Inflammation, prostatitis, proliferative inflammatory atrophy: 'Fertile ground' for prostate cancer development?

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Abstract. Inflammatory processes caused by chemical, physical or biological agents are known to be important cofactors in the pathogenesis of human cancer. In the prostate, epithelial tissue damage followed by cell regeneration in the presence of inflammation is believed to be a key event in neoplastic transformation. According to the 'injury and regeneration' model, inflammatory cells infiltrating the prostate release reactive species in response to bacterial/viral infection, uric acid, or dietary prostate carcinogens. Besides inducing inflammation, tissue injury by these and other agents would promote the appearance of proliferative inflammatory atrophy (PIA). A subset of proliferating atrophic cells – possibly showing stem-cell features – may be exposed to the genotoxic insult of free radicals and to an increased rate of mutations and chromosomal aberrations, ultimately leading to neoplastic initiation, promotion and progression. In the last decade, the link between inflammation and cancer and the hypothesis pointing to PIA as a risk lesion for prostate cancer have been extensively investigated at the pre-clinical, clinical, morphological, cellular and molecular levels. In this article, recent reports describing supportive or negative evidence on the link between prostate inflammation, atrophy and cancer are schematically reviewed.

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1. Inflammation, proliferative inflammatory atrophy and the 'injury-and-regeneration' model of prostate carcinogenesis

Inflammatory processes caused by chemical, physical or biological agents are known to be important cofactors in the pathogenesis of several human cancers. Representative examples of the implication of inflammation in cancer are schistosomiasis-associated bladder cancer, ulcerative colitis-associated colorectal cancer, hepatitis/cirrhosis-associated liver cancer and helicobacterial gastritis-associated stomach cancer. Whereas acute and massive inflammatory processes may lead to cancer rejection by immune cells, milder chronic inflammation appears to facilitate carcinogenesis and tumor progression. This process is based on the interplay between tumor and tumor-associated cells (e.g., M2 macrophages) and involves inflammatory cytokines such as TNF-α, IL1β or IL6, ultimately promoting tumor cell proliferation and progression, stromal deposition and remodeling, tumor angiogenesis and the depression of adaptive immunity (1,2).

In the prostate, epithelial tissue damage followed by cell regeneration in the presence of inflammation is believed to be a key event in neoplastic transformation. According to the 'injury and regeneration' model (3), inflammatory cells and macrophages infiltrating the prostate release nitrogen and reactive oxygen (ROS) species in response to bacterial/viral infection, to endogenous irritant agents such as uric acid, or to dietary prostate carcinogens such as 2-amino-1-methyl-

6-phenylimidazo[4,5-b]pyridine (PhIP; Fig. 1) (3,4). Besides inducing inflammation, tissue injury by these and other agents would trigger epithelial cell regeneration and proliferation, and would lead to the appearance, in place of normal secretory glands, of different kinds of morphologically-diverging glandular structures, collectively named 'proliferative inflammatory atrophy' (PIA) (4).

Prostatic epithelial atrophy is cytologically characterized by decreased cell volume and by an increase of the nucleo-cytoplasm ratio, which results in cells with cuboidal features. Atrophy can involve the prostate in a uniform or patchy manner, and is commonly classified as diffuse or focal. Diffuse atrophy is a regressive process that occurs following total androgen blockade, characterized by a prominent basal cell layer underlying cuboidal cells as well as cytoplasmic vacuolization. Focal atrophy occurs in the form of heterogeneous patches and usually lacks a prominent basal cell layer. However, at times basal cells may become hyperplastic in the peripheral zone of the prostate. Basal cell hyperplasia can be found in association with atrophy, and is frequently adjacent to inflammatory infiltrates (5). As in cancer and in prostatic intraepithelial neoplasia (PIN), focal atrophy is localized mainly within the peripheral zone of the prostate gland. In the past, focal atrophy was classified as simple atrophy (with and without cyst formation variants), sclerotic atrophy and postatrophic hyperplasia (lobular hyperplasia and sclerotic atrophy with hyperplasia variants). Recently, a revision has been made in an attempt to recognize other variants as histologically distinct, such as simple atrophy with cyst formation and partial atrophy, with the goal of minimizing interobserver variability. This could facilitate epidemiological studies of atrophy and cancer (6).

The new system classifies focal atrophy into four distinct morphological entities: simple atrophy, postatrophic hyperplasia, simple atrophy with cyst formation, and partial atrophy. The first two are considered proliferative lesions in a strict sense.

In simple atrophy, at low magnification, the acini are irregular in shape and may be angulated, and are distributed in a configuration similar to that of normal epithelium. Some acini may be dilated, and all lack vascularized papillary infoldings. At high-power magnification, the luminal cells appear cuboidal with scant cytoplasm, which is most often darkly staining (Figs. 2 and 3). In the stroma, chronic inflammation is almost always present, although to quite variable extents. Postatrophic hyperplasia (PAH) consists of a lobular collection of small and round acini, often budding from a central, dilated and atrophic duct. These features are similar to normally-appearing resting breast lobules (PAH is also referred to as 'lobular atrophy'). The glands are lined by low cuboidal cells with scant cytoplasm. This lesion is defined as hyperplastic, since there are usually a large number of small glands within the lesions. Some of the cells may show mild to moderate nucleolar enlargement, thus at times creating serious difficulties in differentiating PAH from carcinoma. Chronic inflammation is frequently detectable in the stroma. Simple atrophy with cyst formation is characterized by rounded glands, in which the luminal cells contain small amounts of clear cytoplasm, that may be either large cyst-like acini (diameter >1 mm), or acini of smaller diameter. In these lesions the intervening stroma is scanty, and very little inflammation is present. Partial atrophy is the fourth category of focal atrophic lesions and represents a peculiar subtype. It is identified by architectural and cytological features. The amount of cytoplasm is less abundant than in normal cells, but more conspicuous than in other subtypes of atrophy. The architectural arrangement of partial atrophy can be similar to simple atrophy or postatrophic hyperplasia. In some lesions, it is possible to recognize fully atrophic acini. It has been proposed that partial atrophy is a separate entity from PIA, since it shows a cytological, molecular and proliferative profile more similar to benign glands, and occurs mostly in the absence of inflammation (6,7).

According to the proposed injury and regeneration model (reviewed in ref. 3), these atrophy lesions arise as a result of epithelial genotoxic injury induced by inflammation (generating reactive oxygen and nitrogen species), environmental toxins (e.g., from the diet) or a combination of both. The injury results in a regenerative response to replace the damaged/killed epithelial cells, and the regenerative lesions have the morphological appearance of the various forms of focal atrophy. Continued exposure to the genotoxic insults of ROS/dietary/environmental toxins results in an increased rate of mutations and chromosomal aberrations in a subset of proliferating atrophic cells – possibly showing a number of features typical of stem cells and a functional intermediate phenotype between basal and secretory cells – and ultimately leads to neoplastic initiation, promotion and progression (3).

De Marzo and coworkers suggested that atrophic cells exposed to massive oxidative DNA damage may subsequently progress to the *in situ* cancer precursor PIN (3). However, this hypothesis does not exclude direct transition from atrophy to carcinoma, nor carcinoma development from high-grade PIN (HGPIN) lesions without associated atrophy.

After the formulation of the injury and regeneration model, the link between inflammation and cancer and the hypothesis pointing to PIA as a risk lesion for prostate cancer (PCa) were extensively investigated at the pre-clinical, clinical, histomorphological, cellular and molecular levels. In the following paragraphs, a selection of recent reports describing supportive or negative evidence for the link between prostate inflammation, atrophy and cancer are schematically presented.

