ABSTRACT
Prostate cancer is among the most common malignancies. It is estimated that 1 in 6 men in the United States will be diagnosed with this disease. Despite the high prevalence and importance of prostate cancer, the molecular mechanisms underlying its development and progression remain poorly understood. This article reviews new information about the roles of oxidants and electrophiles in prostate cancer; the potential importance of chronic inflammation and atrophy in prostate carcinogenesis, and implications for chemoprevention; evidence supporting telomere shortening and genetic instability in the etiology of prostate cancer; and α-methylacyl-coenzyme A racemase (AMACR) as a potential marker for prostate carcinogenesis. These new results show that at least some high-grade prostatic intraepithelial neoplasias (PIN) and early adenocarcinomas appear to arise from proliferative inflammatory atrophy (PIA). Inflammation and other environmental factors may lead to the destruction of prostate epithelial cells, and increased proliferation may occur as a response to this cell death. Such proliferation may be mechanistically related to decreased p27kip1 observed in PIA. The decreased apoptosis associated with these events may also be related to increased expression of Bcl-2. Increased oxidant and electrophile stress in the setting of increased proliferation associated with these events may lead to elevated glutathione S-transferase P1 (GSTP1) expression as a genomic-protective measure. However, aberrant methylation of the CpG island of the GSTP1 gene promoter silences GSTP1 gene expression and protein levels, setting the stage for additional genetic damage and accelerated progression toward PIN and carcinoma. Additional results show that AMACR may be an important new marker of prostate cancer, and its use in combination with p63 staining may provide the basis for an improved method for identification of prostate cancer.
and the use of telomere length as a marker for prostate carcinogenesis; and (4) the use of α-methylacyl-coenzyme A racemase (AMACR) as a tool to aid in the diagnosis of prostate cancer.

**OXIDANTS AND ELECTROPHILES IN PROSTATE CANCER**

The prevalence of prostate cancer appears to be increased among individuals who are exposed to certain oxidants and electrophiles. It is well known that oxygen radicals can directly attack DNA, and that this may result in the accumulation of potentially promutagenic oxidized DNA bases, such as 8-hydroxydeoxyguanosine. Chronic oxidative stress can also result in lipid peroxidation and generation of a wide range of other reactive products with the potential to damage DNA. Consistent with this oxidant stress model of carcinogenesis, dietary agents that have been associated with a protective effect against prostate cancer, such as lycopene, vitamin E, and selenium, are all potent antioxidants.

Disruptions of specific genes may also interact with elevated oxidative stress to increase the risk for development of prostate cancer. Glutathione S-transferase P1 (GSTP1) is a detoxification enzyme that helps to catalyze conjugation reactions between potentially damaging oxidants and electrophiles and glutathione. Expression of GSTP1 is diminished or absent in nearly 100% of human prostate cancers, and this absence is tightly related to hypermethylation of the GSTP1 promoter CpG island. This inactivation may leave cells vulnerable to oxidative DNA damage and/or tolerant to accumulation of oxidized DNA base adducts.

**ROLES OF INFLAMMATION AND ATROPHY IN PROSTATE CARCINOGENESIS**

Increases in oxidant and electrophile stress within a given organ may be derived through a number of different sources. Exogenous sources—which often require activation to the toxic state by enzymes, such as cytochrome p450s—are derived as environmental toxins and dietary factors. Endogenous exposures can come from several sources, such as enhanced production through increased cellular metabolism, mitochondrial dysfunction, or hypoxia. Another endogenous source of reactive oxygen and nitrogen species is derived from phagocytic inflammatory cells that release these compounds in attempts to eradicate infectious organisms (or perceived infectious organisms). Repeated bouts of this immune-mediated oxidant and nitrogenous injury over many years are thought to play a major role in the pathogenesis of cancer in a number of organ systems, including the stomach, colon, and liver. Is there a link between chronic infection/inflammation and prostate cancer?

