce ventral lobar inflammation by treating neonatal Wistar rats with 17 β -estradiol for 2 days and then with testosterone for 14 days in adulthood. This combination of hormonal manipulation produced severe non-infectious prostatitis in both lateral and ventral lobes. Stoker *et al.* demonstrated that perinatal treatment with 17 β -estradiol alone was enough for the development of severe lateral lobe prostatic inflammation in adulthood and that a similar, albeit less common, inflammatory response could be generated by treatment with tamoxifen in the same fashion (130). Recent experiments with this same rodent model have suggested that the prostatitis induced by such hormonal treatments may be autoimmune in origin (131). Seethalakshmi and colleagues harvested splenocytes as well as pure T cells from adult male Wistar rats that were given 17 β -estradiol, as described above, and then adoptively transferred the retrieved cells into syngeneic rats. Recipient rats developed histological prostatitis as evidenced by lymphocytic stromal infiltration and the presence of degranulated mast cells, implicating the immune system in this model of prostatitis. In an effort to further elucidate the mechanisms of prostatic inflammation in hormone-induced rodent models Gilleran *et al.* exposed neonatal Sprague–Dawley rats to either estradiol or estradiol and bromocriptine (a peptide that blocks the release of prolactin by the pituitary gland) (132). They noted that administration of estrogen alone caused a significant prostatic infiltration of CD4+ and CD8+ lymphocytes that was mitigated by bromocriptine. These authors also observed, however, that the estrogen-induced macrophage density within the prostate was not affected by bromocriptine treatment (132). Such

Table IV. Animal models of prostate inflammation

Method of induction	Species	Strain	Phenotype
Spontaneous	Rat	Wistar, Lewis, Copenhagen	Age-dependent lateral lobe prostatitis as early as 13 weeks of age; lymphocytes and macrophages; occasional concomitant bacterial infection
Hormone manipulation	Rat	Wistar, Sprague–Dawley	17 β -estradiol given to adult Wistar rats increases severity of spontaneous prostatitis; when testosterone is simultaneously given prostatitis is attenuated; if 17 β -estradiol is given in neonatal period and testosterone is given in adult life, ventral and lateral lobes develop inflammation with lymphocyte/macrophage infiltrates; reducing elevated prolactin levels in these models only partially reduces inflammation; evidence that prostatitis induced in this way is adoptively transferable
Immune modulation	Rat Mouse	Wistar C57/bl6, SJL, AJ, SNF1, NOD, C3H/HeMs \times 129/J F ₁	Vaccination of homogenates of prostate or male accessory gland combined with complete Freund's adjuvant induces lymphocyte/macrophage prostatitis for >1 month and is adoptively transferable; day of life 3 thymectomy in certain mouse strains produces prostatitis
Infection	Rat	Wistar	Transurethral prostatic introduction of <i>Escherichia coli</i> generates acute (neutrophil) and chronic (macrophage/lymphocyte) model of prostatitis, may require repeated instillation; single injection of <i>Chlamydia trachomatis</i> into vas deferens may generate chronic model
Irritant	Rat	Sprague–Dawley	Transurethral instillation of ethanol and dinitrobenzenesulfonic acid; diet high in isoflavone induces dorsal/lateral lobe prostatitis (lymphocytes/neutrophils)

studies seem to suggest that the elevated prolactin levels that result from estrogen treatment are only partially responsible for the prostatic inflammatory response seen in this model. Interestingly, experiments on Wistar dams (lactating females) treated with atrazine, a herbicide known to suppress prolactin in adult female rats, resulted in not only lower prolactin levels in Wistar dams but also increased severity of prostate inflammation in adult male progeny that suckled from treated females (133). This study also demonstrated that reversal of this phenotype was possible if prolactin was administered to male offspring before post-natal day 9, but not if it was supplemented thereafter. Experiments performed with any of the hormonally induced models of prostate inflammation that aim to study prostate carcinogenesis need to account for the complex hormonal, immune and temporal issues at work in these systems.

