ELSEVIER

NEW CONCEPTS IN TISSUE SPECIFICITY FOR PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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ABSTRACT

Of the hundreds of species of mammals, all of which have prostate glands, only humans and dogs are known to suffer from benign prostatic hyperplasia (BPH) and prostate carcinoma. In humans, prostate carcinoma is common, yet carcinomas of other sex accessory tissues are rare. In addition, different anatomic regions within the prostate gland have very different rates of BPH and carcinoma. In this article, we explore ideas and potential mechanisms relating to these paradoxical findings that may help explain the species, organ, and zone specificity of BPH and prostate cancer. We present an evolutionary argument that attempts to relate a high-fat diet, with its potential for generating oxidative DNA damage, to the species selectivity of prostate cancer. In addition, we outline an argument based on our preliminary studies indicating that chronic inflammation and the associated increase in cell turnover in the setting of increased oxidative stress may help to account for the organ selectivity of genitourinary carcinomas. UROLOGY **53** (Suppl 3A): 29–40, 1999.

In this review, we discuss new concepts relating molecular mechanisms underlying the selective targeting of prostatic cells for spontaneous neoplastic transformation versus cells comprising other male sex accessory tissues. These mechanisms are explored in the context of our recently updated stem cell model of prostate organization and growth.[1,2]

EVOLUTIONARY ARGUMENTS

All mammals have either functioning breasts or a prostate gland. The prostate nurtures and protects sperm during transport to potential ovum fertilization[3] as the breast protects and nurtures offspring. Sex steroid hormones regulate both of these processes by controlling the development and growth of the breast and prostate and by regulating exocrine secretions that are rich in proteins, enzymes, and metal ions. In the prostate, these secretions include proteases such as prostate-specific antigen (PSA), prostatic acid phosphatases (PAPs), immunoglobulins, and zinc. Many components of prostate secretions may protect the male reproductive tract from external invasion by pathogens or serve as olfactory pheromones in marking and imprinting territorial boundaries.[3] Other sex accessory glands, such as the seminal vesicles and bulbourethral gland, also contribute to semen. These are involved in coagulation of the semen, which assists in maintaining high sperm concentrations in the vagina and may also help deter competitive fertilization by sperm from other matings. The rich concentrations of prostaglandins, polyamines, and carbohydrates such as fructose have all been implicated, but not proven, as critical factors in enhancing sperm survival and fertilization.[3]

Of the hundreds of mammalian species, only humans and dogs have a propensity for spontaneously developing benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma. The incidence of BPH is equally high in humans and dogs, but the incidence of prostate cancer is much higher in the human.[4–7] Whereas approximately 10% of prostate cancers may be inherited, over 90% develop as a result of environmental and cultural habits. Age-adjusted prostate cancer incidence and mortality rates vary by >10-fold between different countries, indicating that prostate cancer development is not simply an inherent consequence of ag-

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ing.[8,9] Breast cancer rates also vary across geographic borders, and a graph of the age-adjusted incidence of breast cancer in different countries versus the age-adjusted incidence of prostate cancer yields a nearly straight line.[10] This similar geographic distribution of prostate and breast carcinoma risks suggests the importance of similar dietary or other environmental factors, particularly because migration to new geographic regions results in the adjustment of incidence toward that characteristic of the new location.[11]

Although both prostate and breast carcinoma are common in humans, carcinomas of other sex accessory tissues are rare. For example, <50 cases of primary seminal vesicle carcinoma, <30 cases of carcinoma of the epididymis, and no cases of carcinoma of the bulbourethral glands or vas deferens have been reported.[12–14]

DIET

Homo sapiens appears to have evolved from the great apes, which are capable of consuming great quantities of fresh foliage and fiber every day. Four million years ago, humans evolved and moved down from the trees to seek grain and vegetation on the open savanna.[15] By 1 million years ago, humans were making very crude weapons and functioning as hunters and gatherers.[15] The eating of raw meat provided more highly concentrated energy and improved efficiency over the great apes' requirement for up to 50 pounds of fresh foliage daily. Only approximately 400,000 years ago, humans built the first fires and learned to cook meat, and processed and cooked fat became a prominent part of our diet.[15] Approximately 40,000 years ago, we became farmers and started the domestication and herding of animals, which quickly improved the availability of meat, making it much easier to gain access to this form of protein throughout the year.[15] Therefore, humans evolved for millions of years on a primarily herbivorous diet of fresh greens and then late in their history converted to an omnivorous diet with the emphasis on cooked meat and high fat content (in Western cultures).

