Published in final edited form as:

Prostate. 2009 September 15; 69(13): 1470–1479. doi:10.1002/pros.20983.

Evidence for Field Cancerization of the Prostate

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Abstract

BACKGROUND—Field cancerization, which is not yet well-characterized in the prostate, occurs when large areas of an organ or tissue surface are affected by a carcinogenic insult, resulting in the development of multi-focal independent premalignant foci and molecular lesions that precede histological change.

METHODS—Herein, we review the cumulative body of evidence concerning field effects in the prostate and critically evaluate the methods available for the identification and validation of field effect biomarkers. Validated biomarkers for field effects have an important role to play as surrogate endpoint biomarkers in Phase II prevention trials and as clinical predictors of cancer in men with negative biopsies.

RESULTS—Thus far, field effects have been identified involving nuclear morphometric changes, gene expression, protein expression, gene promoter methylation, DNA damage and angiogenesis. In addition to comparing cancer-adjacent benign tissue to more distant areas or to “supernormal” tissue from cancer-free organs, investigators can use a nested case–control design for negative biopsies that offers a number of unique advantages.

CONCLUSIONS—True carcinogenic field effects should be distinguished from secondary responses of the microenvironment to a developing tumor, although the latter may still lead to useful clinical prediction tools.

Keywords

prostate cancer; field effects; biomarkers

INTRODUCTION

The concept of field cancerization, or the “cancer field effect,” was first proposed in 1953 by Slaughter et al. [1] when describing histological features of oral cancers. The authors of that classic paper concluded that oral cancers resulted from field cancerization based on the frequency of multicentric cancer, which was far above that expected by chance alone, and on the observation of microscopic abnormalities in grossly benign contiguous epithelium. Even though the association between tobacco use and oral cancer had not been established, these features suggested that some type of etiologic stress had caused potentially carcinogenic alterations to an entire field of tissue, from which the cancers arose. Slaughter et al. reported on histological abnormalities in grossly normal tissue; however, contemporary biotechnologies have allowed us to extend the field effect concept to explore molecular abnormalities in tissue that is histologically normal. Such molecular abnormalities have now

been observed in several organs, including colon [2], bladder [3], lung [4], vulva [5], esophagus [6], cervix [7], breast [8], and skin [9].

In this review, we examine the growing evidence that prostate cancer (PCa) is also a result of field cancerization and critically examine the research strategies that are available for the identification and validation of prostate field effect biomarkers. This area of inquiry is of more than academic interest—the identification of prostatic field changes could directly influence patient care as well as research. Due to widespread use of an early detection marker that is measured in serum, prostate-specific antigen (PSA), and the lack of any practical means for imaging lesions, PCa is the only solid tumor that is routinely detected by indirect tissue sampling, that is, without visualization of a clear-cut suspicious lesion. Seventy to 80% of the approximately 1.2 million patients who undergo prostate biopsy each year in the U.S. receive negative results, but cannot be completely reassured because a cancer might have been missed by sampling error. Therefore, predictive biomarkers in benign tissue could provide clinicians with a clear basis for stratifying individuals into those who need repeat biopsy or intensive follow-up and those who do not. As we discuss further below, validated field effects will also provide surrogate endpoint biomarkers for Phase II chemoprevention trials, which are urgently needed to sort through the plethora of candidate interventions for lengthy and expensive Phase III studies.

PROSTATIC LESIONS DEVELOP IN A MULTI-FOCAL PATTERN

Consistent with Slaughter's first observation regarding field cancerization, step-wise sectioning of radical prostatectomy and autopsy specimens has shown that PCa is almost always multi-focal [10]. Molecular and histological evidence supports the presence of two distinct precancerous lesions for PCa; prostatic intra-epithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA) [11,12]. These lesions also occur in a multi-focal pattern, suggesting that they arise independently in response to carcinogenic stimuli. High grade prostatic intra-epithelial neoplasia (HGPIN), which is characterized by non-invasive hyper-proliferative luminal epithelial cells that have malignant morphology and a discontinuous but intact basal cell layer [12], is widely accepted as a precursor to PCa. HGPIN lesions coexist with PCa in the majority of cases and frequently occur in adjacent areas. Moreover, chromosomal and genetic alterations in HGPIN and PCa within the same organ are correlated to a degree, suggesting a common origin. PIA, which is characterized histologically by focal inflammatory infiltrates that are associated with atrophic and proliferative epithelium [11,13,14], are multi-focal and often adjacent to PCa and/or HGPIN [13]. On a molecular level, PIA displays several features associated with prostate carcinogenesis, including increased expression of Ki67, bcl-2, COX-2, and GSTP-1, and decreased p27Kip1 [15].