Immune-induced prostatitis

Taguchi *et al.* reported in the mid 1980s one of the first rodent models of autoimmune prostatitis (134). They demonstrated that 68% of C3H/HeMs \times 129/J F₁ male mice that were thymectomized on day 3 of life developed autoimmune prostatitis after the onset of puberty. This inflammation was confined to the prostate and consisted of a robust lymphocytic infiltrate in the stroma as well as high prostate auto-antibody titers in the serum. Moreover, the authors observed that performing a thymectomy on day 0 or 7 of life did not reproduce this phenotype and that castration at birth eradicated it. The inflammatory reaction was regained in castrated mice, however, if the mice were treated with testosterone during early adulthood. More recently, Bagavant *et al.* reproduced this same autoimmune prostatitis condition by performing day of life 3 thymectomy in a lupus-prone mouse strain (SNF1) (135). Inflammatory reactions in the prostate have also been produced by immunizing rodents with tissue extracts combined with complete Freund's adjuvant. Both prostate and male accessory gland homogenates have been used in C57/bl6, SJL and AJ mice and in Wistar rats to produce autoimmune prostatitis (136,137). Prostatic stromal infiltrates generated with this technique are lymphocytic and mononuclear in composition and can be sustained for >1 month after vaccination. In addition, prostatic inflammation induced in this way is adoptively transferable to non-immunized syngeneic rodents (138). Studies of this model of prostatitis in Wistar rats have suggested that inflammatory damage induced in this setting is the result of increased oxidative metabolism of infiltrating macrophages (139).

Non-obese diabetic (NOD) mice are prone to develop inflammatory reactions in multiple organ sites, including the salivary gland, lachrymal glands, thyroid, parathyroids and adrenal cortex (140). Rivero and associates have demonstrated that after vaccination with male accessory gland extracts and complete Freund's adjuvant 100% of NOD male mice developed severe prostatitis in the dorsal/lateral lobes (141). This response was also observed to occur earlier after immunization than in Wistar rats or C57/bl6 mice. Further experiments by this same group have suggested that CD4⁺ cells are essential for prostate inflammation in NOD mice and that vaccination of a specific steroid-binding protein, prostatein, and complete Freund's adjuvant in this model is capable of eliciting a very similar inflammatory response (142).

Infection-associated prostatitis

To create an infection-based animal model of prostatitis, an infectious organism must be introduced into the prostate through either a direct injection into the prostate or a contiguous urogenital organ or from direct contact by means of transurethral prostatic instillation. Nickel *et al.* created a model of chronic prostatitis by utilizing a standardized protocol of instilling *E.coli* into the prostatic urethra of rats via a calibrated urethral catheter (143). This technique has subsequently been reproduced by other groups (144). Further work by Rippere-Lampe and associates has noted increased efficiency in the creation of prostatic neutrophil infiltrates in this model if *E.coli* with the virulence factor cytotoxic necrotizing factor type 1 is used (144). Injection of *Chlamydia psittaci* into the vas deferens of rats has also been used to create prostate inflammation (145). With this method, Jantos *et al.* were able to provoke an inflammatory reaction in treated rats after a single *Chlamydia* inoculation that peaked at 14 days and was still evident at 60 days post-injection. Seo *et al.* reported an efficiency of 62% for generating histological and microbiological evidence of chronic prostatitis following 4 weeks of repeated transurethral instillation of *E.coli* (serotype Z17, O2:K2:H-) (146). Additionally, they showed that while castration and estrogen therapy only mildly improved the observed prostatitis created by *E.coli* instillation, treatment with either the 5- α reductase inhibitor finasteride or the antibiotic levofloxacin significantly improved the observed prostatic inflammation as well decreased bacterial growth. Infection-based animal models of prostate inflammation must contend with several critical issues: (i) controlled delivery of the organism to the prostate with minimal surrounding tissue disturbance; (ii) creation of an inflammatory prostatic infiltrate that resembles what is commonly found in human prostate tissue (i.e. lymphocytes, mononuclear cells and macrophages); (iii) avoidance of a locally aggressive or serious systemic infection.

Other prostatitis models

Lang *et al.* have shown that transurethral prostatic instillation of ethanol and dinitrobenzenesulfonic acid, as opposed to either agent alone, can induce histological prostatitis for up to 48 h after treatment with concordant evidence of elevated prostate tissue interleukin-1 β levels (147). Recently, Kwon *et al.* reported on studies where 8 week old male Sprague-Dawley rats were fed a diet supplemented with 300 mg/kg of a soy-isoflavone mixture (60% genistein, 19.6% daidzein) daily for 9 weeks (148). They noted that 80% of the treatment group developed significant inflammatory infiltrates of the dorsal and lateral lobes, consisting of neutrophils and lymphocytes. There were no statistically significant differences in serum testosterone and dihydrotestosterone concentrations between the treated group and control animals. The authors speculated that the observed inflammation may be attributable to the weak estrogenic effects of isoflavonoid compounds (148). Overexpression of fibroblast growth factor 8 isoform B driven by a probasin promoter in transgenic mice has been shown to lead to the development of high grade PIN in the dorsal and ventral lobes of 50% of mice (149). Interestingly, Song *et al.* have observed chronic inflammation with T cells in a significant proportion of the mice with high grade PIN (149).