2. Pre-clinical evidence

PhIP, the most abundant heterocyclic amine found in cooked meats and cigarette smoke condensate, has been implicated as a major prostate carcinogen. PhIP is known to act by forming DNA adducts (Fig. 1) (8) and to induce mutations in rat prostate epithelial cells, as well as in mammary glands and in the intestine when administered experimentally (9). In animals exposed to PhIP, Borowsky and coworkers reported significantly more inflammation and the onset of focal atrophy in the prostate as compared to untreated controls. Notably, PIN was shown to occur only in PhIP-treated animals, predominantly within atrophic areas in the ventral prostate (9). Moreover, in the PhIP rat PCa model, inflammation decreased as atrophy increased. On the basis of this evidence, the authors proposed a model based on sequential transition from inflammation to atrophy to PIN. In the same experiment,

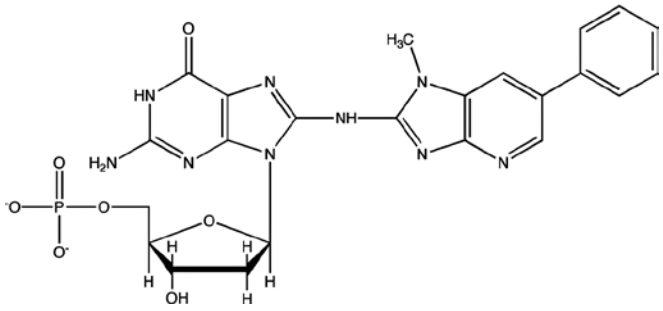

Figure 1. Chemical structure of the C8-deoxyguanosine monophosphate adduct of the dietary prostate carcinogen 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant heterocyclic amine found in cooked meats and cigarette smoke condensate (10).

increased immunoreactivity of the proliferation marker Ki-67 was observed in areas of inflammation and PIN, whereas the π-class glutathione S-transferase P1 (GSTP1) was down-regulated in atypical cells in atrophic areas and in PIN (9). GSTP1 has been described as a 'caretaker' gene, responsible for tissue protection and carcinogen detoxication (4). As described in a following section, GSTP1 expression is silenced by promoter CpG island hypermethylation in >90% of human prostate cancers.

LNCaP human prostate cancer cells, containing a silenced GSTP1 gene, were shown to be sensitive to the cytotoxic activity of PhIP (10). Of note, reconstitution of GSTP1 expression by gene transfection in LNCaP cells confers resistance to PhIP cytotoxicity and decreases PhIP DNA adduct formation by 50% (10). This *in vitro* evidence supports a model whereby suppression of caretaker activity may render prostate cells more vulnerable to the action of genotoxic agents like PhIP, and more prone to neoplastic transformation.

3. Clinical and epidemiological studies

Inflammation, atrophy and prostate cancer. The association between chronic inflammation, focal atrophy and the incidence of prostate cancer has been investigated by MacLennan and coworkers in the frame of a 5-year prospective follow-up study (11). From an initial cohort of 144 subjects with chronic inflammatory findings in initial biopsies, 29 (20%) were subsequently newly diagnosed with PCa during follow-up. By contrast, in 33 patients not showing chronic inflammation in the first biopsies, only 6% (n=2) developed cancer during the 5-year follow-up. Interestingly, the PIA subspecies PAH was detected only in patients with inflammatory findings in the same tissue, and significantly higher inflammation scores were recorded in PAH (score 1.3) and PIN (score 1.2) compared to simple atrophy (score 0.67) and to non-pathological specimens (score 0.72) (11).

The link between inflammation and PCa is indirectly supported by studies demonstrating reduced cancer risk in subjects chronically exposed to non-steroidal anti-inflammatory drugs (NSAIDS). In 2006, Mahmud *et al* reported the results of a case-control study involving a cohort of 1,299 men that demonstrated a significant reduction in the odds of prostate cancer detection associated with the use of aspirin [adjusted odds-ratio (OR)=0.58; 95% CI 0.36-0.91]. Aspirin appeared to be the only active compound, since both non-aspirin NSAIDS and selective cyclooxygenase 2 (COX-2) inhibitors were devoid of a significant protective activity against prostate cancer (12). This study is in agreement with a previous investigation reporting reduced PCa risk in long-term aspirin users (rate ratio, 0.85; 95% CI 0.73-0.99) (13). Although subsequent studies showed contrasting results (14), a very recent meta-analysis, based on 20 studies including 25,768 patients, showed an association between NSAIDS consumption and a moderate decrease of PCa risk (OR=0.92; 95% CI 0.86-0.97) (15).

There is one caveat to ensuring the correct interpretation of these results: direct protection against inflammation injury may be confounded by additional biological properties shown by NSAIDS. For example, NSAIDS are inhibitors of angiogenesis (16) and may prevent within the prostate the 'angiogenic switch' from microscopic *in situ* lesions to frank invasive neoplasia. Inactivation of Bcl-2 and AKT, or up-regulation of the p75NTR tumor suppressor by NSAIDS, represent additional confounding factors.

Relationship between histological inflammation and clinical prostatitis. The evidence reviewed so far points to a relationship between prostate inflammation, PIA and cancer. In this context, the link between histological findings of prostatic inflammation and the possible clinical manifestation of inflammatory lesions is a critical issue: to what extent does chronic tissue inflammation manifests itself clinically, and can it be detected in the form of symptomatic chronic prostatitis?

The contemporary classification system for prostatitis includes four distinct clinical entities:

Category I: acute bacterial prostatitis, which is an acute prostate bacterial infection affecting patients with local (pain, voiding disturbances, pyuria and bacteriuria) and systemic (fever and malaise) signs/symptoms.

Category II: chronic bacterial prostatitis (CBP), which is characterized by recurrent episodes of urinary tract infection stemming from a prostate chronically infected by a uropathogen, causing urological pain and voiding symptoms.

Category III: chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), which is a disease characterized by urological pain as its major component, associated or not with voiding and/or sexual disfunction. Patients with the inflammatory 'a' subtype have leukocytes in their expressed prostatic secretions or semen, whereas the noninflammatory 'b' subtype occurs in the apparent absence of inflammation.

Category IV: asymptomatic inflammatory prostatitis (AIP), which is detected mainly by histology in asymptomatic subjects usually undergoing prostate biopsy.

Thus, the latter is the only class of prostatitis diagnosed by histological findings. Being asymptomatic, AIP is less prone to be detected, and is rarely the target of appropriate cancer chemopreventive strategies.

In 97 patients showing symptoms of CP/CPPS, only 5% showed moderate or severe histological findings of inflammation in a total of 368 biopsies (17). These data apparently exclude a close relationship between clinical and histological prostatitis. However, a subset analysis of a patient population enrolled in the REDUCE trial showed a weak but statistically significant relationship between the degree of chronic

inflammation and lower urinary tract symptoms (LUTS) (18). A very recent study supports the findings of the REDUCE subset analysis. In benign hypertrophy (BPH) patients showing high-grade inflammatory infiltrates, increased symptom scores were reported (19). However, the fact that increased inflammation was correlated with higher prostatic volumes in BPH patients may represent a major bias and an obstacle to correct evaluation of the results of both trials. Indeed, LUTS may be linked to prostate enlargement rather than or in addition to chronic inflammation, and the International Prostate Symptom Score (IPSS) is not an optimal investigation tool for prostatitis, since it focuses on voiding symptoms and takes into no account pelvic or ejaculatory pain and additional symptoms linked to clinical prostatitis. The NIH-Chronic Prostatitis Symptom Index (NIH-CPSI) represents in our opinion a more suitable tool for such evaluation.

Clinical chronic prostatitis and the risk of cancer. In 2002, a meta-analysis by Dennis and coworkers performed on 11 studies, including a total of 1,648 cancer cases and 1,824 controls, found a significant association between a clinical history of prostatitis and PCa, with an OR of 1.6 (95% CI 1.01-2.45), increased to 1.8 (95% CI 1.05-2.98) when the analysis was restricted to population-based case-control studies (20). Although the authors admit the possible confounding influence of detection and recall biases in the analyzed studies, these data point to a potential role of a history of symptomatic prostate inflammation in subsequent cancer development.