Multi-focality by itself is not sufficient evidence for a field effect because cells from a single tumor focus could conceivably spread to other locations within the organ through intraductal or other pathways. However, studies of somatic mutation and loss-of-heterozygosity (LOH) patterns have shown that PCa and HGPIN foci are genetically as well as morphologically heterogeneous, consistent with the concept that individual lesions arise from a disturbed field and develop divergent genetic identities due to stochastic mutation and clonal selection processes [16]. Further support for the importance of diffuse genomic instability in prostate carcinogenesis comes from analyses of telomere dysfunction in both preneoplastic and neoplastic tissue [12,17]. Telomere shortening has been observed commonly in PCa and, to a slightly lesser extent, in HGPIN lesions [17,18]. Interestingly, telomere length appears to be shorter in HGPIN foci located near cancer than in foci that are more distant; and in addition, there is considerable heterogeneity in telomere length among HGPIN foci within individual patients [12]. Together, these observations suggest that telomere dysfunction

plays a role in driving the early development of PCa, by creating a field of genetic instability that facilitates the multi-step accumulation of critical genetic aberrations. Other generalized processes besides telomere dysfunction, such as suboptimal hormone signaling, inflammation, oxidative stress and DNA repair defects may play a role in creating a fertile field for development of multi-focal neoplastic lesions in the prostate.

TISSUE ADJACENT TO PROSTATE CANCER IS ABNORMAL

Histologically “normal” tissue and precancerous foci adjacent to PCa are found to be morphologically and genetically distinct from distant tissue. Table I describes some of the genetic, epigenetic, cytomorphologic and gene/protein expression abnormalities identified thus far that support the existence of a field effect in prostate carcinogenesis. This tabulation is not necessarily complete since the terms “field effect” or “field cancerization,” which we included in Medline searches, may not appear explicitly in some articles. Our definition of field effect requires documentation of aberrant biomarker expression in histologically normal glands, and thus excludes biomarkers expressed solely in pre-cancerous lesions.

Cytomorphological Abnormalities

Studies of nuclear morphology using digital image analysis have identified subtle changes in the histologically benign tissue adjacent to PCa. Mairinger et al. compared nuclei from 67 cases of BPH treated by open prostatectomy to nuclei extracted from benign areas surrounding PCa. Nuclei were physically extracted from paraffin-embedded tissue sections and morphometric features were analyzed digitally after Feulgen staining. A multivariable classifier based on nuclear chromatin texture features discriminated these two types of benign nuclei with 90% sensitivity and 97% accuracy [19]. Veltri et al. [20] combined nuclear chromatin texture with nuclear size, shape and DNA content to calculate a quantitative nuclear grade (QNG) for normal adjacent nuclei (NN) and PCa nuclei (CaN) in radical prostatectomy specimens of men with biochemical recurrence. Analyzed independently, both the QNG-NN and the QNG-CaN were able to predict time to distant metastases in PCa patients with biochemical recurrence, suggesting that the “normal” nuclei were as distinctive in aggressive disease as the PCa nuclei.

Montironi et al. [21] combined architectural and cytological features, as observed by pathologists, to construct a Bayesian belief network for classifying benign, premalignant and malignant prostate tissue. The eight traits inputted into the network were gland pattern changes, cribriformity, basal cell nuclei recognition, basal cell nuclei prominence, secretory cell nuclei enlargement, secretory cell nuclei stratification, secretory cell cytoplasm appearance and secretory cell nucleolar prominence. From these eight morphologic traits the network was able to distinguish normal tissue adjacent to PCa from normal tissue associated with inflammation and normal tissue not associated with any disease. Unfortunately, the methods for analyzing nuclear morphological features vary greatly among research groups and no specific method has been fully validated as yet. More standardized protocols for staining, analysis and quantification of these nuclear morphological changes need to be established before these markers can become widely used in the research or clinical setting.

