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Prostatic Intraepithelial Neoplasia (PIN) and Other Prostatic Lesions as Risk Factors and Surrogate Endpoints for Cancer Chemoprevention Trials

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Abstract The most efficient strategy for chemoprevention clinical trials are short-term studies which focus on surrogate endpoint biomarkers (SEBs) in high-risk target populations. High-grade prostatic intraepithelial neoplasia (PIN) is the most likely precursor of prostate cancer, and is found in a significant number of routine contemporary needle biopsies without cancer. The frequency and extent of PIN are decreased with androgen deprivation therapy, suggesting that it is a suitable endpoint biomarker for modulation. Potential SEBs for screening chemopreventive agents for prostate cancer in short-term Phase II trials include (1) histologic premalignant lesions, such as high-grade PIN; (2) biochemical markers, including prostate-specific antigen (PSA) serum concentration; and (3) morphometric markers, including nuclear texture, shape, and roundness; size and number of nucleoli; and number of apoptotic bodies; (4) proliferation markers, including MIB-1 and PCNA; (5) genetic markers, including nuclear DNA content (ploidy), oncogene c-*erb*B-2 (HER-2/*neu*) expression, fluorescence in situ hybridization for chromosome 8; and PSA-producing cells in the blood detected by reverse transcriptase polymerase chain reaction; and (6) differentiation markers, such as microvessel density as a determinant of angiogenesis. Each of these endpoint biomarkers is measured easily and accurately in serum or in tissue specimens such as formalin-fixed, paraffin-embedded needle biopsies, and may be modifiable by intervention. The clinical utility of these biomarkers as modulatable endpoints in prostate cancer chemoprevention needs to be demonstrated in future clinical trials. J. Cell. Biochem. 25S:156–164. © 1997 Wiley-Liss, Inc.

Key words: carcinogenesis; chemoprevention; prostate cancer; prostatic intraepithelial neoplasia; prostatic neoplasms; surrogate endpoint biomarkers

High-grade prostatic intraepithelial neoplasia, PIN is the most likely precursor of invasive prostatic carcinoma [1,2]. Premalignant lesions such as high-grade PIN identify patients at high risk for developing invasive cancer, and these are ideal target populations for chemopreventative trials. Chemoprevention is a strategy to reduce cancer risk in susceptible individuals by administering natural or synthetic drugs with little or no toxicity which suppress, delay, or reverse the process of carcinogenesis [3]. Thus, chemoprevention seeks to treat the earliest stages of carcinogenesis when reversibility may be feasible. Recently, the National Cancer Institute sponsored workshops to design clinical trial strategies for chemoprevention, and introduced the concept of surrogate endpoint biomarkers as intermediate trial endpoints. Intermediate endpoints include histologic and biochemical alterations, and also proliferation, differentiation and genetic biomarkers [4]. Studies using cancer as an endpoint must treat large groups of subjects, recognizing that only a small number will develop cancer early in the course of study. Surrogate endpoints would shorten the duration of chemoprevention trials, rather than waiting many years to accumulate sufficient numbers of subjects with invasive cancers to determine if the intervention was successful [5].

SURROGATE ENDPOINT BIOMARKERS

The use of surrogate endpoint biomarkers (SEBs) promises rapid results in clinical prevention trials, but progress in chemopreventive drug development is slowed by lack of agreement on which SEBs can be substituted for

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cancer incidence reduction. SEBs make it possible to carry out many studies on fewer subjects for shorter periods of time. Useful markers are directly associated with the evolution of neoplasia, and develop at high frequency in abnormal cells of susceptible individuals. If SEBs are modified by a particular intervention regimen in short-term studies, this strengthens the rationale for carrying out long-term studies.