A study by Roberts *et al* based on 409 cases and 803 controls confirmed a relatively strong association of PCa with acute bacterial prostatitis (OR=2.5; 95% CI 1.3-4.7), but not with CBP (OR=1.6; 95% CI 0.8-3.1) or CP/CPPS (OR=0.9; 95% CI 0.4-1.8) (21). Commenting on their results, the authors suggested that perhaps their study was characterized by inadequate statistical power and by potential misclassification biases, making it difficult to make a conclusive statement on the link between chronic prostatitis and cancer.

A large prospective study reported in 2006 by Sutcliffe and coworkers, based on data collected in the frame of the Health Professional Follow-up Study, included 5,732 patients with and 29,854 patients without prostatitis (22). Although the study failed to show an overall link between prostatitis and prostate cancer, it demonstrated a positive significant association between these conditions in the younger male population. The age-adjusted relative risk (RR) for prostate cancer among patients treated for prostatitis at a young age (30-39 years) was 1.33 (95% CI 1.06-1.66), and lost significance either at later ages or among men of the same age span screened for prostate cancer. Since the latter evidence suggested the existence of a detection bias in the patient population, subsequent subset analyses were performed by considering only men screened for prostate cancer. In this context, a significant association was found between PCa and a history of clinical prostatitis among men diagnosed for PCa <59 years of age (22).

In summary, analysis by age revealed significant links that may have been overlooked in studies performed on a non-stratified population. Sutcliffe *et al* explained this positive association mainly in terms of the differential distribution of prostate conditions in different age groups (e.g., BPH vs. bacterial prostatitis), or of the lesser varied accumulation of mutually-confounding carcinogenic exposures in younger men. An additional explanation, as yet to be confirmed, for the stronger link between PCa and prostatitis in patients of a young age may be the higher rate of testosterone secretion in such patients, potentially exposing young individuals to stronger proliferative signals in the regenerative phase of prostate carcinogenesis.

4. Morphological evidence

In the last few years, several research groups have investigated the relationship between PIA, HGPIN and PCa at the morphological level. Discussing the results of a prospective follow-up study, Postma and coworkers could not point to a significant association between an initial finding of PIA and the incidence of PCa or HGPIN monitored in subsequent diagnostic biopsies (23). Billis and coworkers investigated the topographical relation of inflammatory or non-inflammatory atrophy with adenocarcinoma in 172 needle biopsies. The authors expected to observe higher frequencies of inflammatory atrophy in cores with evidence of adenocarcinoma, but failed to demonstrate any significant link between these lesions (24). Therefore, the hypothesis that cancer may stem from atrophy should not be based on a mere linear quantitative relationship between the extent of atrophic and cancer lesions, i.e., on the assumption that a higher number of atrophic glands linearly correlates with a higher probability of cancer findings in a given patient. However, this does not rule out the hypothesis that, given a gland of normal phenotype and an atrophic gland, the latter represents fertile ground for prostate cancer development. As shown in the following sections, this fertile ground may consist of a number of alterations at the genetic and molecular levels, recalling many distinctive features of PCa or HGPIN and potentially facilitating the drift of atrophic cells towards a phenotype characterized by poorly controlled cellular proliferation and proneness to malignant transformation and progression.

Inflammatory atrophy appears to occur more frequently in association with PCa rather than with other benign prostatic lesions. According to Tomas *et al*, analysis of the distribution frequencies of PIA vs. 'proliferative atrophy' (PA) in PCa and BPH lesions in radical prostatectomy specimens showed that the inflammatory subtype was significantly more frequent in prostates with carcinoma, whereas non inflammatory PA (most frequently cystic atrophy) was more frequently found in prostates with BPH (25).

Two independent studies characterized the morphological transition between PIA and HGPIN. Putzi and De Marzo showed that high-grade PIN merged with PIA in 42.5% of HGPIN lesions, and was adjacent or near PIA in 46% of PIN lesions. Merging between carcinoma and PIA was not reported, but clusters of cells showing mild nuclear atypia were observed within atrophic lesions, and 23% of carcinoma lesions were observed to merge with high-grade PIN (26). The evidence that morphological transitions between PIA and HGPIN frequently occur within the same acinus or duct has been recently confirmed by a study by Wang and coworkers, also performed on radical prostatectomy tissues (27). From a series of 50 prostatectomy-derived specimens, 35 showed merging between PIA and HGPIN; 198 merging structures

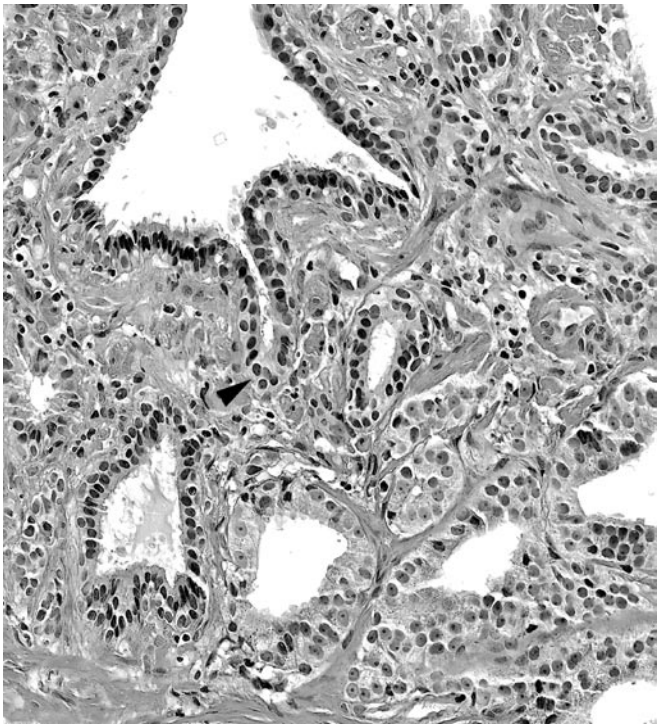

Figure 2. Simple atrophy merging with an early adenocarcinoma lesion. Atrophic areas, characterized by darker nuclei of more regular shape, are in close contact with the adenocarcinoma. Within atrophic lesions, cells showing mild nuclear atypia are visible (arrowhead).

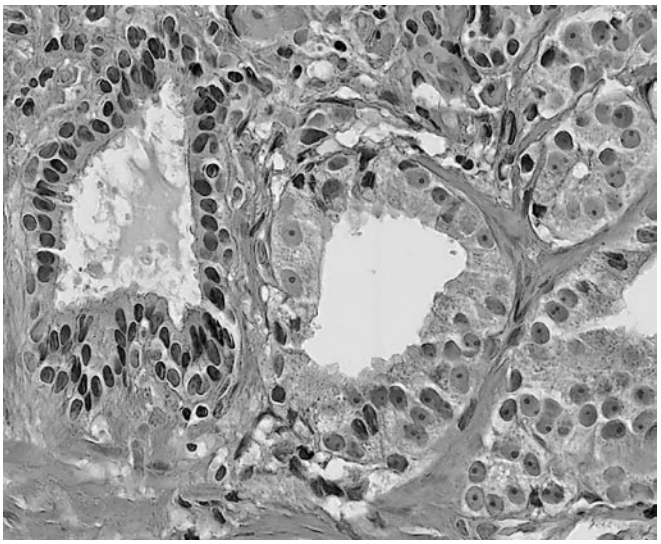

Figure 3. Detail from Fig. 2, showing a PIA lesion (left) in tight contact with a neoplastic acinus, characterized by nuclei varying in size and showing prominent nucleoli.

were detected, representing 17% of a total of 1,188 analyzed HGPIN lesions. In the same report, merging between PCa and PIA was reported in 14 out of 50 prostatectomy specimens (27), confirming previous observations of Montironi and coworkers (28). Interestingly, clusters of atypical epithelial cell hyperplasia were occasionally observed within atrophic lesions. These atypical clusters are probably related to the foci of mild nuclear atypia observed by Putzi and De Marzo in their morphologic transition study (26). Common features between these similar (if not identical) morphological entities are variability in cell size and shape, nuclear enlargement and hyperchromasia, and occasional prominent nucleoli.