ALTERED GENE OR PROTEIN EXPRESSION

Multiple studies have shown that the gene/protein expression profile of normal prostate tissue adjacent to PCa is distinguishable from normal tissue that is not associated with malignancy. Chandran et al. [22] compared PCa, adjacent normal tissue and normal tissue obtained from organ donors using cRNA microarrays. As expected, tumors showed differential up-regulation of proliferation-related genes compared to adjacent normal; however, a similar pattern of up-regulation was observed in adjacent normal areas when

they were compared to organ donor tissue. Furthermore, the tumor-to-adjacent normal contrast failed to detect differences in the expression of oncogenes, signal transducers and immune response genes that were only apparent when tumors were compared to organ donor tissue. Yu et al. [23] reported similar results—90% of normal tissue samples adjacent to tumor were predicted to be tumor based on a 671-gene signature derived from a comparison of tumor to organ donor tissues. Recently, a simpler 8-gene expression panel was shown to discriminate between normal and cancer specimens even though nearly half of the cancer specimens were estimated to contain less than 15% actual cancer cells [24].

Gene expression changes are easily quantifiable and have potential to be good field effect markers. However, mRNA profiling can be problematic in prostate biopsies due to formalin fixation, sparse tissue and the admixture of epithelial and stromal elements. This problem has been overcome in breast cancer prognostic testing, where the Oncotype DX™ gene expression profile of biopsy tissue is routinely used in estrogen receptor positive patients [25]. Compared to prostate needle biopsies, breast tissue biopsies are larger and the cancer can be easily macro-dissected to facilitate reproducible results. As technical advances are made in RNA extraction and gene expression profiling from small quantities of formalin-fixed biopsy tissue, an RNA-based diagnostic or prognostic from normal prostate biopsies may soon become a reality.

Ananthanarayanan et al. [26] examined protein expression of two cell proliferation proteins, Mcm-2 and Ki67, and two apoptotic proteins, cleaved caspase-3 and Bcl-2, in near and distant normal and HGPIN glands. In near normal tissue Mcm-2 and cleaved caspase-3 were increased compared to distant normal. In near HGPIN, protein levels of all the markers, Mcm-2, Ki67, cleaved caspase-3 and bcl-2, were increased compared to distant HGPIN foci. Another study reported increased proliferation, as indicated by Ki67 and topoisomerase II immunopositivity, in normal, atrophic or HGPIN areas adjacent to PCa as compared to paired areas more than 5 mm away [27].

Similar field effects have been demonstrated for other markers including alpha-methylacyl-CoA racemase (AMACR) [27,28], the estrogen response protein pS2 [29], Akt-1 [30], apoptotic calcium channel receptor proteins (P2X₁, P2X₂, P2X₇) [31], and androgen receptor (AR) [32]. EPCA (early prostate cancer antigen) - a nuclear matrix protein - has been the subject of more detailed investigation. Dhir et al. [33] reported that EPCA staining intensity was significantly greater in negative biopsies from men who later had PCa detected than in organ donor prostates without PCa. A second study observed EPCA staining in epithelial glands adjacent to cancer in 47 of 50 radical prostatectomy specimens versus 0 of 10 prostates removed due to bladder cancer; however EPCA expression was reported only in PIA and PIN lesions rather than histologically normal areas [34]. More recently, Hansel et al. [35] conducted a study using biopsies only, and found that EPCA expression was more pronounced in negative biopsies from men who later were found to have PCa than in men with persistently negative biopsies. The latter study attempted to quantify the association between EPCA staining and cancer risk, but this effort was limited by the small number of cases involved who had subsequent cancer. It also remains unclear why EPCA, a protein first isolated from the nuclear matrix, is detected predominantly in the cytoplasm and cell membrane rather than the nucleus and why it can be detected in plasma, even among individuals not considered at elevated risk for PCa [36].