A panel of endpoint biomarkers was recommended by the Consensus Panel at the 1994 NCI meeting on Quantitative Pathology Prevention Trials: Standardization and Quality Control of Surrogate Endpoint Biomarker Assays for Colon, Breast, and Prostate [12]. The markers considered promising for screening chemopreventive agents for prostate cancer in short-term Phase II trials by this panel include: (1) histologic premalignant lesions, such as high-grade prostatic intraepithelial neoplasia; (2) biochemical markers, including prostate-specific antigen (PSA) serum concentration; and (3) morphometric markers, including nuclear texture, shape, and roundness; size and number of nucleoli; and number of apoptetic bodies; (4) proliferation markers, including MIB-1 and PCNA; (5) genetic markers, including nuclear DNA content (ploidy), oncogene C-*erb*B-2 (HER-2/*neu*) expression, and fluorescence *in situ* hybridization for chromosome 8; and PSA-producing cells in the blood detected by reverse transcriptase polymerase chain reaction; and (6) differentiation markers, such as microvessel density as a determinant of angiogenesis. Each of these SEBs is measured easily and accurately in serum or in tissue specimens such as formalin-fixed, paraffin-embedded needle biopsies, and may be modifiable by intervention (Table I). Also, the efficacy of each as a possible prognostic factor has been established and confirmed. It should be noted, however, that none of these markers has been tested as a SEB in prostate cancer chemoprevention, and their clinical utility needs to be confirmed.

TABLE I. Potential Surrogate Endpoint Biomarkers for Prostate Chemoprevention Clinical Trials

Histologic Premalignant Lesions
High Grade Prostatic Intraepithelial Neoplasia (PIN)
Biochemical Markers
Prostate-Specific Antigen (PSA)
Morphometric Markers
Nuclear Texture, Shape, and Roundness
Size and Number of Nucleoll
Number of Apoptotic Bodies
Proliferation Markers
MIB-1
Proliferating Cell Nuclear Antigen (PCNA)
Genetic Markers
Nuclear DNA Content (ploidy)
Oncogene c-*erbB-3 (HER-2 / neu) Expression*
Fluorescene *In Situ Hybridization for Chromosome 8*
RT-PCR for PSA-Expressing Cells in Serum
Differention Markers
Microvessel Density (Angiogenesis)

PREMALIGNANT LESIONS OF THE PROSTATE

Prostatic intraepithelial neoplasia (PIN) refers to the precancerous end of the morphologic continuum of cellular proliferations within prostatic ducts, ductules, and acini [1,6,7]. PIN is divided into two grades (low grade and high grade) to replace the previous three-grade system (PIN 1 is considered low grade, and PIN 2 and 3 are considered high grade). The continuum from low-grade PIN to high-grade PIN and early invasive cancer is characterized by basal cell layer disruption, progressive abnormalities in markers of secretory differentiation, increasing nuclear and nucleolar abnormalities, increasing proliferative activity, increasing microvessel density, increasing genetic instability, and increasing DNA content [1]. Autopsy studies indicate that PIN proceeds carcinoma by 10 years or more. Low-grade PIN first emerges in men in the third decade of life.

The clinical significance of recognizing PIN is based on its strong association with prostatic carcinoma [8]. High-grade PIN is present in up to 16% of contemporary 18 gauge needle biopsies in urology office practice [9]; by comparison, the American Cancer Society National Cancer Detection Project identified PIN and cancer in 5.2% and 15.8% of men, respectively, from a series of 330 biopsies from men participating in an early detection project [10]. PIN has a high predictive value as a marker for adenocarcinoma, and identification in biopsy specimens warrants further search for concurrent invasive carcinoma. Davidson et al. [8] found adenocarcinoma in 35% of subsequent biopsies from cases with biopsy-proven PIN, compared with 13% in a control group without PIN. High-grade PIN, patient age, and serum PSA concentration were all highly significant predictors of cancer, but PIN provided the highest risk ratio

(14.93). Others have also reported a high predictive value of PIN for cancer, ranging from 38% to 100% [1]. High-grade PIN is considered the most likely precursor of invasive carcinoma, according to a recent consensus conference of the American Cancer Society. These data underscore the strong association of PIN and adenocarcinoma, and indicate that diagnostic follow-up is needed.

Biopsy remains the only definitive method for detecting PIN and early cancer. PIN is often found in the vicinity of carcinoma; its identification in prostate biopsy specimens warrants further search for concurrent invasive carcinoma. If all procedures fail to identify co-existent cancer, close surveillance and follow-up biopsy are indicated. Follow-up is suggested at 3 or 6 month intervals for 2 years, and thereafter at 12 month intervals for life [1]. Identification of PIN in the prostate should not influence or dictate therapeutic decisions.