The evolution of these dietary practices was rapid and late, and may have outpaced the time required for biologic evolution to provide a biochemical adjustment. This may be one possible reason for the positive correlation between breast, prostate, and colorectal carcinoma rates and levels of dietary animal fat in different populations. The association of fat content and red meat with cancer has long been known and may result from oxidative DNA damage exceeding protection and repair.[16] In addition, it has recently been recognized that the mysterious steroid receptor-like transcription factors, termed orphan receptors, may be regulated by ligands such as fats. This provides a molecular mechanism whereby dietary fats can more directly affect cell growth and function.[17] In addition, the physiologic or pathologic results may be dependent on the specific type of fat and the cell types with which it interacts. The effects of specific fats on orphan receptor signaling and oxidative damage is one of the frontiers of prostate carcinogenesis. Diet makes a difference, and now we are beginning to find out why. We propose that these effects of fats on carcinogenesis vary in different sex accessory tissues and in different zones of the prostate.

RELATION OF BENIGN PROSTATIC HYPERPLASIA TO PROSTATE CANCER

The geographic distribution of clinically apparent prostate cancer does not correlate with the prevalence of pathologic BPH. However, the incidence of BPH in various countries appears to increase with age in a manner similar to "latent" prostate cancer, which is defined by small incidentally identified histologic prostate cancer lesions.[18] In contrast, as previously mentioned, clinically apparent prostatic adenocarcinoma has a far different geographic variation in incidence than the 2 more benign diseases, BPH and latent carcinoma. Indeed, BPH and clinical prostate cancer are primarily entities of 2 different areas of the prostate; the transition zone has a high incidence of BPH and a low incidence of carcinoma, whereas the peripheral zone has a high incidence of carcinoma and a low incidence of BPH.[19,20] It appears that the normal prostate evolves through phases of prostatic intraepithelial neoplasia (PIN) and possibly other atypically proliferating lesions before producing a prostate adenocarcinoma.[21] Most peripheral zone cancers occur without the intervening step of BPH. Also, the development of BPH does not involve PIN. BPH can be an overgrowth or hyperplasia of both the epithelial and stromal compartments. Very large BPH glands are primarily rich in epithelial growth, but in some BPH glands the stromal elements are predominant. It is clear that there is close interaction between the epithelium and stroma in relation to their induction of growth by bidirectional stromal–epithelial interactions.[22] What is the role of stromal cells in carcinogenesis in the prostate?

CELL TYPE SPECIFICITY

In regard to cellular differentiation, it has been shown that specificity resides in the cytoskeleton and nuclear matrix composition. The main cytoskeleton intermediate filaments of epithelial cells are keratins, of which there are >20 varieties, and their composition has long been used to identify

different epithelial cell types.[23–25] William B. Isaacs (unpublished observations) was the first to identify keratins in basal cells of the dog prostate, and subsequently a variety of different anti-keratin antibodies have been developed that identify basal cells and secretory luminal cells of the prostate. The use of keratin antibodies is one of the most frequently applied markers for identifying prostate cancer, in which the basal cell component is lost[26] and basal cell-specific cytokeratin immunohistochemical staining therefore disappears. The cytoplasmic intermediate filament keratins are also attached to a nuclear matrix component, which is the skeletal aspect of the nucleus. The nuclear matrix is a residual component of the nucleus equivalent to the cytoskeleton. Its outer layer is a protein layer composed of lamins. There are 3 major types of lamins, which are related to keratins.[27] The laminal proteins vary with embryonic development and are markers of certain cell types.

The internal components of the nuclear matrix organize the DNA into tissue-specific loop domains, with active genes being associated with the matrix and inactive genes extending out into loop components.[28,29] The DNA loop components are anchored at their base to the nuclear matrix, and each loop comprises approximately 60,000 base pairs; there are about 50,000 such loops in a mammalian nucleus. The loop base is the site of DNA replication, and the loop domains are replicons that function by being reeled down through these fixed synthetic sites during S phase. Steroid hormone receptors also bind to the nuclear matrix and are part of the tissue specificity.[30] Indeed, the organization of DNA loops in the seminal vesicle and ventral prostate are different, as is the protein composition of the nuclear matrix.[29]

In summary, the cytoskeleton and nuclear matrix make up a tissue matrix system extending from the extracellular matrix through to the DNA. This system is highly tissue specific and defines the organization of functional domains of DNA.

When BPH develops in humans there is a change in nuclear matrix composition, and some of these changes are the same as those that occur in adenocarcinoma.[31] This suggests that many similar events occur in both BPH and cancer.[31] However, additional changes occur in the nuclear matrix of carcinoma that are not seen in BPH.[31] It has, therefore, been proposed that BPH and prostate cancer undergo similar changes, but because they occur in different zones they do not necessarily have a cause-and-effect relation but rather develop in parallel. How might the molecular determinants of tissue and cell specificity, i.e., the intermediate filaments and nuclear matrix, be involved in the organ and site specificity of carcinogenesis?