**Prostatic Inflammation**. The word *prostatitis* literally means inflammation of the prostate, a condition that is known to be extremely common in men as they age. *Clinical prostatitis* is a term that has been used to describe a wide range of genitourinary symptoms that often do not correlate with histologic prostatitis. The lack of specificity in the definition of clinical prostatitis and the difficulty in rendering the diagnosis have hampered efforts to either support or refute potential causal links between this condition and the development of prostate cancer. Currently, there are suggestive but not definitive data on the relation between inflammation of the prostate and the development of malignancy. Results from observational studies have suggested that a history of clinical prostatitis may be associated with increased risk for the development of prostate cancer. Major difficulties with prior studies on the association between prostatitis and prostate cancer are the high likelihood of recall bias and the complexity in establishing the diagnosis.

In the study by Leitzman et al., as well as other reports cited by those investigators, ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with reduced prostate cancer and/or metastatic prostate cancer risk. This association supports the contention that chronic inflammation may be important in the pathogenesis of the disease, although the ability of some anti-inflammatory agents to directly downregulate androgen receptor expression may also play a role. Interestingly, the risk for prostate cancer has been found to be increased in men with a history of gonorrhea or syphilis, suggesting that chronic inflammation associated with sexually transmitted infections may be associated with prostate carcinogenesis. As further potential support for the concept that chronic inflammation may promote prostate cancer, recent studies that have identified alleles that confer a heritable increased risk of prostate cancer implicate both the MSR-1 gene and the RNAse L gene in these processes. Although definitive results have not been obtained, it is intriguing that mutations in these genes in animal models result in an increased susceptibility to certain types of bacterial and other infections.

The potential role of inflammation in prostate cancer and the possibility that regular NSAID ingestion may reduce risk for this malignancy raise the possibility that cyclooxygenase (COX), and in particular COX-2, may play a role in the development of prostatic carcinoma. COX-2 is the inducible isoform of COX that converts arachidonic acid to proinflammatory prostaglandins. We recently examined the expression of COX-2 protein and
messenger RNA (mRNA) in prostate tissue containing various lesions and in prostate cancer cell lines. In the cell lines LNCaP, DU-145, and PC-3, COX-2 protein expression was undetectable under basal conditions, although levels could be induced by treatment with phorbol esters in PC-3 cells.\textsuperscript{20} In contrast to most other studies in the literature on COX-2 and prostate cancer, immunohistochemical analysis in our laboratory of human prostates indicated no consistent overexpression of COX-2 in either established cancer or high-grade prostatic intraepithelial neoplasia (PIN), as compared with adjacent normal tissue. Positive staining was seen only in scattered cells (<1%) in both tumors and adjacent normal tissue.\textsuperscript{20} In some areas of chronic inflammation, macrophages stained strongly for COX-2. We also found a consistent increase of staining for COX-2 in a fraction of the epithelial cells in proliferative inflammatory atrophy (PIA) lesions (see discussion of PIA below). Western blotting and quantitative reverse transcriptase-polymerase chain reaction analyses confirmed these patterns of expression.\textsuperscript{20} Thus, although COX-2 inhibition may be related to a reduction of prostate cancer, it does not appear to be mediated by elevated COX-2 in the high-grade PIN or the cancer cells themselves. However, COX-2 inhibition may be related to elevated levels of COX-2 in PIA cells and/or macrophages, or via non-COX-2-related mechanisms that these agents can also possess.

**Prostatic Atrophy.** Focal prostatic glandular atrophy has been suggested as a precursor to prostatic adenocarcinoma.\textsuperscript{21–23} and it occurs in close association with chronic inflammation.\textsuperscript{23,24} Prostatic atrophy is identified as a reduction in the volume of preexisting glands and can be divided into 2 major patterns: diffuse and focal.