There is a marked decrease in the prevalence and extent of high-grade PIN in prostates after androgen deprivation therapy compared with untreated prostates [11]. This decrease is accompanied by epithelial hyperplasia, cytoplasmic clearing, and prominent acinar atrophy, with decreased ratio of acini to stroma. These findings indicate that the dysplastic prostatic epithelium is hormone dependent. In the normal prostatic epithelium, luminal secretory cells are more sensitive to the absence of androgen than basal cells, and these results show that the cells of high-grade PIN share this androgen sensitivity. The loss of normal, hyperplastic, and dysplastic epithelial cells with androgen deprivation is probably due to acceleration of programmed single cell death (apoptosis) with subsequent exfoliation into acinar lumens.

PIN is a model system for the study of the chemoprevention of prostate cancer. This premalignant lesion can be safely and easily monitored by repeat biopsy. Findings would be immediately applicable to long-term chemopreventive treatment for patients with PIN.

PROSTATE-SPECIFIC ANTIGEN

Prostate-specific antigen (PSA) is the most important, accurate, and clinically useful biochemical prostate marker; produced by and specific for prostatic tissue, it is an excellent candidate SEB for chemoprevention trials, and may be the most practical because it is measured from serum rather than from tissue. This 34 kD serine protease is manufactured by the epithelial cells and secreted into the prostatic ductal system, where it catalyzes the liquefaction of the seminal coagulum after ejaculation. Serum levels are normally below about 4.0 ng/ml, but vary according to patient age; any process which disrupts the normal architecture of the prostate allows diffusion of PSA into the stroma, where it gains access to the blood through the microvasculature. Elevated serum PSA concentrations are seen with prostatitis, benign prostatic hypertrophy (BPH), and transiently following biopsy, but the most clinically important elevations are seen with prostatic adenocarcinoma. Although cancer produces less PSA per cell than benign epithelium, the greater number and density of malignant cells and the associated stromal disruption accounts for the elevated serum PSA concentrations.

The major form of measurable PSA in the serum is a complex between the PSA molecule and α-1-anti-chymotrypsin; there is a higher proportion of complexed PSA in the serum of patients with cancer than in other patients, and this serum fractionation may be diagnostically useful. New microassays for serum PSA allow detectability as low as 0.1 ng/ml.

In tissue sections of normal and neoplastic prostate, PSA expression is easily demonstrated immunohistochemically, and helps the pathologist distinguish high-grade prostate cancer from transitional cell carcinoma, colonic, carcinoma, granulomatous prostatitis, lymphoma, and other histologic mimics. It also allows the site of tumor origin to be identified in metastatic adenocarcinoma. PSA expression is usually greater in low-grade tumors than in high-grade tumors, but shows significant heterogeneity from cell to cell. Up to 1.6% of poorly differentiated cancers will be negative for both PSA and prostatic acid phosphatase.

MORPHOMETRIC MARKERS

Numerous morphometric markers have provided valuable prognostic information in prostate cancer, including size and number of nucleoli; nuclear texture, shape, and roundness, and the number of apoptotic bodies. Morphometric studies should employ objective, quantitative morphometric techniques, preferably computer-assisted. A recent study successfully separated prostate cancers with favorable and unfavorable prognoses based on a discriminant function derived from five chromatin texture-related features. The Consensus Panel [12] recognized that there are no accepted stan-

dards for morphometric studies, and considers this an important and significant area for future investigation.

PROLIFERATION MARKERS

The rate of cell proliferation is a useful prognostic factor and SEB in many cancers. We will use the markers MIB-1 and proliferating cell nuclear antigen (PCNA) rather than others such as mitotic index, S-phase fraction, Ki-67, DNA polymerase-α, ^{3}H-thymidine, or 5-bromo-2′ deoxyuridine incorporation, as recommended by the Consensus Panel convened by the National Cancer Institute in 1994 [12]. The utility of proliferation fraction as an SEB and a prognostic factor in prostate cancer is limited by the low and narrow range of growth fractions, varying from 0.4%–9.1% in one study.