GENERAL FEATURES OF STEM CELLS

Although stem cells were first identified in bone marrow, solid organs that undergo continual and rapid cell replacement, such as the gastrointestinal tract and epidermis, also require stem cells. More recently, it has been recognized that the epithelium from most other organs also continuously replaces itself,[32,33] albeit more slowly; thus stem cells appear to exist in these organs as well. Unique stem cell features include: (1) long [^{3}H]thymidine label retention times/slow turnover rates; (2) vast proliferative capacity; (3) pleuripotentiality; and (4) self-renewal.[34] Recently, novel experimental systems have been developed that possess many features of the stem cell hierarchy of both squamous epithelium[35,36] and mesodermal tissues.[37]

Stem cells do not appear to give rise directly to mature functional cells. Rather, they give rise to a population of amplifying cells that divide rapidly but that have much more limited proliferative capacity.[34] These transiently proliferating cells (TP cells) differentiate into mature cells that are programmed to die.

STEM CELL THEORY OF CANCER

Pierce and coworkers[38–40] have proposed that cancer cells represent the malignant counterpart of normal tissue stem cells. In this scheme, tumors are caricatures of tissue renewal, with some tumor cells representing stem cells and others representing terminally differentiating cells that lose proliferative and neoplastic potential. Buick and Pollak[41] also contributed to this concept and hypothesized that the molecular-genetic and stem cell theories of neoplasia development could be integrated by predicting that oncogene expression in normal cells is tightly regulated in relation to differentiation, a prediction that is proving to be accurate.[42]

EVIDENCE FOR STEM CELLS IN THE NORMAL PROSTATE

In several solid organs, cell types are organized so that stem cells, TP cells, and mature terminally differentiated cells occupy discrete locations.[33,43,44] Although there is still debate as to the existence, location, and nature of prostatic stem cells, our group and others have presented models suggesting that a similar stem cell–driven hierarchical arrangement is responsible for epithelial cell renewal in the adult prostate.[1,2,18,45–47]

The majority of prostatic epithelial cells in the adult gland are androgen dependent for survival[48] such that castration leads to the loss of up to 90% of all epithelial cells via programmed cell death.[48,49] The remaining epithelial cell population is andro-

gen independent for survival. However, at least some of these surviving epithelial cells remain androgen sensitive, because the subsequent administration of exogenous androgens to the castrated animal results in regeneration of the prostate to normal size and morphology. This process of involution and subsequent androgen-induced regeneration can be cycled numerous times. Based on these results, Isaacs and Coffey[18] postulated a stem cell model of prostate organization whereby slowly proliferating androgen-independent reserve stem cells give rise to a second population of more rapidly cycling androgen-independent but androgen-responsive amplifying cells (this proposed amplifying population is analogous to the TP cells seen in other organ systems and will be referred to as such). Although these TP cells actively cycle, their capacity for self-renewal is limited. By amplifying their number they would appear to reduce the potential for accumulation of mutations in the longer-lived stem cells. The TP cells respond to androgens by producing more mature secretory cells with very limited proliferative potential that subsequently undergo terminal differentiation into androgen-dependent secretory cells.[18]

CELLULAR COMPARTMENTALIZATION OF PROSTATIC EPITHELIUM

Prostatic epithelium consists of 2 defined compartments, basal and secretory.[50–52] In the basal compartment, 1 or 2 layers of cells are situated between the basement membrane and the overlying secretory cells.[50,51] The secretory compartment consists of a luminal layer of columnar cells that rest upon the basal cells. Basal cells can also be distinguished phenotypically from secretory cells, because they uniquely express specific cytokeratins, including keratins 5 and 14, and lack expression of secretory markers such as PSA and prostate-specific acid phosphatase.

Several lines of evidence suggest that prostate secretory cells arise from basal cells. *First*, immunohistochemical and radiolabeling experiments show that the bulk of the proliferating pool in the normal-appearing human prostate is restricted to some basal cells.[53,54] *Second*, cells with characteristics intermediate between those of basal cells and secretory cells are present in the developing and adult prostate.[45] In addition, double immunolabeling has shown that individual cells are present that have cytokeratin expression profiles of both basal and secretory cells.[55,56] Indeed, it has been suggested that these latter cells may represent the "amplifying" or TP compartment in the prostate.[55,56]

OTHER EMERGING POTENTIAL STEM CELL/CANCER CELL MARKERS OF THE PROSTATE

Reiter *et al*.[57] recently identified a gene called prostate stem cell antigen (PSCA) with homology to stem cell antigen 2, a marker for the earliest phase of hematopoietic development. This antigen is present in a normal subset of basal cells and is highly expressed in prostate carcinomas. Because the encoded protein appears to be expressed on the cell surface, PSCA may be a useful marker in the localization and isolation of putative prostate stem cells and in the diagnosis, prognosis, and treatment of prostate cancer.

ALTERATIONS OF STEM CELL COMPARTMENTALIZATION IN PRENEOPLASTIC LESIONS

Molecular alterations in cell cycle control that result in increased overall proliferation must be present in human neoplasms.[58–61] High-grade PIN is the presumed precursor lesion to many prostatic adenocarcinomas.[21,62–64] As in precursor lesions of cancer of the colon and cervix, there is an overall increase in the proliferative fraction in PIN. Importantly, the compartmentalization of proliferating cells is altered such that the ratio of proliferating secretory-type cells to proliferating basal cells is greatly increased.[53,54] This alteration in the normal compartmentalization of proliferation has recently been termed topographic infidelity of proliferation (TIP)[2] and also appears in other preneoplastic conditions, such as those of the colon[65] and cervix. Neither the mechanisms nor the consequences of such altered proliferation in cancer precursor lesions are understood. In addition, little is known regarding the molecular mechanisms of how the compartmentalization of proliferation and differentiation are coordinated in normal tissue and altered in prostatic preneoplasia. We have recently begun to examine molecular markers that may help regulate these processes and have focused on the cell cycle regulators.