5. Chromosomal alterations and oncogene/tumor suppressor involvement

Four studies by two independent groups have shown that cells within PIA lesions may contain somatic chromosomal alterations known to be typical hallmarks of prostate cancer and PIN.

By fluorescence *in situ* hybridization, it was shown that focal atrophy is characterized by increased chromosome 8 centromeric signals, by loss of chromosome 8p and gain of chromosome 8q24 (Fig. 4) (29-31). In a study by Yildiz-Sezer *et al*, loss of 8p was found in 3.6% of atrophic cell nuclei in a control group of non-prostate cancer patients. This aberration was markedly increased in cancer patients, being found in 10.3% of cells within non-neoplastic tissue, 14.2% of PIA lesions, 17.1% of PIN and 21.2% of cancer tissue (29). Increased 8p loss (1.8-fold) was also demonstrated by Macoska and coworkers in atrophic lesions of prostate cancer patients as compared to benign tissue (31). In that study, atrophic lesions were described as open glands showing very little visible cytoplasm. This description corresponds to the PIA subspecies simple atrophy.

The implications of the loss of the short arm of chromosome 8, known to be a key early event in prostate cancer progression, are of great significance in prostatic tissue (32). Putative prostate tumor-suppressor genes located in the 8p22 region are *NKX3.1*, the lipoprotein-lipase (LPL) gene and the macrophage scavenger receptor 1 gene (*MSR1*).

The homeobox gene *NKX3.1* encodes for a prostate-restricted homeodomain protein frequently deleted in prostate carcinoma and contributing to prostate carcinogenesis by cooperating with other tumor suppressors. *NKX3.1* plays an essential role in normal prostate organogenesis, as its loss of function leads to defects in protein secretion and in ductal morphogenesis. By a variety of mechanisms, *NKX3.1* expression is reduced in non-invasive and early-stage human prostate cancer, thus suggesting that its decreased expression is one of the most frequent and earliest steps in prostate oncogenesis. In addition, *NKX3.1* mutant mice are predisposed to prostate carcinoma (33).

Of note, significantly reduced levels of NKX3.1 protein and RNA have been documented in PIA as compared to normal-appearing epithelium (34). Importantly, although chromosome deletions may coincide with decreased levels of NKX3.1, Bethel and coworkers have shown that this phenomenon is not strictly correlated to 8p allelic loss in PIA (34). The authors discuss this finding by postulating that small regional deletions may indeed occur in the NKX3 locus, and would not be detected by the LPL probe used in the experiment. Interestingly, although the authors found in the same study a significant increase in the percentage of cells in atrophy that harbored three or more signals for 8c compared with normal cells (2.4% for atrophy vs. 1.2% for normal; p=0.024), no TMA cores containing prostate atrophy were

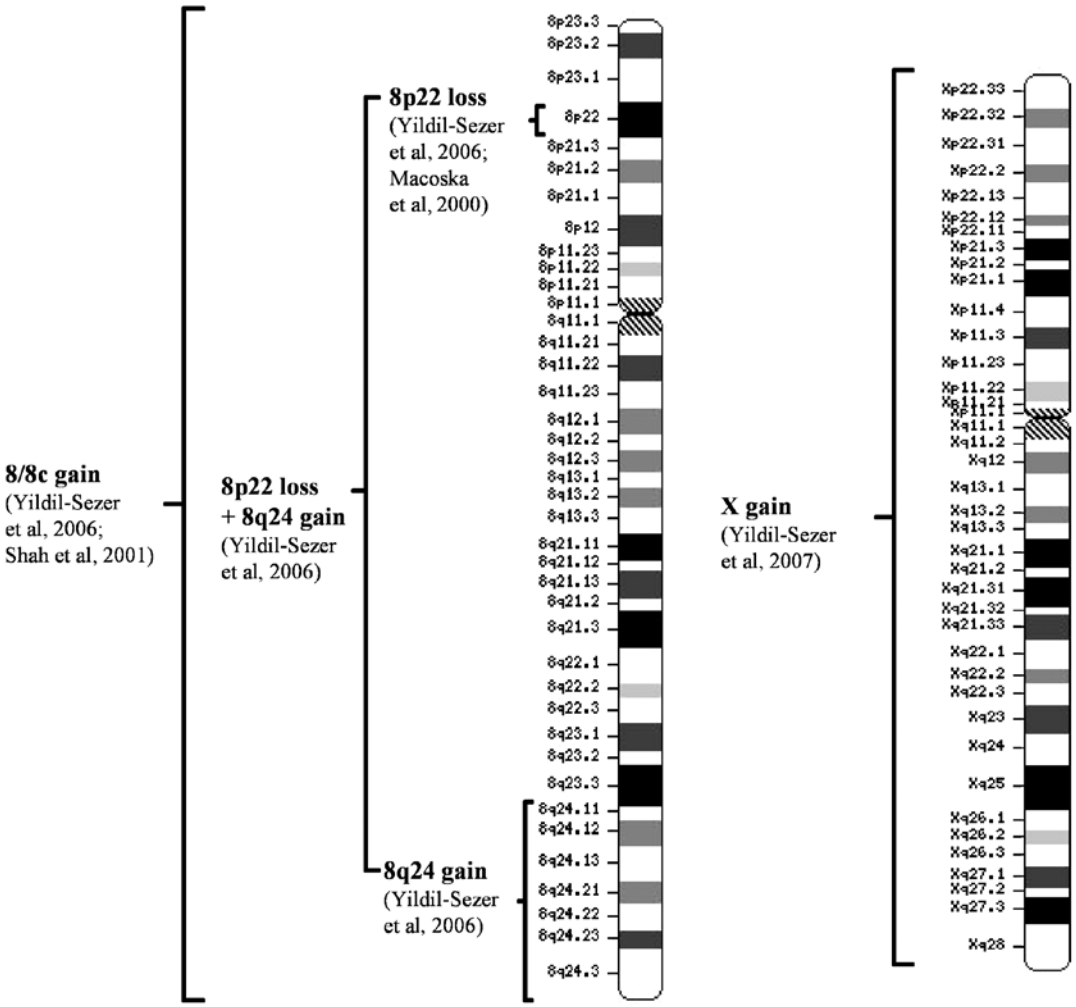

Figure 4. Chromosomal alterations common to proliferative inflammatory atrophy, PIN and prostate cancer. The figure summarizes the results of studies by Yildil-Sezer *et al* (29,45), Shah *et al* (30) and Macoska *et al* (31). Chromosome diagrams are modified from: http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606.

considered to have gained the chromosome 8 centromere in a clonal fashion (34).

LPL plays a crucial role in fat metabolism by hydrolyzing triglycerides in chylomicrons and VLDLs. In a Japanese population, Narita and coworkers reported that probands with the CG+GG genotypes of a Ser447stop single nucleotide polymorphism within exon 9 of the *LPL* gene had an increased risk of prostate cancer compared to those with a CC genotype (OR=1.6; 95% CI 1.06-2.4). This link was stronger in patients with high-grade cancer (OR=2.8; 95% CI 1.2-6.4) or metastatic disease (OR=2.3; 95% CI 1.04-5). However, the risk was not significant in subjects with low- to intermediate-grade cancer or non-metastatic disease (35).