Diffuse prostatic atrophy results from a decrease in circulating androgens and involves the entire prostate in a relatively uniform manner. In contrast, focal atrophy is not known to be related to decreased concentrations of circulating androgens, and it occurs as patches of atrophic epithelium within a background of surrounding normal-appearing epithelium. Focal prostatic atrophy occurs primarily, although by no means exclusively, in the outer part of the gland, referred to as the peripheral zone—which is also the preferred site for carcinoma.\textsuperscript{24}

It is not yet completely clear how focal prostatic atrophy may give rise to prostate cancer, but recent studies have provided some important clues. Paradoxically, most focal prostate lesions appear to be proliferative rather than quiescent, as implied by the name atrophy. Although proliferation is increased, apoptosis is not.\textsuperscript{23,25,26} These findings are consistent with the view that focal atrophy may represent either a de novo proliferative lesion or a regenerative lesion arising as a response to cellular loss. Because the lesions are proliferative and generally associated with inflammatory infiltrates, the term proliferative inflammatory atrophy, or PIA, was introduced.\textsuperscript{23}

Results from studies carried out in our laboratory several years ago provided information about the morphology of cells in PIA of the prostate as well as expression of specific molecules and markers that have been associated with prostatic carcinogenesis. Most PIA lesions were considered simple atrophy and consisted of glands with variable acinar caliber. Several lesions were classified as postatrophic hyperplasia (PAH)\textsuperscript{25,27} and were composed of foci of crowded glands with small-caliber, round, atrophic acini that often had a larger duct-like structure that appeared to be the origin of the smaller glands. Some lesions were mixed simple atrophy and PAH. All cases contained at least some chronic inflammation, reflected by infiltration of lymphocytes and macrophages. Most were CD3\textsuperscript{+} T cells, CD20\textsuperscript{+} B cells, and CD68\textsuperscript{+} macrophages. Over half (60%; 33 of 55) of the lesions had at least focal acute inflammation as well.

Staining for proliferation-associated markers supported an association between PIA and prostate cancer. In PIA, secretory-type cells showed elevated Ki-67 staining. All lesions also showed reduced levels of p27\textsuperscript{Kip1}, a cyclin-dependent kinase inhibitor.\textsuperscript{23} As in the study of Ruska et al.,\textsuperscript{25} which used terminal deoxynucleotidyl transferase nick end–labeling, the level of apoptosis was low in regions of PIA.\textsuperscript{23} There was also an overall increase in the level of Bcl-2 staining in all of these lesions in comparison with the adjacent normal epithelium.\textsuperscript{23} Finally, all PIA lesions showed elevated levels of GSTP1, glutathione S-transferase A1,\textsuperscript{28} and COX-2\textsuperscript{20} in many, although not all, of the secretory-type cells, suggesting a stress-induced response in these cells.\textsuperscript{23}

**Molecular Alterations in Prostatic Atrophy**

In terms of genetic alterations in PIA, recent studies have shown changes in chromosome 8 in atrophic epithelium.\textsuperscript{29,30} These changes were present at a frequency that was similar to what was seen in high-grade PIN and carcinoma.\textsuperscript{30} In a study of human prostates in Japan, mutations in the p53 gene could be found in PAH.\textsuperscript{31} The mutations were identified as rare alleles in the microdissected cell population by single-stranded conformational polymorphism analysis; they occurred at a similar frequency (≈5%) to that found in high-grade PIN but did not occur in the normal-appearing epithelium.\textsuperscript{31} We recently used laser capture microdissection of human clinical specimens where we found that GSTP1 CpG island hypermethylation
was not detected in normal epithelium (0 of 48) or in hyperplastic epithelium (0 of 22), but was found in 4 of 64 (6.3%) PIA lesions. In addition, similar to studies using nonmicrdissected cases, hypermethylation was found in 22 of 32 (68.8%) high-grade PIN lesions and in 30 of 33 (90.9%) adenocarcinoma lesions.

In addition to molecular markers, we have shown morphologic transitions between PIA and high-grade PIN, where approximately 40% of high-grade PIN lesions merge directly with PIA. Cells with nuclear atypia—that is, intermediate between that found in normal prostate and PIN—were identified in the regions of transition. In addition, small carcinoma lesions were also frequently found very close to prostate atrophy. Others had previously reported direct outgrowths of atrophic epithelium that merged into what was considered early carcinoma lesions. Although we did not find this in our initial research, we have recently identified several cases where it does appear that PIA can merge directly with early infiltrating carcinoma lesions. It should be noted, however, that others have reported a lack of a specific association between prostate atrophy and prostate cancer. Thus, additional studies are needed to determine which elements of the PIA-to-cancer hypothesis may be correct.