Two other potential effect markers—COX-2 and PCA3—have been evaluated for possible clinical utility. In an analysis of 60 archival radical prostatectomy specimens (23 with biochemical recurrence), Cohen et al. showed that COX-2 protein was expressed more strongly in benign glands adjacent to tumor in patients who later had biochemical recurrence than in those who did have a recurrence [37]. It should be noted that Cohen et al. also

observed elevated COX-2 protein in PCa glands of patients who later recurred. Therefore, it is difficult to distinguish whether the elevated COX-2 expression in adjacent normal is a true field effect or a result of paracrine signaling from the PCa. Moreover, published studies remain mixed as to whether COX-2 protein levels in PCa are elevated, adding further uncertainty to COX-2 as a field effect marker [15]. PCA3 is a noncoding RNA from chromosome 9q21-22 that is unequivocally expressed in PCa with minimal expression in normal epithelium or stroma [38]. However, Popa et al. [39] found that normal glands adjacent to PCa also exhibited focal expression of PCA3 by in situ hybridization in 8 of 28 specimens. Although the molecular function of PCA3 is still unclear, a quantitative urine test for PCA3 is readily available [38]. Because of the perceived specificity of PCA3, the PCA3 urine test is potentially helpful in determining the presence of detectable PCa in patients with elevated PSA and negative biopsies. However, if PCA3 is indeed found in high-risk normal glands before significant PCa is present, this will have to be considered when interpreting results of the urine test.

EPIGENETIC CHANGES

PCa and many other cancers display characteristic epigenetic changes in global chromatin methylation and methylation of specific genes. Nakayama et al. [40] assessed methylation patterns in GSTP1 in epithelial cells isolated by laser capture microdissection from areas designated as normal, nodular BPH, PIA, HGPIN and PCa. Although CpG island methylation in the promoter region of GSTP1 was detected in a subset of PIA lesions and most PIN or PCa, no such methylation was found in normal or BPH areas. The BPH areas were in the transition zone and were most likely distant from neoplastic lesions, whereas the distance of the normal areas from neoplasia was not specified. However, another group of investigators microdissected normal epithelial and stromal cells adjacent to PCa, as well as tumor cells and tumor-associated stroma from five radical prostatectomy cases [41]. Moderate to extensive methylation of GSTP1 and RAR β 2 promoters was detected in this histologically normal epithelial and stromal tissue; tumor cells and tumor-associated stroma were more heavily methylated. [*Note: tumor-associated stromal cells clearly participate directly in the carcinogenic process itself and therefore we do not consider them to be indicative of true field effects.*] Henrique et al. [42] performed quantitative methylation-specific PCR on normal, HGPIN and PCa laser-microdissected tissues from 30 patients and, in the normal epithelium, reported rare GSTP1 promoter methylation, but low-level methylation in over half of the subjects for APC and RAR β 2.

Mehrotra et al. studied methylation in ex vivo core biopsies taken at varying distances (1–4 mm) from a PCa focus in a small group of 8–11 evaluable patients. None of 8 cases had methylated GSTP1 in any of these cores; however, methylated APC was observed in adjacent normal tissue from 1 of 8 cases and methylated RAR β 2 or RASSF1A in 2 of 11 and 3 of 11 cases, respectively [43]. Extensive RASSF1A methylation also has been observed in laser capture microdissected normal glands adjacent to HGPIN in cancer-free biopsies [44]. Unexpectedly, methylation was highly prevalent in completely benign control biopsies, however. This study involved complete bisulfite sequencing of all 16 CpG islands in the RASSF1A promoter region, which was probably more sensitive than the methylation-specific PCR method used in other studies. However, selection bias could also be a factor because many of the negative biopsy controls may have harbored undetected PCa. In a single interesting report, Krop et al. [45] studied methylation of HIN-1 (high in normal-1), a putative tumor suppressor in several types of tissue. Seventy-one percent of normal tissue samples adjacent to PCa showed HIN-1 promoter methylation versus no samples showing methylation among those taken from cases of BPH without cancer.