The *MSR1* gene encodes a homotrimeric class A scavenger receptor, whose expression is largely restricted to macrophages. Interestingly, *MSR1* is overexpressed 15-fold in tumor-associated M2 macrophages as compared to M1 macrophages (36). *MSR1* is capable of binding many ligands, including modified lipoproteins and Gram-negative and Gram-positive bacteria. Recent evidence suggests an anti-inflammatory role for this receptor. The R293X (nonsense) and H441R (missense) inactivating mutations, located within regions encoding the collagen-like and the scavenger receptor cysteine-rich carboxy-terminal domains of MSR1, respectively, have been detected in a number of families with putative hereditary prostate cancer (37). Although other studies yielded contradictory results (summarized in ref. 4), a meta-analysis by Sun and coworkers showed that *MSR1* mutations can have a reproducible albeit modest effect on prostate cancer risk in African Americans (38). Since MSR1 is involved in the host response to infectious agents, inactivating mutations or deletions of the *MSR1* gene may reduce the ability of macrophages to kill infectious agents, thus leading to chronic inflammation.

Besides loss of 8p, Yildiz-Sezer *et al* demonstrated a significant increase of nuclei with 8q24 gain in atrophic lesions (11.5%), PIN (12.7%) and cancer (15.2%) compared to normal tissue (6.2%). Lower rates of 8q24 gain (2.3%) were observed in nuclei from a control group consisting of non-cancer patients (29). A major consequence of this aberration – frequently found in PCa – may be increased expression of both the PSCA prostate cell surface antigen and the *c-myc* oncogene. PSCA is overexpressed in PCa, co-amplifies with *myc* in advanced-stage disease, and is increasingly overexpressed as the Gleason grade and metastatic features increase. Interestingly, PSCA-targeted monoclonal antibodies inhibit PCa growth and metastasis in animal models (reviewed in ref. 39).

Increased c-myc signals were detected in atrophy and cancer, but not in PIN (29). *c-myc* has been found to be overexpressed and/or amplified in prostate cancer, and a high frequency (85%) of 8q and *c-myc* amplifications is found in prostate cancer metastases. Moreover, additional increases of c-myc (AI-c-myc) have been associated with a higher Gleason score and poorer survival in men with Stage $pT_3N_0M_0$ prostate cancer (reviewed in ref. 40). In a study based on histopathological examination of surgical specimens from a cohort of 52 patients, Qian *et al* demonstrated that AI-c-myc was strongly associated with high Gleason score, and thus with prostate cancer progression, and proposed c-myc as a potential marker of survival in Stage $T_{(2-3)}N_{(1-3)}M_{(0)}$ prostate cancer (40).

C-myc is pivotal in key signalling pathways in prostate cancer. For example, (i) endothelin-1-induced androgen receptor transcription appears to be mediated by c-myc in LNCaP prostate cancer cells (41); (ii) the TMPRSS2-ERG fusion, among the most frequent (>95%) TMPRSS2-ETS factor alterations described in PCa thus far, can modulate the growth of PCa cells by up-regulating *c-myc* and by abrogating the differentiation of prostate epithelium (42); (iii) *myc* overexpression may cause telomerase reactivation and telomere stabilization which, in turn, would allow permanent proliferation and suppression of senescence programs (43). An important caveat to the potential link between 8q gain and *c-myc* overexpression comes from a recent study in which myc protein expression was not fully correlated with 8q24 gain in cancer, and appeared to not be significantly enhanced in atrophy (44). However, myc protein staining was found to be shifted from the basal to the luminal compartment in atrophy as compared to normal epithelium (44). The authors discussed this finding by hypothesizing that activation of myc in luminal cells may serve to 'reprogram' these cells into cancer stem-like cells, in line with the model indicating partially differentiated luminal cells as target progenitors cells for prostate neoplastic transformation.

Of note, loss of 8p concomitant with gain of 8q24, a common alteration in PIN and in low-grade, high-grade and metastatic prostate cancer, was reported in 0.5% of nuclei in PIA lesions (PIN, 1.49%; PCa, 3%), with a 2-fold increase vs. surrounding normal tissue and a 100-fold increase compared to non-cancer patient specimens (29). Gain of 8c was also reported in increased rates in PAH as compared to benign prostatic tissue, but also as compared to simple atrophy and PIN lesions (30). In the same study, Shah and coworkers reported a strong topographic association between PAH and PCa (30). Gain of 8c, concomitant or not with 8p loss, was also reported in increased rates in simple atrophy compared to benign prostatic tissue (31).

An additional chromosomal alteration was demonstrated in PIA by Yildiz-Sezer and coworkers. In samples taken from a cohort of 20 subjects, gain of Xc was observed by FISH in atrophic areas in 68.4% and in cancer tissues in 90% of prostate cancer patients, with no evidence of Xc gain in controls. Gain of the whole chromosome X was also found in atrophic tissues in 70% of patients by CGH (45) (Fig. 4). Interestingly, in the majority of patients, similar percentages of nuclei with Xc gain were assessed in atrophic and cancer tissues belonging to the same subject/specimen (45).

Chromosome X contains a putative prostate cancer susceptibility Xq27-28 locus, named *HPCX* (46). Another possible consequence of X chromosome gain could be amplification of *PAGE4*, a cancer-testis antigen mapping in Xp11.23, commonly expressed in normal prostate epithelium but not in surrounding stroma and overexpressed in cancer specimens (47). Of note, cDNA microarray analysis showed that the expression of lipoprotein lipase is down-regulated in PAGE4-overexpressing NIH-3T3 cells, but not in non-transfected PC3 prostate cancer cells, likely harboring a monoallelic deletion of *LPL* (47).

Importantly, the X chromosome contains the androgen receptor (AR), whose mRNA levels were found to be increased in prostate cancer cells as compared to benign tissue biopsies (48). The AR increase in HGPIN was found to be intermediate, between the levels observed in benign and cancer tissues (48). In a study by Roepke *et al*, polysomy of the X chromosome with the corresponding *AR* gene was detected in 9 out of 80 prostate cancer cases (11%), and was correlated with pathological classification and tumor volume (49).

In conclusion, the data presented above confirm that PIA may contain cell subsets displaying an enhanced degree of genetic instability leading to important chromosomal changes similar, if not identical, to those found in HGPIN and in prostate cancer.

Although clonal alterations were not identified in atrophy, some of the changes reported (8c gain, 8p loss and 8q gain) are similar to changes found as clonal alterations in the truly neoplastic appearing cells in invasive carcinoma, and at times in HGPIN. In this respect, Valdman and coworkers suggested that non-clonal DNA alterations are a sign of genomic instability arising with increased frequency in atrophy as compared with normal-appearing epithelium, and could later be selected for use during the process of neoplastic transformation (50).

6. Molecular evidence

De Marzo and coworkers conducted a comprehensive immunohistochemical study of PIA lesions, focusing on the expression of a panel of proteins involved in cell growth, differentiation and senescence, carcinogen detoxification or apoptosis regulation, or playing a role as markers of prostate cell injury or basal cell integrity (51).

The basal cell-specific 34βE12 cytokeratin showed expected poor or absent staining in atrophic secretory-like cells, also showing weak staining for PSA and prostate-specific acid phosphatase (51).

Subsequently, van Leenders *et al* showed that increased numbers of luminal cells (40 vs. 2.4% of normal cells) in PIA expressed keratin 5 (52). In these cells, p27 was virtually absent. Moreover, approximately 40% of luminal PIA cells expressed c-MET, compared to 2% of normal cells. Thus, cells phenotypically intermediate between basal and secretory cells were shown to be increased in PIA lesions.

As far as PSA is concerned, a report from Billis *et al* showed a significant positive correlation between the extent of prostatic atrophy and either total or free serum PSA levels (53).

A marked increase in the fraction of cells staining for the proliferation marker Ki-67 was shown to occur in PIA (51). This result, also supported by evidence of the up-regulation of the antiapoptotic protein Bcl-2 and by the previously documented lack of increased apoptosis in PIA (54), points to a net increase in cell proliferation within PIA lesions.

Differential expression patterns of the p16, p27 and p53 growth and tumor suppressors were reported in proliferative inflammatory atrophy.