Monoclonal antibody Ki-67 recognizes a human nuclear antigen expressed in the S, G2, and M phases of all cycling human cells and absent in G0 and early G1; although Ki-67 remains popular to evaluate proliferative activity in frozen sections, it has been replaced in archival studies by its counterpart, MIB-1 which produces accurate and reproducible immunohistochemical results in paraffin embedded sections. Ki-67 expression weakly correlated with time to tumor progression after hormonal therapy. Results with MIB-1 and prostate cancer appear to be comparable to Ki-67. PCNA (or cyclin) is a non-histone nuclear protein, an accessory of DNA polymerase. Closely linked to the cell cycle, its expression is maximal during S phase. The PCNA labeling index in prostate cancer is 1.6–15%, with heterogeneity and expression in different parts of the tumor. Montironi et al. noted a decrease in expression of PCNA in normal epithelium, PIN, and cancer in successive cell layers in the gland periphery to the lumen, suggesting progressive terminal differentiation as cells move toward the lumen [13]. Immunohistochemical methods for these markers are well defined and commercially available, and routinely used in our immunohistochemical laboratory for diagnostic purposes.

GENOMIC MARKERS

Nuclear DNA Content (Ploidy)

DNA content analysis of prostate cancer by flow cytometry and static image analysis may provide independent prognostic information which supplements histopathologic examination [14–30]. Patients with diploid tumors have a more favorable outcome than those with aneuploid tumors; for example, among patients with lymph node metastases treated with radical prostatectomy and androgen deprivation therapy, those with diploid tumors often survive 20 years or more, whereas those with aneuploid tumors usually die within 5 years [25,26]. However, the ploidy pattern of prostate cancer is often heterogeneous, creating potential problems with sampling error. An international DNA cytometry conference reviewed the literature and concluded that the clinical significance and biologic basis of DNA ploidy needs further investigation [28].

The Consensus Panel of the National Cancer Institute [12] felt that the evidence linking nuclear DNA content and prognosis was sufficiently compelling to recommend it as a useful SEB in chemoprevention trials, although the technique is limited by inexact quality standards and interpretive differences. Digital image analysis appears to have a high level of concordance (about 85%) with radical prostatectomy specimens evaluated by flow cytometry [26], so the problems of sampling error are limited.

Oncogene C-*erb*B-2 (HER-2/*neu*) Expression

The c-*erb*B-2 oncogene codes for a transmembrane growth factor receptor with 43% homology to epidermal growth factor receptor (EGFR), but is distinct from EGFR in its chromosomal location and specificity for single transduction and ligand binding. The function of the c-*erb*B-2 oncoprotein is uncertain; it is thought to play a role in cell growth and differentiation, possessing an intracellular domain with tyrosine-specific kinase activity and an extracellular domain. Results of immunohistochemical studies have been variable, ranging from 0–92% staining in hyperplastic prostatic tissue and 0–100% of prostate cancer; the discordant results are attributed to differences in tissue handling and antibody reagents [31–41]. The Consensus Panel convened by the National Cancer Institute in 1994 [12] acknowledged the work of two of its members in endorsing c-*erb*B-2 expression as an SEB; Veltri et al. [39] found expression to be a strong univariate predictor of cancer progression in a series of 124 cases followed for a mean of 8.6 years, and Grizzle et al. and Myers et al. [41] observed coarse and punctate cytoplasmic and membrane staining in a significant number of cancers and in PIN.

Other studies have suggested that c-*erb*B-2 is overexpressed in human prostate cancer, al-

though this has been refuted [31–41]. Activated oncogenes such as *ras* appear infrequently in early prostate cancer, but increased expression seems to be correlated with higher tumor grade and aneuploid status [42]. One or more tumor suppressor genes are apparently involved in prostatic carcinogenesis; existing data are not yet mature enough for the panel to recommend use of a genetic marker as a SEB. Inactivation of p53, a tumor suppressor gene on chromosome 17p, occurs in up to 25% of advanced primary prostate cancers, and in up to 50% of metastases, but is rare in early cancers, suggesting that it may play a role in late progression [43]. Loss of expression of the retinoblastoma gene of chromosome 13q is seen in a minority of prostate cancers, usually in advanced stages [44].