To summarize, several studies support the existence of a field effect for gene silencing through hyper-methylation in prostate carcinogenesis. The genes implicated include GSTP1, APC, RASSF1A, HIN-1 and especially, RARb2. Some conflicting results may have occurred due to differences in methods for selecting tissue or quantifying methylation.

OTHER BIOMARKERS (DNA DAMAGE, ANGIOGENESIS)

Malins et al. [46] characterized a “cancer DNA phenotype” based on Fourier-transformed infrared spectroscopy (FT-IR) analysis of DNA extracted from PCa. These spectral profiles represent structural alterations to DNA bases and the phosphodiesterdeoxyribose backbone, some of which may be attributable to oxidative damage. The comparison of FT-IR spectra revealed strong similarities between tumor DNA and DNA from adjacent normal areas [47]. The cancer profile was also identified in normal prostate from a subset of older men (aged 55–80), but not in any prostate samples from younger men (aged 16–36). Interestingly, these investigators also reported that an FT-IR profile in adjacent normal tissue could differentiate between PCas with or without distant meta-stasis formation, suggesting that, apart from having a role in carcinogenesis, these DNA characteristics may also reflect a direct effect of the tumor on its microenvironment, one that indicates metastatic potential [48]. A field effect involving mitochondrial DNA damage in prostatic epithelial cells has also been reported recently [49]. Parr et al. interrogated the entire mitochondrial genome, and reported that mutation rates in PCa and adjacent normal tissue, particularly in coding regions, were nearly equivalent, and that both rates were substantially greater than those seen in samples from men with negative prostate biopsies. The authors speculate that this may represent an early effect of reactive oxygen species associated with defective mitochondrial-nuclear signaling and aberrant cell survival.

Siegal et al. [50] quantified microvessel density (MVD) in tissue zones at varying distances from a PCa focus. The MVD was significantly greater in normal tissue at the edge of a cancer than that located 2.5 mm or more away. Montironi et al. [51] also found evidence that changes in capillary density and architecture occur much earlier in the carcinogenic process than previously thought. We note that in studies that are cross-sectional in time, it is difficult to distinguish between a carcinogenic field effect and a neovascular response to the nearby tumor, possibly mediated by diffusible growth factors.

Over-expression of ETS transcription factors due to chromosomal rearrangement has been found in 60–80% of PCa [52]. Interestingly, one of the more common rearrangements, TMPRSS2-ERG-fusion-A, recently has been found by real-time RT-PCR in normal prostate tissue [53]. Although not the focus of this study, 2 of 45 normal areas of prostate contained the TMPRSS2-ERG fusion. Rechecking of those areas failed to show PCa, leading the authors to conclude that the fusion might be indicative of future cancer, in other words, a field effect. Mosquera et al. [54] used FISH to evaluate TMPRSS2-ERG fusion in 143 patients with HGPIN; the majority had paired PCa samples. Sixteen percent of the HGPIN cases were positive for the fusion and all of these cases showed fusion in their paired PCa as well. Although this study indicates that the TMPRSS2-ERG fusion may have an early role in prostate carcinogenesis, further work needs to be done to determine whether a field effect exists for this important genetic aberration.

CHALLENGES IN IDENTIFICATION OF FIELD EFFECTS IN THE PROSTATE

Progress in identifying, assessing and tracking field effects in the prostate has been slower than some other cancers because the prostate is a solid organ. Development of epithelial cancers in hollow, accessible organs, such as the oropharynx, esophagus, lung, colon, and bladder, can be visually monitored by endoscopies and two-dimensionally mapped to follow pre-cancerous lesions and malignancy patterns. On the other hand, prostate needle biopsies

randomly sample a very small percentage of three-dimensional space, making it impossible to follow individual lesions. Furthermore, biopsy of the benign prostate is normally considered too invasive a procedure to perform strictly for research purposes. Therefore, we are forced to take advantage of tissue specimens collected during routine clinical care, organ donation or autopsies. Three specific study designs are feasible. The pros and cons of each one are discussed in the remainder of this article.