The cyclin-dependent kinase inhibitor and putative tumor-suppressor p27 was found to be down-regulated in PIA, whereas p16/CDKN2 was up-regulated in the same lesions as compared to normal epithelium (55). The sudden rise of p16 in PIA mimics the pattern observed in PIN (56) and PCa (57), and may reflect a cellular response aimed at maintaining tissue homeostasis in reaction to redundant growth signals.

The tumor suppressor p53 is overexpressed in PIA lesions as compared to normal-appearing prostatic acini (58). Moreover, a positive correlation betwen p53 expression and Ki-67 was found in COX-2-positive PIA lesions. Overexpression of p53, which is infrequent in prostate cancer and virtually absent in HGPIN (59), may be triggered in response to enhanced ROS-induced DNA damage in inflammatory tissues.

Interestingly, p53 point-mutations, mostly of the missense type and located in known mutational 'hotspots' of the gene (e.g., codon 273 in exon 8 and codon 158 in exon 5) have been identified in human post-atrophic hyperplasia (60). However, these mutations do not appear to be clonal, and are likely the result of increased DNA damage and instability. Nevertheless, by impairing the activity of p53, these point-mutations may lead to neoplastic transformation within PAH lesions, as observed in a variety of other human cancers.

Inactivation of p53 has been proposed as the main mechanism whereby the putative prostate oncogenic virus BK (BKV) plays a role in early cancer progression, as hypothesized by Das *et al* (61). This polyomavirus, 75% homologous to JCV and present in subclinical form in the urinary tract of >90% of the human population, expresses a large tumor antigen, homologous to the SV40 large-T, that promotes cell transformation by interfering with the p53 and pRB tumor suppressors (61). Inactivation of p53 appears to occur not through down-regulation, but rather through cytoplasmic sequestration of the protein by BK large-T. Whereas BKV gene sequences have been demonstrated in both normal and PIA ducts of cancerous prostate specimens, BK large-T was found exclusively in atrophic cells, where it co-localized with cytoplasmic p53. Of note, BKV was present at a lower frequency in normal prostates than in cancerous prostates, and large-T was detected only in specimens containing PIA and PIN lesions, where it co-localized with wild-type p53 (61).

In the De Marzo study described above (51), the π-class glutathione S-transferase P1 was found to be markedly overexpressed in a variable fraction of atrophic cells within PIA lesions. This evidence is in apparent contrast with down-regulation caused by CpG island hypermethylation within the promoter region of the *GSTP1* gene, observed in approximately 90% of prostate cancer lesions and 70% of HGPIN (51). However, in 6% of the atrophic areas, presumably occurring in cells not expressing GSTP1, the *GSTP1* gene was also found to be inactivated by CpG hypermethylation (62). Thus, the epigenetic knockdown of *GSTP1* is indeed present in PIA, albeit in a 'patchy' pattern. In the same study, hypermethylation was found to be absent in normal epithelium, but present in 69% of HGPIN lesions and virtually ubiquitous in prostate cancer (62).

Similar to GSTP1, glutathione S-transferase-α and COX-2 were markedly up-regulated in restricted areas of PIA, most likely as a consequence of enhanced tissue stress. In contrast, these proteins were detectable at baseline levels in PCa, HGPIN and normal glands (63,64). COX-2 overexpression was confirmed by additional studies in both post-atrophic hyperplasia and simple atrophy (65). Sustained COX-2 expression may also be a consequence of increased expression of the transcription factor C/EBPβ in PIA, involved in the inducible expression of the *COX-2* gene (66). A stabilizing role in the sustained overexpression of COX-2 may be played by the *COX-2* mRNA ELAV-like HuR protein, overexpressed in PIA lesions, HGPIN and PCa (67).

7. Conclusions and research perspectives

'Cancer forerunner' (68,30), 'PIN precursor' (69), 'background lesion of prostate cancer' (70) and 'proposed early pre-neoplastic lesion' (71,72) are some of the phrases used in the last few years to define proliferative inflammatory atrophy. Although evidence at the pre-clinical, clinical, genetic and molecular levels increasingly supports the 'injury-and-regeneration' model for prostate carcinogenesis, additional studies are required to ultimately confirm or refute the role of PIA in the early stages of prostate oncogenesis.

At the pre-clinical level, investigation of the kinetics of neoplastic transformation in animal models may aid in elucidating in detail the various steps of prostate cancer development. This will help to confirm, also at the morphological and molecular levels, that prostate neoplastic transformation involves the transition of affected glands through defined stages, and that PIA represents a key early event in this process.

At the clinical level, studies performed in large patient populations may provide conclusive results regarding the relationship between histological findings of proliferative inflammatory atrophy and cancer. In particular, dissecting patient populations in cohorts that are homogeneous, e.g., in terms of age and type of prostatic inflammatory evidence, may help to remove a number of confounding factors that have to date limited our understanding of the clinical relevance of PIA lesions. Moreover, studies describing the morphological merging between PIA and PIN/cancer did not provide conclusive information on the possible role of PIA as a precursor of PIN or PCa. Follow-up investigations performed with repeated biopsies or repeated non-invasive imaging series (if appropriate markers and technologies are available) will shed new light on prostate cancer development kinetics. Additional research efforts should focus on the relationship between clinical chronic prostatitis in its bacterial and abacterial forms and the development of PIA lesions. This will help to further characterize and better define the potential early etiological agents of prostate cancer. Moreover, if a relationship between clinical chronic prostatitis and atrophy is confirmed, symptom-directed early diagnosis of proliferative pre-neoplastic lesions will greatly facilitate the design of focused chemoprevention strategies, with invaluable benefits in terms of public health. In this respect, the spectrum of chemopreventive agents, until now restricted to NSAIDS, may broaden to include antibacterial drugs like fluoroquinolones, anti-viral compounds and dietary supplements or herbal extracts of proven efficacy.

At the morphological level, the discovery of foci of atypical epithelial cell hyperplasia within PIA lesions represents, in our opinion, an interesting area of further investigation. The phenotype, growth kinetics and molecular alterations within cells belonging to these foci should be studied in order to characterize their potential role as early cellular 'units' drifting towards neoplastic transformation. Basal cell hyperplasia is also a lesion whose relationship with inflammation and atrophy, described by Thorson *et al* (5), should be further investigated, in part due to its possible role as a precursor of basal cell carcinoma (72).

In a previous section, we reported that two studies failed to establish a relationship between PIA and HGPIN/PCa findings (23,24). The fact that simple linear relationships were not at first ascertained should not discourage researchers from performing new studies using more sophisticated mathematical tools and models. For example, threshold functions applied to prostate histopathology may be conceived and elaborated. The 'D-score' histomorphometric qualitative threshold function, conceived to predict the progression of endometrial intraepithelial neoplasia to endometrial cancer, has shown enhanced predictive power as compared to alternate systems (73,74).

At the genetic level, research may focus on the search for additional chromosomal alterations in PIA lesions. Several loci in chromosome 1 have been linked, with various degrees of strength, to PCa [e.g., CAPB in 1p36, HPC1 in 1q24-25 (contains *RNAse-L*, implicated in PCa and interferon-response in viral diseases), PCAP in 1q42.2-43]. Additional investigation may focus on chromosomes 10 [loss of heterozygosis (LOH) of 10q, containing the PTEN tumor suppressor gene, is reported in PCa and metastasis (75)] and 12 [PCa may show LOH of 12p, containing the candidate genes *p27Kip* and *ETV6* (75)]. The somatic or inherited aberrations and the genes/molecular pathways involved therein should be studied in the proliferative subset of atrophic lesions (simple atrophy, PAH).

In conclusion, the fact that PIA is a very common finding in the human prostate does not in our opinion diminish its possible role as an early cancer precursor or, more cautiously, as 'fertile ground' for neoplastic transformation. Indeed, the studies reviewed herein confirm that specific genetic and molecular changes in PIA may be carried forward in subsequent HGPIN and carcinomas, thereby supporting lineage continuity between putative pre-malignant and malignant stages of prostate cancer.