Modulation of inflammation in animal models of prostate cancer

Known modulators of inflammation have had varying effects on prostate carcinogenesis in animal models. For example, in the TRAMP model of prostate cancer, where probasin promoter-driven SV40 T-antigen expression induces prostate adenocarcinoma, immunotherapy consisting of irradiated tumor cell vaccine and anti-CTLA-4 antibodies has been shown to markedly decrease the incidence of prostate tumors (15 versus 75% control) (150). CTLA-4 is a T cell surface antigen that plays an important role in attenuating T cell activity (151). Mice treated with anti-CTLA-4 antibodies display a robust immune response to a variety of antigens (152). Tumor abrogation in this application of the TRAMP model therefore is, not surprisingly, accompanied by increased lymphocytic inflammatory infiltrates. Further studies with the TRAMP model by Gupta *et al.* have demonstrated a remarkable reduction in the rate of prostate tumorigenesis and metastasis formation between TRAMP mice fed a control diet and those fed a diet supplemented with the COX-2 inhibitor celecoxib (100 versus 25% and 65 versus 0%, respectively) (153). These findings suggest a critical role for the pro-inflammatory enzyme COX-2 in prostate cancer development and progression in the TRAMP model. Interestingly, the putative anti-inflammatory drug finasteride has been shown to inhibit the formation of spontaneously occurring prostate tumors in ACI rats (154). Homma *et al.* have noted the interesting finding that rats fed finasteride at a dose of 20 p.p.m./day for 60 weeks versus those fed 200 p.p.m./day for 60 weeks or a control diet had a 15% reduction in prostate tumor incidence. No differences with respect to inflammation severity were noted between the groups. These findings may have special relevance for future human prostate cancer trials with finasteride (155,156). As we have seen, it is critical to match drug mechanism with animal model system when evaluating the inflammatory modulating ability of specific compounds. Any of the above discussed methods of inducing inflammation may be used to study prostate carcinogenesis in established animal models (123,124). Future studies that focus on the inter-relationship between inflammation and cancer development, via the examination of known animal models of prostate cancer for inflammation as well as the evaluation of established animal models of prostatitis for cancer modulation, may provide an interesting outlook on prostate carcinogenesis. It is intriguing to consider what may result from inducing prostate inflammation in animal models that are known to develop PIN-type lesions (i.e. *nkx3.1*-deficient mice) (157). Given the hypothesis that chronic inflammation in the setting of a specific molecular defect may lead to prostate carcinogenesis, the induction of inflammation in genetically altered animal models may be of value. In humans prostatic inflammation over years, perhaps decades, is thought to modulate carcinogenesis. The precise duration of inflammation needed to modify prostate carcinogenesis in animal models has yet to be determined.

Current and/or future human studies might help address the role of inflammation in prostate carcinogenesis by assessing human sera and tissue for biomarkers of inflammation, examining the association of specific genetic variations and abnormalities with the presence of prostate cancer and studying the incidence of prostate cancer in clinical trials involving anti-inflammatory drugs. One might speculate that a large trial that randomized men before the peak incidence of prostate

cancer (i.e. 40–45 years of age) to receive either an anti-inflammatory drug or placebo chronically for a significant duration (i.e. ~5–10 years) might be instructive. At this time, however, the optimal drug, dose and duration of treatment for this type of trial are unknown.

Conclusion

Novel insights into the development of human prostate cancer have recently emerged that implicate the process of chronic inflammation in prostate carcinogenesis. Epidemiological studies of prostatitis and STIs and genetic epidemiological investigations of key somatic genetic alterations and germline variants have formed the foundation of the proposed link between inflammation and prostate cancer. Advances in molecular pathology and in our understanding of inflammatory toxicology have bolstered this hypothesis even further. Animal models of prostate inflammation provide a unique laboratory venue in which the development of prostate cancer can be studied. The future examination of known animal models of prostate cancer and prostate inflammation, with an eye towards characterizing the relationship between the two, may uncover new perspectives on prostate carcinogenesis and reveal novel targets for prevention and therapy.

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Received July 29, 2004; revised and accepted October 12, 2004