$P27^{kip1}$ EXPRESSION IS TOPOGRAPHICALLY ORGANIZED IN NORMAL HUMAN PROSTATES

Progression through the cell cycle is controlled by cyclin:cyclin-dependent kinase complexes and their various inhibitors, such as the cip/kip family of cyclin-dependent kinase inhibitors, including $p27^{kip1}$ and p21/waf1/cip1.[66] Recent studies have indicated that $p27^{kip1}$ expression is downregulated in carcinomas of the breast, colon, lung, and prostate, and that levels may correlate with clinical outcome.[1,67–78] We have recently tested the hypothesis

that $p27^{kip1}$ downregulation renders cells capable of serving as the TP compartment in the prostate.[1] In nonneoplastic normal-appearing prostate, moderate to strong $p27^{kip1}$ staining was present in >85% of secretory cell nuclei, whereas in the basal cell compartment $p27^{kip1}$ expression was commonly significantly reduced or absent. Although prostatic ducts/acini have generally been considered to contain 2 cell layers, we found a variably present third zone of cells.[1] We postulate that these $p27^{kip1}$-negative cells are either proliferating actively or are proliferation competent and poised for rapid growth after additional mitogenic stimulation. Whether this third layer corresponds to the intermediate cells, as defined by specific cytokeratin expression,[55,56] remains to be determined.

$P27^{KIP1}$ IS DOWNREGULATED IN HIGH-GRADE PIN AND INVASIVE CARCINOMA

In all cases of high-grade PIN, we found downregulation of $p27^{kip1}$ as compared with adjacent benign prostatic epithelium.[1] In invasive carcinomas, there was also significant downregulation of $p27^{kip1}$ as compared with adjacent benign epithelium.[1] Others have also recently reported downregulation of $p27^{kip1}$ in the vast majority of prostatic carcinomas.[73–78] Although $p27^{kip1}$ levels in prostatic carcinoma may help predict clinical outcome,[74,75,77,78] this is still at least somewhat controversial.[76] Our studies of high-grade PIN suggest that alterations in the regulatory control of $p27^{kip1}$ levels occur early in prostatic carcinogenesis and may be a key mechanism for cell cycle dysregulation in the vast majority of clinical prostate cancers.[1]

DNA DAMAGE, GLUTATHIONE S-TRANSFERASE PI, AND PROSTATE CARCINOGENESIS

Prostate cancer development is accompanied by somatic genomic changes, including deletions, amplifications, and point mutations.[79–81] The pi class glutathione S-transferase (GSTPI), has been proposed to function in the defense against carcinogens.[82–84] By immunohistochemistry of normal-appearing prostate tissue, GSTPI expression is largely, but not exclusively, restricted to the basal compartment. In contrast, GSTPI is not expressed in the vast majority of prostatic adenocarcinomas[85–89] or in high-grade PIN.[90] Lee *et al.*[85] found that promoter hypermethylation of the GSTPI gene is present in nearly all prostate carcinomas, whereas no hypermethylation is detected in adjacent normal prostatic epithelium. In addition, approximately 70% of high-grade PIN lesions also show hypermethylation of the GSTPI promoter.[90] Thus, GSTPI promoter hypermethylation appears to arise early in prostatic carcinogenesis. This abnormal methylation of the GSTPI promoter appears to explain the absence of GSTPI expression in prostatic neoplasia.[85]

TISSUE SPECIFICITY OF BPH AND CANCER

BPH, as the name implies, is a benign disorder that develops predominantly in the transition zone of the prostate. Although BPH is associated at times with a lesion termed adenosis or atypical adenomatous hyperplasia (AAH), BPH does not appear to progress directly to carcinoma. Whether adenosis/AAH is a precursor of low-grade carcinoma is presently uncertain, although recent molecular analysis that shows that some adenosis/AAH lesions have loss of chromosome 8p is certainly suggestive that at least some may be preneoplastic.[91] Nevertheless, carcinomas that arise in the transition zone, and presumably at times from adenosis/AAH, are usually of low Gleason grade and low malignant potential as compared with the more aggressive tumors that develop in the peripheral zone[92,93] (presumably from high-grade PIN).