Near-Normal Versus Far-Normal

It is reasonable to presume that early carcinogenic events are not homogeneously distributed throughout the prostate and that frank malignant or pre-malignant lesions represent zones in which the greatest amount of damage has occurred and clonal selection has progressed. Therefore, the simplest approach for identifying prostatic field effects involves comparison of histologically benign tissue located either near or far from neoplastic lesions. Most of the studies summarized above used this approach, which ordinarily requires only a set of formalin-fixed paraffin-embedded radical prostatectomy specimens. The approach is more complex than it seems initially. Tissue sections, stained with hematoxylin and eosin, must be carefully mapped by an expert pathologist to indicate each focus of neoplasia and to exclude areas affected by atrophy or artifact. The multifocal nature of prostate cancer creates overlapping fields such that a benign area can be close to one lesion and far from another. A definition of what is “near” and what is “far” must be established, although this will be inherently arbitrary. Tissue sections allow for evaluation of distance from a lesion in two dimensions; it may be necessary to check adjacent sections or blocks to ensure that a “far-normal” area is not in fact adjacent to a lesion at another level. Finally, the near versus far approach relies on histological mapping of large areas, and therefore is generally amenable only to the use of formalin-fixed specimens (see Ref. [39] for an exception that uses ex vivo biopsies from whole fresh prostates).

Formalin fixation degrades RNA and promotes protein cross-linking, which introduces challenges for the quantitative analysis of potential biomarkers. The situation is complicated by variation in the time from surgical removal to fixation, time in fixation before embedding, and time in storage of archival blocks. Variability in fixation is more of a problem for prostatectomy samples as opposed to biopsies, because the former are not placed immediately into formalin and their larger size can result in uneven penetration of the fixative. Recent advances in real-time PCR measurement of short, fragmented RNA sequences have improved the utility of FFPE samples for gene expression analysis [55,56]. Laser capture microdissection has become indispensable for these studies to ensure that one has a homogeneous population of histologically benign cells and that a putative field effect is not due to a small subset of infiltrating tumor cells.

Normal Versus “Super-Normal”

Many studies refer to histologically benign tissue from a tumor-containing prostate as normal when in fact the field effect concept suggests that it is not actually normal in the strictest sense. Therefore a second approach for the identification of field effects is to compare normal tissue from prostate glands containing PCa to glands in which PCa is presumed to be absent. The latter, which we refer to as super-normal prostate, can be obtained from bladder cancer surgery that removes the entire prostate (cystoprostatectomy), or from organ donors. Cystoprostatectomy specimens are relatively rare but are generally easy to obtain. Organ donor prostates, on the other hand, are relatively more abundant but more complicated to obtain. An important advantage of cystoprostatectomies is that these patients have an age distribution that is similar to PCa patients, whereas organ donors tend to be younger. Regardless of their source, super-normal prostates must be thoroughly sectioned and examined microscopically to rule out the presence of significant tumors.

Case–Control Studies

Perhaps the most relevant and powerful approach to the identification of field effects is to conduct nested case–control studies of negative biopsies in which a “case” who is subsequently diagnosed with PCa is compared to a control who does not develop PCa during follow-up. This study design is diagrammed in Figure 1.

The term “nested” implies that both cases and controls arise from (i.e., are nested within) a single defined cohort. Careful attention to the selection of cases and controls can yield some important dividends. The strengths and weaknesses of this study design are summarized in Table II.

As noted, this design makes efficient use of samples and allows one to compute a multivariable-adjusted odds ratio expressing the magnitude of association between the biomarker level and risk. One can also easily use ROC curve analysis to test how much the biomarker adds to routine clinical predictors of PCa risk among patients with negative biopsies. Some field effect biomarkers will decay as storage time increases (see Ref. [34]); therefore matching cases to controls on the date of the index negative biopsy adjusts for this as well as equalizing the calendar period at risk for PCa detection.