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References

1. Mantovani A, Romero P, Palucka AK and Marincola FM: Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 371: 771-783, 2008.
2. Mantovani A, Allavena P, Sica A and Balkwill F: Cancer-related inflammation. Nature 454: 436-444, 2008.
3. De Marzo AM, Platz EA, Sutcliffe S, *et al*: Inflammation in prostate carcinogenesis. Nat Rev Cancer 7: 256-269, 2007.
4. Palapattu GS, Sutcliffe S, Bastian PJ, Platz EA, De Marzo AM, Isaacs WB and Nelson WG: Prostate carcinogenesis and inflammation: emerging insights. Carcinogenesis 26: 1170-1181, 2005.
5. Thorson P, Swanson PE, Vollmer RT and Humphrey PA: Basal cell hyperplasia in the peripheral zone of the prostate. Mod Pathol 16: 598-606, 2003.
6. De Marzo AM, Platz EA, Epstein JI, *et al*: A working group classification of focal prostate atrophy lesions. Am J Surg Pathol 30: 1281-1291, 2006.
7. Przybycin CG, Kunju LP, Wu AJ and Shah RB: Partial atrophy in prostate needle biopsies: a detailed analysis of its morphology, immunophenotype and cellular kinetics. Am J Surg Pathol 32: 58-64, 2008.
8. Purewal M, Fretland AJ, Schut HA, Hein DW and Wargovich MJ: Association between acetylator genotype and PhIP DNA adduct formation in colon and prostate of inbred Fischer 344 and Wistar Kyoto rats. Cancer Lett 149: 53-60, 2000.
9. Borowsky AD, Dingley KH, Ubick E, Turteltaub KW, Cardiff RD and Devere-White R: Inflammation and atrophy precede prostatic neoplasia in a PhIP-induced rat model. Neoplasia 8: 708-715, 2006.
10. Nelson CP, Kidd LC, Sauvageot J, *et al*: Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. Cancer Res 61: 103-109, 2001.
11. MacLennan GT, Eisenberg R, Fleshman RL, Taylor JM, Fu P, Resnick MI and Gupta S: The influence of chronic inflammation in prostatic carcinogenesis: a 5-year follow-up study. J Urol 176: 1012-1016, 2006.
12. Mahmud SM, Tanguay S, Bégin LR, Franco EL and Aprikian AG: Non-steroidal anti-inflammatory drug use and prostate cancer in a high-risk population. Eur J Cancer Prev 15: 158-164, 2006.
13. Jacobs EJ, Rodriguez C, Mondul AM, Connell CJ, Henley SJ, Calle EE and Thun MJ: A large cohort study of aspirin and other nonsteroidal anti-inflammatory drugs and prostate cancer incidence. J Natl Cancer Inst 97: 975-980, 2005.
14. Daniels NA, Chen YH and Bent S: Antibiotic and anti-inflammatory use and the risk of prostate cancer. BMC Res Notes 2: 57, 2009.
15. Jafari S, Etminan M and Afshar K: Nonsteroidal anti-inflammatory drugs and prostate cancer: a systematic review of the literature and meta-analysis. Can Urol Assoc J 3: 323-330, 2009.
16. Tarnawski AS and Jones MK: Inhibition of angiogenesis by NSAIDs: molecular mechanisms and clinical implications. J Mol Med 81: 627-636, 2003.
17. True LD, Berger RE, Rothman I, Ross SO and Krieger JN: Prostate histopathology and the chronic prostatitis/chronic pelvic pain syndrome: a prospective biopsy study. J Urol 162: 2014-2018, 1999.
18. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC and Rittmaster RS: The relationship between prostate inflammation and lower urinary tract symptoms: examination of baseline data from the REDUCE trial. Eur Urol 54: 1379-1384, 2008.
19. Robert G, Descazeaud A, Nicolaiew N, *et al*: Inflammation in benign prostatic hyperplasia: a 282 patients immunohistochemical analysis. Prostate 69: 1774-1780, 2009.
20. Dennis LK, Lynch CF and Torner JC: Epidemiologic association between prostatitis and prostate cancer. Urology 60: 78-83, 2002.
21. Roberts RO, Bergstralh EJ, Bass SE, Lieber MM and Jacobsen SJ: Prostatitis as a risk factor for prostate cancer. Epidemiology 15: 93-99, 2004.
22. Sutcliffe S, Giovannucci E, De Marzo AM, Leitzmann MF, Willett WC and Platz EA: Gonorrhea, syphilis, clinical prostatitis and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 15: 2160-2166, 2006.
23. Postma R, Schroeder FH and van der Kwast TH: Atrophy in prostate needle biopsy cores and its relationship to prostate cancer incidence in screened men. Urology 65: 745-749, 2005.
24. Billis A, Freitas LL, Magna LA and Ferreira U: Inflammatory atrophy on prostate needle biopsies: is there topographic relationship to cancer? Int Braz J Urol 33: 355-360, 2007.
25. Tomas D, Kruslin B, Rogatsch H, Schaefer G, Belicza M and Mikuz G: Different types of atrophy in the prostate with and without adenocarcinoma. Eur Urol 51: 98-103, 2007.
26. Putzi MJ and De Marzo AM: Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. Urology 56: 828-832, 2000.
27. Wang W, Bergh A and Damber JE: Morphological transition of proliferative inflammatory atrophy to high-grade intraepithelial neoplasia and cancer in human prostate. Prostate 69: 1378-1386, 2009.
28. Montironi R, Mazzucchelli R and Scarpelli M: Precancerous lesions and conditions of the prostate: from morphological and biological characterization to chemoprevention. Ann NY Acad Sci 963: 169-184, 2002.
29. Yildiz-Sezer S, Verdorfer I, Schaefer G, Rogatsch H, Bartsch G and Mikuz G: Assessment of aberrations on chromosome 8 in prostatic atrophy. BJU Int 98: 184-188, 2006.