Oncogene c-*erb*B-2 expression is easily determined immunohistochemically in tissue sections, and the methods employing microwave antigen retrieval appear maximally sensitive in identifying expression of this marker [41].

Fluorescence In Situ Hybridization for Chromosome 8

Fluorescence in situ hybridization (FISH) analysis of interphase cells with centromere-specific and region-specific probes is useful for the detection of numerical chromosomal anomalies in solid tumors, including prostatic carcinoma, which is often difficult for conventional cytogenetic analysis [45–48]. When applied to histologic sections, this method allows study of multiple foci of normal epithelium, PIN, and carcinoma within a single prostate specimen, and make the evaluation of matched metastatic sites possible [45].

We recently reported that the overall frequency of numeric chromosomal anomalies in PIN and carcinoma foci was remarkably similar (50% and 51%, respectively), suggesting that they share a similar underlying pathogenesis [49]. Overall, the mean number of abnormal chromosomes increased in PIN to carcinoma foci, and within each whole mount prostate, the carcinoma foci contained more anomalies than paired PIN foci. These findings suggest that PIN is a precursor of carcinoma [1,6,7]. However, within five prostates, one or more PIN focus clearly contained more anomalies than concurrent carcinoma foci, indicating that some PIN foci may have a divergent pathogenesis or that foci of carcinoma may occasionally be derived from other precursor lesions such as atypical adenomatous hyperplasia [49].

We found that gain of chromosome 8 was the most frequent numeric anomaly in PIN and prostatic carcinoma. Other studies have also demonstrated gain of the chromosome 8 centromere by FISH, and loss of portions of the 8 p-arm by PCR in specimens of PIN and carcinoma, [50] suggesting that alterations of this chromosome and/or a tumor suppressor gene(s) (TSG) on short arm may be important for the initiation or early progression of prostate cancer. Supporting this hypothesis, and consistent with previous reports, [51–53] gain of chromosome 8 also correlated with carcinoma stage and grade. Multiplication of the 8 q-arm is often accompanied by 8 p-arm allelic loss. A likely genetic mechanism underlying both the FISH and molecular genetic observations is the presence of multiple isochromosomes 8q in tumor cells. The cumulative findings suggest that gain of chromosome 8 is a marker of clinically aggressive prostatic carcinoma.

RT-PCR FOR PSA-EXPRESSING CELLS IN SERUM

There is a low incidence of micrometastatic occult prostatic carcinoma in serum and pelvic lymph nodes which cannot be detected by routine hematoxylin and eosin staining [54]. Using immunohistochemical studies directed against cytokeratin, Moul et al. found lymph node micrometastases in 3% of patients with clinically localized prostatic adenocarcinoma [54], similar to the results of Gomella et al. [55]. In another study, circulating PSA-immunoreactive cells in the blood were identified by flow cytometry in all cases of adenocarcinoma with distant metastases and 47% of lower stage adenocarcinomas [56]. Reverse transcriptase polymerase chain reaction studies to detect PSA mRNA revealed PSA-positive cells circulating in the peripheral blood of 4 of 12 patients with adenocarcinoma with pelvic lymph node metastases [57]. Katz et al. demonstrated the clinical utility of this test in predicting stage, and referred to it as "molecular staging" [58].

DIFFERENTIATION MARKERS

Microvessel Density (Angiogenesis)

Angiogenesis (neovascularity or vessel density) is a necessary prerequisite for tumor

growth and progression in most cancers, including prostatic adenocarcinoma. It appears to be stimulated by factors released from cancer cells, inflammatory cells, and the extracellular matrix. Vessel density is increased in PIN and cancer compared with normal and hyperplastic prostatic epithelium, and is an independent predictor of pathologic stage, malignant potential, and metastasis. Significant differences remain in evaluating vessel density, but the Consensus Panel convened by the National Cancer Institute in 1994 [12] expected that standards will soon be adopted to allow rational comparison of results from different centers.