It has been shown previously that there is an increased rate of proliferation in BPH,[94,95] but the ratio of proliferating basal cells to secretory cells is intact compared with normal-appearing prostate epithelium[1,53]; thus there is no TIP in BPH. We have hypothesized that the target epithelial cell type for abnormal growth regulation in BPH is predominantly the basal cell.[2] Because basal cells generally express much higher levels of gene products that appear to have genome protection features, such as GSTPI (protection against electrophilic carcinogens),[82,84] we proposed that basal cells are largely protected from the acquisition of multiple genomic changes and hence neoplastic transformation.[2] This is consistent with the literature indicating that unlike PIN and cancer, there is no evidence that BPH is a clonal disorder and only rare genetic abnormalities have been identified in BPH, indicating a lack of genomic instability.[96–99]

On the other hand, high-grade PIN and prostate cancer are thought to be clonal disorders, with the bulk of the available data supporting the concept that PIN is the precursor lesion to many clinically significant peripheral zone prostatic carcinomas.[63] We propose that high-grade PIN, and hence the majority of invasive prostatic carcinomas, are derived from secretory cells, but not terminally differentiated secretory cells. We suggest that the known small subset of TP cells in the secretory compartment, which have not yet undergone terminal differentiation, undergoes a lesion in the cell cycle that prevents or prolongs the normal course of rapid and permanent exit from cell division.[2] A strong candidate for such an abnormality in cell

cycle regulation is $p27^{kip1}$.[1] Another previously proposed candidate is BCL-2,[100–103] which can be found in the secretory compartment of high-grade PIN and which may prolong the life of these cells or prevent apoptosis in response to DNA-damaging agents.

Although secretory cells have the capacity to induce GSTPI expression,[85] it appears that at some point during the progression of normal prostate secretory cells through high-grade PIN to carcinoma, the GSTPI promoter becomes silenced by hypermethylation.[90] Thus, our recent model suggests that during the development of high-grade PIN and in carcinoma, these abnormal secretory cells practice unsafe replication,[2] i.e., they synthesize DNA in the absence of a normal battery of DNA replication–protective mechanisms, such as GSTPI. This permits the accumulation of genetic errors leading to neoplastic transformation. Certain features of normal stem cells that are maintained or enhanced in the aberrant cells aid full neoplastic transformation. These features include apoptosis suppression and immortality. The latter is most likely the result of telomerase expression. Additional genomic changes accumulate due to continued unsafe proliferation and finally result in invasion and metastasis.

Still paradoxical is what is responsible for the different target cell selectivity in these different zones. We have pointed out that basal cells in the prostate transition zone show differences in differentiation markers, such as patterns of intermediate filament[104–106] and nuclear matrix protein expression,[31] versus other prostate zones, thus implying different underlying biologic properties for basal cells from the transition zone compared with those from other zones.

WHICH MECHANISMS ACCOUNT FOR THE TISSUE SPECIFICITY OF CANCER IN THE HUMAN MALE GENITOURINARY TRACT?

We have been attempting, most recently, to shed light on the apparent paradox that although the seminal vesicles and prostate share the same blood supply, have the same genome, have the same androgen receptor, and are subjected to the same dietary carcinogens, they have different rates of carcinoma. We explore this in light of both current concepts regarding the development of cancer in general and specific concepts relating to the male genitourinary system.

For carcinoma to develop, 3 events must occur: (1) target cells must be capable of proliferation; (2) target cells must actively proliferate; and (3) target proliferating cells must undergo heritable genomic alterations that provide a survival advantage. In other words, DNA lesions occurring in dividing cells enable the lesions to be converted into permanent heritable genomic alterations such as mutations, which are thought to be central to the development of malignant neoplasia.[107,108] In terms of the capability for proliferation, all epithelia are known to turn over. What varies is the rate of turnover of the epithelia and the frequency of proliferative events in at-risk cells. In many organs, cell turnover itself does not predispose to cancer. For example, the rate of epithelial turnover in the small intestine, which contains many billions of cells, is high and comparable to that of the large intestine. However, small intestinal carcinomas, as opposed to colorectal carcinomas, are a rarity. In this regard, it has been shown that the rate of cell turnover in the seminal vesicles is approximately 6-fold lower than that in the prostate as measured by [^{3}H]thymidine incorporation.[94] Meyer *et al.* postulated that this low rate of proliferation in the seminal vesicles might account for the rarity of seminal vesicle carcinomas.[94]

We have recently begun to examine this as well. In normal-appearing seminal vesicle epithelium and prostate, we find a relatively increased amount of cell proliferation in the prostate, as measured by Ki-67 labeling (A.M. De Marzo, unpublished observations). However, because we agree that the difference is on the order of 5- to 10-fold, we submit that this cannot explain by itself the great difference in the incidence of cancer in these 2 organs. Rather, we now raise the possibility that there is an abnormal increase in proliferation in the prostate, in the setting of increased oxidative stress, that leads to an increased risk of carcinoma. We postulate that the prostate is subjected to injurious agents that the seminal vesicle is not subjected to, and these agents can act focally within the gland to markedly increase the proliferation of target secretory cells of the prostate.

CAN THE DRAMATIC DIFFERENCE IN THE RATES OF ACUTE AND CHRONIC INFLAMMATION HELP ACCOUNT FOR THE DIFFERENCES IN THE INCIDENCE OF CARCINOMA IN THE PROSTATE AND SEMINAL VESICLES?