30. Shah R, Mucci NR, Amin A, Macoska JA and Rubin MA: Postatrophic hyperplasia of the prostate gland: neoplastic precursor or innocent bystander? Am J Pathol 158: 1767-1773, 2001.
31. Macoska JA, Trybus TM and Wojno KJ: 8p22 loss concurrent with 8c gain is associated with poor outcome in prostate cancer. Urology 55: 776-782, 2000.
32. Matsuyama H, Pan Y, Oba K, *et al*: The role of chromosome 8p22 deletion for predicting disease progression and pathological staging in prostate cancer. Aktuelle Urol 34: 247-249, 2003.
33. Abate-Shen C, Shen MM and Gelmann E: Integrating differentiation and cancer: the Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis. Differentiation 76: 717-727, 2008.
34. Bethel CR, Faith D, Li X, *et al*: Decreased NKX3.1 protein expression in focal prostatic atrophy, prostatic intraepithelial neoplasia and adenocarcinoma: association with Gleason score and chromosome 8p deletion. Cancer Res 66: 10683-10690, 2006.
35. Narita S, Tsuchiya N, Wang L, *et al*: Association of lipoprotein lipase gene polymorphism with risk of prostate cancer in a Japanese population. Int J Cancer 112: 872-876, 2004.
36. Martinez FO, Gordon S, Locati M and Mantovani A: Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol 177: 7303-7311, 2006.
37. Xu J, Zheng SL, Komiya A, *et al*: Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 32: 321-325, 2002.
38. Sun J, Hsu FC, Turner AR, *et al*: Meta-analysis of association of rare mutations and common sequence variants in the MSR1 gene and prostate cancer risk. Prostate 66: 728-737, 2006.
39. Chin JL and Reiter RE: Genetic alterations in prostate cancer. Curr Urol Rep 5: 157-165, 2004.
40. Qian J, Hirasawa K, Bostwick DG, *et al*: Loss of p53 and c-myc overrepresentation in stage $T_{(2-3)}N_{(1-3)}M_{(0)}$ prostate cancer are potential markers for cancer progression. Mod Pathol 15: 35-44, 2002.
41. Lee JG, Zheng R, McCafferty-Cepero JM, Burnstein KL, Nanus DM and Shen R: Endothelin-1 enhances the expression of the androgen receptor via activation of the c-myc pathway in prostate cancer cells. Mol Carcinog 48: 141-149, 2009.
42. Sun C, Dobi A, Mohamed A, *et al*: TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. Oncogene 27: 5348-5353, 2008.
43. Cerni C: Telomeres, telomerase and myc. An update. Mutat Res 462: 31-47, 2000.
44. Gurel B, Iwata T, Koh CM, *et al*: Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis. Mod Pathol 21: 1156-1167, 2008.
45. Yildiz-Sezer S, Verdorfer I, Schaefer G, Rogatsch H, Bartsch G and Mikuz G: Gain of chromosome X in prostatic atrophy detected by CGH and FISH analyses. Prostate 67: 433-438, 2007.
46. Xu J, Meyers D, Freije D, *et al*: Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20: 175-179, 1998.
47. Iavarone C, Wolfgang C, Kumar V, Duray P, Willingham M, Pastan I and Bera TK: PAGE4 is a cytoplasmic protein that is expressed in normal prostate and in prostate cancers. Mol Cancer Ther 1: 329-335, 2002.
48. Lévesque MH, El-Alfy M, Cusan L and Labrie F: Androgen receptor as a potential sign of prostate cancer metastasis. Prostate 69: 1704-1711, 2009.
49. Roepke A, Erbersdobler A, Hammerer P, Palisaar J, John K, Stumm M and Wieacker P: Gain of androgen receptor gene copies in primary prostate cancer due to X chromosome polysomy. Prostate 59: 59-68, 2004.
50. Valdman A, Jenkins RB, Lan F and De Marzo AM: Histopathology and Molecular Biology of Prostate Atrophy. In: Current Clinical Oncology: Prostate Cancer: Signaling Networks, Genetics and New Treatment Strategies. Pestell RG and Nevalainen MT (eds). Humana Press, Totowa, NJ, pp1-15, 2008.
51. De Marzo AM, Marchi VL, Epstein JI and Nelson WG: Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am J Pathol 155: 1985-1992, 1999.
52. Van Leenders GJ, Gage WR, Hicks JL, van Balken B, Aalders TW, Schalken JA and De Marzo AM: Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. Am J Pathol 162: 1529-1537, 2003.
53. Billis A, Meirelles LR, Magna LA, Baracat J, Prando A and Ferreira U: Extent of prostatic atrophy in needle biopsies and serum PSA levels: is there an association? Urology 69: 927-930, 2007.
54. Ruska KM, Sauvageot J and Epstein JI: Histology and cellular kinetics of prostatic atrophy. Am J Surg Pathol 22: 1073-1077, 1998.
55. Faith D, Han S, Lee DK, Friedl A, Hicks JL, De Marzo AM and Jarrard DF: p16 is upregulated in proliferative inflammatory atrophy of the prostate. Prostate 65: 73-82, 2005.
56. Henshall SM, Quinn DI, Lee CS, *et al*: Overexpression of the cell cycle inhibitor p16INK4A in high-grade prostatic intraepithelial neoplasia predicts early relapse in prostate cancer patients. Clin Cancer Res 7: 544-550, 2001.
57. Halvorsen OJ, Hostmark J, Haukaas S, Hoisaeter PA and Akslen LA: Prognostic significance of p16 and CDK4 proteins in localized prostate carcinoma. Cancer 88: 416-424, 2000.
58. Wang W, Bergh A and Damber JE: Increased p53 immunoreactivity in proliferative inflammatory atrophy of prostate is related to focal acute inflammation. APMIS 117: 185-195, 2009.
59. Johnson MI, Robinson MC, Marsh C, Robson CN, Neal DE and Hamdy FC: Expression of Bcl-2, Bax and p53 in high-grade prostatic intraepithelial neoplasia and localized prostate cancer: relationship with apoptosis and proliferation. Prostate 37: 223-229, 1998.
60. Tsujimoto Y, Takayama H, Nonomura N, Okuyama A and Aozasa K: Postatrophic hyperplasia of the prostate in Japan: histologic and immunohistochemical features and p53 gene mutation analysis. Prostate 52: 279-287, 2002.
61. Das D, Wojno K and Imperiale MJ: BK virus as a cofactor in the etiology of prostate cancer in its early stages. J Virol 82: 2705-2714, 2008.
62. Nakayama M, Bennett CJ, Hicks JL, Epstein JI, Platz EA, Nelson WG and De Marzo AM: Hypermethylation of the human glutathione S-transferase-pi gene CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: a detailed study using laser-capture microdissection. Am J Pathol 163: 923-933, 2003.
63. Parsons JK, Nelson CP, Gage WR, Nelson WG, Kensler TW and De Marzo AM: GSTA1 expression in normal, preneoplastic and neoplastic human prostate tissue. Prostate 49: 30-37, 2001.
64. Zha S, Gage WR, Sauvageot J, *et al*: COX-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. Cancer Res 61: 8617-8623, 2001.
65. Wang W, Bergh A and Damber JE: Chronic inflammation in benign prostate hyperplasia is associated with focal upregulation of cyclooxygenase-2, Bcl-2 and cell proliferation in the glandular epithelium. Prostate 61: 60-72, 2004.
66. Wang W, Bergh A and Damber JE: Increased expression of CCAAT/enhancer-binding protein beta in proliferative inflammatory atrophy of the prostate: relation with the expression of COX-2, the androgen receptor, and presence of focal chronic inflammation. Prostate 67: 1238-1246, 2007.
67. Barbisan F, Mazzucchelli R, Santinelli A, *et al*: Overexpression of ELAV-like protein HuR is associated with increased COX-2 expression in atrophy, high-grade prostatic intraepithelial neoplasia and incidental prostate cancer in cystoprostatectomies. Eur Urol 56: 105-112, 2009.
68. Mikuz G, Algaba F, Beltran AL and Montironi R: Prostate carcinoma: atrophy or not atrophy that is the question. Eur Urol 52: 1293-1296, 2007.
69. Sciarra A, Di Silverio F, Salciccia S, Autran Gomez AM, Gentilucci A and Gentile V: Inflammation and chronic prostatic diseases: evidence for a link? Eur Urol 52: 964-972, 2007.
70. Woenckhaus J and Fenic I: Proliferative inflammatory atrophy: a background lesion of prostate cancer? Andrologia 40: 134-137, 2008.
71. Montironi R, Mazzucchelli R, Lopez-Beltran A, Cheng L and Scarpelli M: Mechanisms of disease: high-grade prostatic intraepithelial neoplasia and other proposed preneoplastic lesions in the prostate. Nat Clin Pract Urol 4: 321-332, 2007.
72. Montironi R, Mazzucchelli R, Stramazzotti D, Scarpelli M, Lopez Beltran A and Bostwick DG: Basal cell hyperplasia and basal cell carcinoma of the prostate: a comprehensive review and discussion of a case with c-erbB-2 expression. J Clin Pathol 58: 290-296, 2005.
73. Baak JP, Orbo A, van Diest PJ, *et al*: Prospective multicenter evaluation of the morphometric D-score for prediction of the outcome of endometrial hyperplasias. Am J Surg Pathol 25: 930-935, 2001.
74. Hecht JL, Ince TA, Baak JP, Baker HE, Ogden MW and Mutter GL: Prediction of endometrial carcinoma by subjective endometrial intraepithelial neoplasia diagnosis. Mod Pathol 18: 324-330, 2005.
75. Dong JT: Chromosomal deletions and tumor suppressor genes in prostate cancer. Cancer Metastasis Rev 20: 173-193, 2001.