METHODOLOGIC ISSUES OF SURROGATE ENDPOINT BIOMARKER STUDIES IN HUMAN CHEMOPREVENTION TRIALS

The problem facing the measurement of these genetic, proliferation and differentiation markers during clinical trails in humans is the size of tissue that can be obtained for analysis. Recent advances in the development of immunohistochemistry, in situ hybridization [45], and other microassay techniques make such measurements feasible on serial sections of needle biopsies of the prostate. The goal of any prostate chemoprevention clinical trial should also be to apply these techniques to the study of the multistep process of carcinogenesis in vivo and to use these assays to determine surrogate endpoint biomarkers for the cellular effects of the chemopreventative agent.

STRATEGIES FOR PROSTATE CANCER CHEMOPREVENTION CLINICAL TRIALS

Many planned and ongoing phase III chemoprevention trials are tremendously expensive because their cancer-incidence endpoints require thousands of subjects and decades of studies. These costly trials have not always enjoyed the design benefit of a solid scientific basis for their selection of agent doses and schedules. A scheme for future validation might include a) a first generation of study in which non-randomized short-term trials in high-risk subjects determine the feasibility and prequalify a panel of markers; b) a second generation in which non-toxic dose and schedule trials use modulations of promising surrogate endpoint biomarkers as study endpoints; and c) a third generation in which long-term phase III trials employ optimal doses determined in the second generation and validate candidate biomarkers against cancer incidence. Assessment of modulation of biomarkers should allow smaller sample sizes and shorten the duration of chemopreventative trials.

Chemoprevention trials designed to prevent, inhibit, or reverse high-grade PIN may be confounded by the presence of underlying but undetected prostate cancer. This difficult problem could be partially resolved by either requiring a second biopsy without cancer before entry into the study (preferably sextant biopsies with special attention to areas of ultrasound or digital rectal exam abnormality); or including enough subjects in the study and control groups, that risk of coexistent cancer at the time of initiation is equal between the two groups [59]. As with any large scale contemporary clinical trial an experienced statistician should assist in designing the study to assure an appropriate sample size and level of significance.

In phase III clinical trials patients should be randomized in a placebo controlled double blind fashion. Periodic re-evaluation including physical examination, rebiopsy, and surrogate intermediate endpoint biomarkers will be necessary. If subsequent biopsy reveals prostate cancer these patients will need to be considered for definitive treatment. Those with PIN or no malignancy will need to continue on an observation protocol. A sample chemoprevention clinical trial schema is shown in Figure 1.

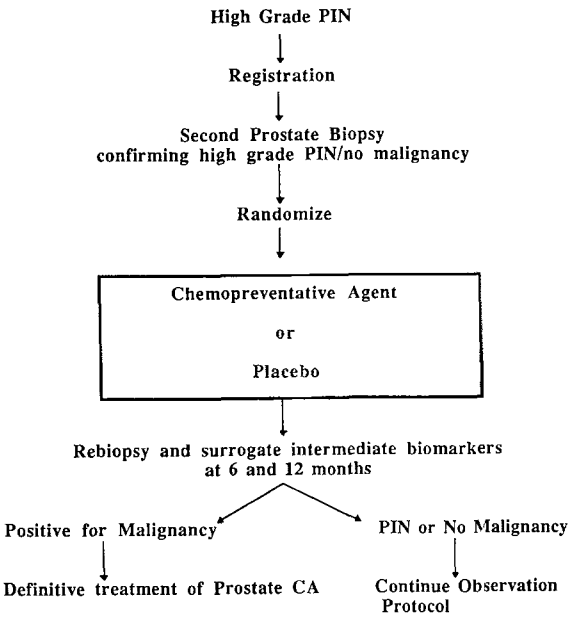

Fig. 1. A sample chemoprevention clinical trial schema.

CONCLUSIONS

High-grade PIN is considered the most likely precursor of invasive prostatic carcinoma and is a model system for the study of chemopreventative agents. Chemoprevention trial strategies which focus on subjects with precursor lesions such as high-grade PIN, as well as changes in surrogate intermediate endpoint biomarkers may result in more efficient clinical trials.

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