It has been suggested for decades that chronic inflammatory reactions in tissues place them at increased risk for the development of carcinomas. Recently, these concepts have been revisited and greatly expanded.[107–113] In terms of abnormally increased epithelial cell proliferation in the prostate, inflammatory reactions have been implicated.[114] The relation between chronic inflammation and the etiology of prostate carcinoma has been suggested recently in an insightful review that argues from an epidemiologic and mechanistic point of

view that we should systematically examine the potential relation between long-standing inflammation and prostate carcinogenesis.[115]

It has been known for many decades that the prostate gland is often the target of acute and chronic inflammatory responses; the leading precipitant of visits to urologists is not cancer or BPH but prostatitis.[116] In light of our present argument with regard to the relative risks of prostate and seminal vesicle carcinoma, we would like to point out the little discussed fact that primary clinical seminal vesiculitis is rare.[13]

The dramatic differences in the incidence of clinically apparent inflammatory lesions in the prostate and seminal vesicles are also reflected microscopically. Histopathologic analysis reveals that inflammation is not present in the vast majority of human seminal vesicles. We routinely receive intact seminal vesicles with radical prostatectomy specimens. In review of thousands of cases, we have never seen significant acute or chronic inflammation of the seminal vesicles (J.I. Epstein, A.M. De Marzo, unpublished observations). The work of others[117] and our recent unpublished observations (A.M. De Marzo, D.S. Coffey) suggest that essentially all prostates removed surgically for either prostate or bladder cancer have multiple foci of chronic inflammation and often have foci of mixed acute and chronic inflammation as well. Our recent work also suggests that in chronic inflammatory lesions the prostate epithelium is hyperactive and undergoing a dramatic increase in proliferative rate (A.M. De Marzo, W.G. Nelson, J.I. Epstein, D.S. Coffey, unpublished observations), suggesting tissue damage as a result of the inflammatory response with resultant compensatory cell replacement. This is coupled with the oxidative stress from the inflammatory cells themselves.[110] We propose that this proliferative response renders cells at high risk for DNA damage and hence the development of neoplasia. The possible chemoprotective effect of antioxidants such as vitamin E[118] may indeed act by protecting prostate cells from DNA damage occurring in the setting of inflammatory lesions. In addition, Sharma *et al.*[119] have found that diet can play a role in the development of prostatitis in the rat. They showed that a soy-rich diet was protective against the development of lobe-specific prostatitis. Can this finding be related to the known lower rates of prostate carcinoma in Asian populations, which consume much larger quantities of soy protein than do Western populations?

Although the precise target of the inflammatory response in the prostate remains unknown in the majority of cases, novel infectious agents may be involved,[120,121] as detected by isolation and sequencing of prokaryotic ribosomal DNA. We are only beginning to examine this potential association of chronic inflammation with prostate cancer, but the difference between the presence of inflammation in the prostate and the seminal vesicles is striking. It would certainly be of interest to determine whether the prokaryotic rDNA sequences found in the prostate correlate with the presence of carcinoma and whether these sequences are absent in the seminal vesicles.

Our preliminary studies also suggest another mechanism whereby the seminal vesicle might be protected against the development of cancer. We recently questioned whether the expression of apparent cancer-protective enzymes, such as GSTPI, is different in the prostate and the seminal vesicles. Our preliminary studies indicate a major difference in the expression of GSTPI in the secretory layer of cells in the seminal vesicles as compared with those of the prostate. Figure 1 shows a human seminal vesicle tissue section that was stained for GSTPI. It is clear that both the secretory and basal layers of the seminal vesicle are strongly positive for GSTPI. This is in contrast to prostate tissue, which was present on the same section, in which the normal-appearing secretory cells are GSTPI negative (Fig. 1, bottom inset).[85–88]

Taken together, we argue that human seminal vesicle epithelium is protected from undergoing neoplastic transformation by at least 3 mechanisms. *First*, the cells of both the basal and secretory compartments of the seminal vesicles express high levels of GSTPI, which may naturally render them protected against DNA damage. *Second*, seminal vesicle epithelial cells naturally replace themselves very slowly, and hence the DNA is at less risk for acquiring errors during replication. Third, the absence of acute and chronic inflammatory responses in the seminal vesicles may place the relative risk of carcinoma development at a very low level compared with the prostate.