All of the above-described results are consistent with the hypothesis that prostatic cells in PIA lesions that are exposed to inflammatory oxidants induce GSTP1 expression as a defense against oxidative genome damage. Cells with defective GSTP1 genes become vulnerable to oxidants and electrophiles that can inflict genomic damage, which, in turn, promotes transformation to PIN and prostate cancer cells. PIN and prostate cancer cells with defective GSTP1 genes remain vulnerable to oxidative stress and promote malignant progression. Such speculation has potentially important implications for the design of new prostate cancer prevention strategies, including (1) restoring GSTP1 expression via treatment with inhibitors of CpG methylation, (2) compensating for inadequate GSTP1 activity via treatment with inducers of general GST activity, and (3) reducing the risk of genome damage by avoidance of exogenous carcinogens and decreasing oxidative stress.

In summary, available results support the view that all forms of focal prostate glandular atrophy are proliferative, and that the vast majority are associated with inflammation. Many of the proliferating cells appear to have an immature secretory cell phenotype similar to that in PIN and prostate cancer. These common lesions may arise in the setting of increased oxidative stress, possibly derived from the proximate inflammatory cells. These findings are all consistent with the view that PIA may represent a precursor to PIN and prostate cancer.

**TELOMERE LENGTH: PROSTATE CANCER RISK AND DIAGNOSIS**

The ends of all eukaryotic chromosomes contain unique structures referred to as telomeres. Telomeres are composed of repeats of 6 base pairs as well as several different binding proteins that serve to protect chromosome ends from being recognized as double-strand breaks and from illegitimate recombination. Telomeres cannot be fully replicated during cell division and are thus subjected to progressive shortening, unless they are lengthened by the enzyme telomerase or a poorly characterized alternative pathway referred to as alternative lengthening of telomeres. Most somatic cells lack telomerase activity and undergo shortening of the telomeric DNA with cell division and presumably with aging in the organism. Telomere shortening appears to limit the lifespan of human cells and is believed to signal the onset of cellular senescence. Continued proliferation requires restoration and/or preservation of at least a minimal telomere length. This can be achieved by telomerase activity, which can be detected in most tumor cells.

As telomeres become shorter in experimental cell cultures, most normal cells undergo senescence, a process that permanently arrests the cells from further cell division. In cells that are treated with oncogenic simian virus 40 or other means to inactivate the p53 and pRb pathways, there is additional cell division and telomere shortening. At some point where telomeres become critically short, a “crisis” is ultimately reached that is characterized by a wide range of chromosomal abnormalities, including telomere end-to-end fusions, chromosome breaks, and translocations. Given the inverse relation between telomere length and genetic stability, it might be expected that shortened telomeres would be associated with increased risk for cancer, and this has been shown recently to be the case in animal models.

In human prostate cancer, Sommerfeld et al. measured telomere lengths in matched samples of normal benign prostatic hyperplasia (BPH) and in prostate cancer tissue taken from radical prostatectomies. The telomeres from prostate cancer tissue were significantly and consistently shorter than the telomeres from cells in either the adjacent normal tissues or BPH tissues. Many other cancer types also contain short telomeres. More recently, our group has developed a quantitative method that permits assessment of telomere lengths in individual cells in situ. We used this approach to show...
that telomeres were shortened in prostatic tumor cells versus normal prostatic epithelial cells.