Having a potential culprit in hand, we agree with Platz[115] that the examination of prostate inflammation and its association with carcinoma may prove to be a fruitful area of investigation. In addition, the potential relation between inflammation and prostate cancer raises possibilities with regard to chemoprevention with anti-inflammatory agents. Some of these agents, such as the nonsteroidal anti-inflammatory drugs, have a known chemoprotective effect in other human organs in which there is an association between carcinoma and long-standing chronic inflammation, such as the colon/rectum, stomach, and esophagus.[122]

CONCLUSIONS

In summary, this article explores ideas and mechanisms regarding the seemingly paradoxical

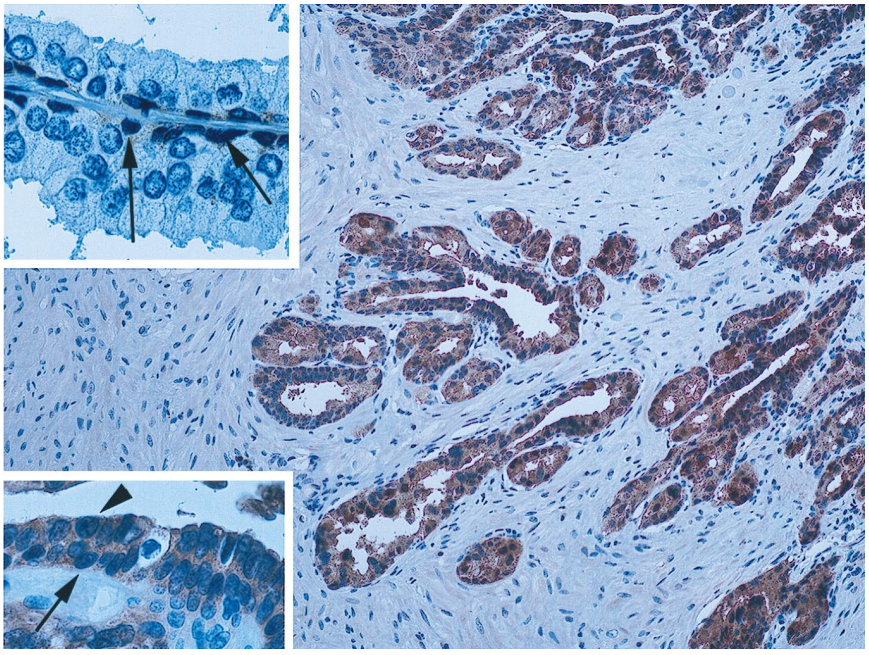

FIGURE 1. *High levels of glutathione S-transferase pi (GSTPI) expression in human seminal vesicle epithelial cells. Photomicrograph of a section of formalin-fixed and paraffin-embedded human seminal vesicle stained with a polyclonal antibody against GSTPI as described.[90] Note intense staining of most seminal vesicle epithelial cells. Surrounding stroma shows only weak, nonspecific immunoreactivity (original magnification × 100). Bottom inset shows a high-power view. Arrow indicates basal compartment and arrowhead indicates luminal compartment (original magnification × 400). Top inset shows high-power view of adjacent prostate epithelium present in the same tissue section. Note basal cell-specific immunostaining (arrows) for GSTPI in the prostate epithelium (original magnification × 400).*

species and organ specificity of steroid hormone–dependent tumors of the prostate. The evolutionary argument presented attempts to relate a high-fat diet with its associated oxidative DNA damage to the species selectivity of prostate cancer. In addition, an argument whereby chronic inflammation and its associated increase in cell turnover in the setting of increased oxidative stress was presented, which may help to account for the organ selectivity of genitourinary carcinomas.

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QUESTIONS AND ANSWERS: DONALD S. COFFEY, PhD

Michael Marberger, MD: Benign prostatic hyperplasia (BPH) occurs in the transition zone, whereas prostate cancer occurs mainly in the peripheral zone. Therefore there is localized predominance of disease. How does this fit into the stem cell theory?

Dr. Coffey: The incidence of cancer in the transition zone is low. However, BPH does not transform into cancer; this never occurs. We have found that what causes the stem cell to move toward the epithelium is tissue specificity in the various areas of the prostate. This will be the next frontier for us to work on. However, we think that we have determined how these cells are capable of becoming genetically unstable. This is important. If a cancer is stained using 15 different antibody enzymes, the cells will express every possible combination of these markers: one cell will express prostate-specific antigen (PSA), another will not; one cell will express acid phosphatase with no PSA, another cell both of these, and yet another cell neither of them. This is called tumor cell heterogeneity, and it is this diversity that produces variants that are resistant to every therapy we have available. If BPH cells are stained for the same 15 markers, they are uniform, as is the healthy prostate. Thus every cancer becomes resistant to every drug treatment presented, whereas normal cells never develop resistance to cancer chemotherapy drugs or androgen withdrawal. This diversity is caused by genetic instability, and this occurs as the glutathione S-transferase (GST) pi disappears and the cells replicate in an unsafe area, accumulating damage. This is part of the picture and it is like turning on the evolutionary process. What triggers the change in the stem cells in the different areas of susceptibility, whether it is stroma–epithelium interactions or something else, is an area about which nothing is known. However, it is important that the diversity and clonal expansion seen in cancer do not occur in BPH, and this model explains why this is so.

Dr. Marberger: Does this also apply to the seminal vesicles?

Dr. Coffey: There are more ways to absorb activated oxygen than through GST pi. One of these is that on oxidation, some lipids form lipid fusion particles. In aged tissue these particles build up in cells and seminal vesicles are full of them; prostate cancer is not. We believe that seminal vesicles are using a different protective system, as well as GST pi, to absorb free radicals.