Additional very recent results from our laboratory have added to our information on the relation between telomere shortening and prostate cancer. We have demonstrated that telomeres are shortened in luminal cells in the vast majority of lesions of high-grade PIN, but they retain normal lengths in underlying basal cells (Figure 1).49

A puzzling aspect of results on the relation between telomere length, telomerase activity, and prostate cancer is that telomerase activity has been shown to be increased in prostatic malignancies.46,50,51 This might be expected to be associated with longer telomeres and improved genetic stability. Although it is quite understandable that neoplastic cells containing short telomeres would activate telomerase to maintain telomere function, the reason that telomeres remain short in cancer is still a paradox.

The importance of telomere length in prostate cancer and other cancers has prompted investigation into its utility as a diagnostic and prognostic marker.52,53 Most relevant in the present context are results from Donaldson et al.53 that showed that death and disease recurrence in men with prostate cancer was significantly associated with reduced telomere DNA content, presumably reflecting shortened telomeres. Further study is needed, however, to elucidate the full extent of the relation between telomere length and prostate cancer over time.

In summary, results obtained by several different groups have shown that telomere shortening is a prevalent biomarker in human prostate neoplasia, and that it occurs early in the process of prostate carcinogenesis. Thus, genetic instability may be driven by telomere dysfunction in human intraepithelial carcinoma.

**α-METHYLACYL-COENZYME A RACEMASE: A NEW MOLECULAR MARKER FOR PROSTATE CANCER**

The importance of early and accurate diagnosis and prognosis of prostate cancer has prompted a search for better markers focused on the molecular mechanisms underlying tumor behavior (eg, altered cell cycle progression, apoptosis, neuroendocrine differentiation, and angiogenesis).54 Recent studies using complementary DNA microarray analysis have demonstrated widespread differences in gene expression patterns between benign and malignant growth of the prostate gland.55–65 Such gene expression analysis of prostate tissues should help to disclose the molecular mechanisms underlying prostate malignant growth and identify molecular markers for diagnosis, prognosis, and therapy.
Recent studies from our institution have focused on the gene for AMACR. AMACR plays an important role in the β-oxidation of branched-chain fatty acids and fatty acid derivatives; it catalyzes the conversion of several (2R)-methyl-branched-chain fatty acyl-coenzyme A to their (S)-stereoisomers.

Most importantly in the present context, AMACR is consistently upregulated in prostate cancer (Figure 2). Analysis of mRNA levels for AMACR revealed that it was increased about 9-fold in clinical prostate cancer specimens versus normal prostate tissues. Detailed immunohistochemical analysis indicated that AMACR was increased in tissues from both prostate cancers and high-grade PIN. Both untreated metastases and hormone-refractory prostate cancers were generally strongly positive for AMACR. We have seen no relation between AMACR upregulation and prostate cancer grade; AMACR appears to mark the presence of prostate cancer but not necessarily its aggressiveness.

Luo et al. extended the utility of this marker for prostate cancer diagnosis by combining immunocytochemistry for AMACR with staining for the nuclear protein, p63, a basal cell marker in the prostate that is absent in prostate cancer. Combined staining for p63 and AMACR resulted in a pattern that greatly facilitated the identification of malignant prostate cells. AMACR's consistency and magnitude of cancer cell–specific expression may render it an important new marker of prostate cancer. Its use in combination with p63 staining may lead to a method for improved identification of prostate carcinomas.

CONCLUSION

The results briefly summarized in this article are consistent with the following model for the development of high-grade PIN and early adenocarcinoma from PIA. Chronic and acute inflammation, in conjunction with dietary and other environmental factors, targets prostate epithelial cells and results in their injury or destruction. Increased proliferation occurs as a regenerative response to lost epithelial cells and may be mechanistically related to decreased p27Kip1, which we have shown to be decreased in PIA. The decreased apoptosis associated with these events may also be related to increased expression of Bcl-2 that we have also demonstrated in this tissue. The proposed increased in oxidative stress associated with these events may lead to elevated GSTP1 as a genomic protective...
measures. However, aberrant methylation of the GSTP1 gene promoter may decrease GSTP1 gene expression and protein levels. This, coupled with critically short telomeres, sets the stage for additional genetic damage and accelerated progression toward PIN and/or directly to carcinoma.

REFERENCES