E. David Crawford, MD: It is my understanding that in cancer the basal cell layer is lost. This is why many of the pathology tests done on biopsies use high-molecular-weight cytokeratin stains for the basal layer. Is this correct?

Dr. Coffey: Yes, it is correct, and I see your point. If basal cells moved up into the secretory cell layer and started to divide and expand, they would cease to be basal cells. In this instance, high-molecular-weight keratin would not be present. This is what

the model says will happen. The replicatory process is now toward secretory cells and basal cells are not needed because these secretory cells now have stem cell–like properties.

Dr. Crawford: Therefore, the basal cells are still present, but they have different properties. If a patient receives androgen deprivation therapy, the majority of the cells will undergo apoptosis, leaving a group of "basal cells" that are androgen independent.

Dr. Coffey: First, the cancer arises from expansion of the epithelial secretory cells. This is why PSA is produced at such high levels. These cells are now stem cells in themselves, but are highly variable. Those with the ability to grow without androgen will continue to replicate with androgen deprivation therapy. This is a major problem.

I do not want to give the impression that all of this is clearly understood. Cancer is a stem cell problem.

Dr. Crawford: I assume that you are familiar with the Goldie–Colman hypothesis that the longer a cancer exists, the more genetic "hits" it receives?

Dr. Coffey: Of course, and you would predict in this case that as these cells replicate without protection, the number of genetic "hits" would increase.

Dr. Crawford: However, hormone therapy is actually a relatively effective treatment for prostate cancer. There are not many cancers in which widespread disease can be treated with a simple therapy, allowing patients to live for 3 years. The clinical challenge that I see is that patients present with prostate cancer earlier and earlier, but I know they are going to fail therapy. They do not have metastatic disease, but increasing PSA levels after failed local therapy. This is now the most common presentation. Is there a rationale based on this hypothesis to treat people only when they become symptomatic or to treat earlier with androgen therapy, which works, or even use chemotherapy as well?

Dr. Coffey: First, when men have their testes removed and are dying of prostate cancer, PSA levels continue to increase. This indicates that PSA is being produced by cells, in this case secretory cells, that are not controlled by androgens. Normally, when the testes are removed PSA levels decrease rapidly, but rebound.

What does this mean for cancer patients? When this work was started in the 1970s, we were using animal models, which, although informative, were different from humans. The work is now being done in human tissues. However, we found that if a tumor was induced in an animal and allowed to increase in size, there comes a point where it cannot be cured by surgery or hormone therapy. As long as the tumor was small, hormone therapy was effective in increasing the life of the animal. Based on this we concluded that early hormone therapy is essential to extend the life of patients. I still believe that this is true, but when is early? I think that using PSA as a marker is enabling us to define when early is, and the use of adjuvant therapy and other modalities is just confirming that treating early is best.

Dr. Crawford: The majority of people seen clinically with metastatic prostate cancer have a hard prostate on rectal examination and positive bone scans. They are treated with hormonal therapy and die, but with treatment their prostates are no longer palpable. Does this mean that local tumors respond differently than metastatic tumors, and if so, are the new treatment strategies (adjuvant and neoadjuvant therapies) beneficial?

Dr. Coffey: The bottom line is as follows. If you perform warm autopsies in patients who have died of advanced prostate cancer, the prostate is enlarged but the metastatic tumors are hockey puck size. Tumors of this size are not seen in the primary site. This means that prostate cancer cells that move out of the prostate have a different ability to grow. The Mayo Clinic group then showed that prostate cancer is multifocal, i.e., it is not one type of cancer. Using genetic markers they showed that the tumors are different and that the ones killing patients are smaller tumors. Prostate cancer is a complicated disease and cannot be discussed as one entity; it is multifocal, just as it is heterogeneous. We now need to examine individual cells and I think that this is where telomerase will become very useful.

Dr. Marberger: You showed the similarities between prostate cancer and breast cancer. Does breast cancer fit the same pattern?

Dr. Coffey: Yes. The same thing is happening, but different brake systems are in action: sometimes it is p21, sometimes p16, and sometimes p27. These are cyclin-dependent kinase inhibitors, and they inhibit the switch governing cell cycle activity. It will be necessary to learn how these are regulated.

Dr. Crawford: What will be the role of telomerase, the enzyme of immortality, in diagnosing prostate cancer? Is there any work on eliminating or neutralizing telomerase?

Dr. Coffey: When speaking to the general public, I put it like this. PSA is a smoke alarm. If your smoke alarm goes off and you do not check it, you will get into trouble, although the fact that a smoke alarm goes off does not guarantee that there is a fire. Telomerase is a fire alarm. When telomerase is turned on, cells become immortal and the only cells that do this apart from sperm, ova, and some stem cells are cancer cells. Thus, prostate cancer expresses telomerase; BPH and healthy prostate, apart from the stem cells, do not.