Many clinical investigators are unfamiliar with the epidemiological principles inherent in the nested case–control design, and implementation of a pure design runs into some practical constraints. Technically, in order for the odds ratio to be an accurate reflection of the true relative risk, the controls should be sampled from the members of the cohort who were at risk during the same period as the case yet remained free of a cancer diagnosis. This design aspect, referred to as incidence density control sampling, requires that the likelihood of PCa detection be equal for a case and its matched controls during the period at risk *except* for any risk association carried by the biomarker in question. However, a large number of variables can affect the likelihood of PCa detection after a negative biopsy; it is difficult to obtain adequate data on all of them and also difficult to control for so many variables within a relatively small set of study samples. In a large cohort of men undergoing repeat biopsy, we recently identified nine clinical variables with independent predictive significance for PCa detection [57]. Furthermore, incidence density sampling allows cases to be selected as controls and vice versa. Since prostate biopsy usually involve random needle cores, in many instances, patients with negative biopsies harbor an undetected malignancy that was missed by chance (spatial sampling error). Therefore, some controls may have synchronous PCa at the time of biopsy, and the contrast between these controls and matched cases may be minimal. This will attenuate the observed case–control differences, particularly when the case has PCa detected relatively soon after the index negative biopsy. One can expect the difference between cases and controls to expand as the interval between index biopsy and PCa detection increases; the difference thus begins to reflect true differences in the biological risk-status of the gland.

Since field effect analysis of prostate biopsy specimens is limited by the amount of material, several of the markers discussed in this review are unlikely to be applicable to negative biopsies. For example, analysis of MVD has only been done in radical prostatectomy (RP) specimens and is unlikely to be useful in prostate biopsies. Other markers, such as cytomorphological abnormalities and FT-IR-detected DNA abnormalities, have currently only been examined in RP specimens, but should be applicable to biopsy material. As discussed above, gene expression analysis from biopsies is challenging and not routine. However, if a change in a specific gene(s) was identified and validated as a true field effect, it would not be unreasonable to produce a diagnostic test similar to the Oncoype DX™ test.

SUMMARY AND SIGNIFICANCE

The field effect biomarkers discussed above have the potential to become valuable tools for research and patient care. The main clinical application will be in prediction of PCa among men with initially negative biopsies. It is also possible that study of the tissue adjacent to a tumor will reveal characteristics indicative of its invasive or metastatic potential [48]. Development of these clinical applications will require biomarkers that are reproducible and cost-effective, and that have documented evidence for independent improvement in predictive ability beyond currently used clinical variables. Well-validated field effect markers will also be valuable as surrogate endpoint biomarkers in Phase II prevention trials, either in prostate biopsies or in neoadjuvant pre-radical prostatectomy trials. Since prostate biopsies are performed blindly, there is no way to reliably re-sample premalignant areas in order to observe treatment effects, and no way to ensure that a negative biopsy means that the prostate gland is entirely free of cancer. Therefore, it would be of great benefit if potentially cancerous changes could be identified in “normal” prostate tissue acquired by needle biopsy before and after treatment.

It should be emphasized that malignancy-associated changes in histologically normal tissue, broadly speaking, may be due either to a common underlying carcinogenic process (which we define as a field effect), or to the response of adjacent tissue to carcinogenic changes in the vicinity. These two possibilities might be resolved by time-to-event studies in animal models with long enough follow-up. A biomarker that is reflective of a tissue response to nearby malignancy could be useful for clinical prediction but would probably not be useful as a surrogate endpoint in clinical trials. One area for future work—apart from the continued search for PCa field effects and their validation—lies in the possible exploitation of random fine needle aspirate samples (FNA), which have shown promise as a research tool in breast cancer prevention [58]. Prostate FNA has fallen out of favor in clinical practice, but its less invasive nature may facilitate studies that require repeat sampling of the gland in men with normal PSA and digital rectal exams, for whom biopsy is not indicated [59].

Acknowledgments

Grant sponsor: National Cancer Institute; Grant number: RO1 CA90759; Grant sponsor: National Institutes of Health; Grant number: RO3 CA131595; Grant sponsor: Department of Defense Prostate Cancer Research Program; Grant number: PC050393.

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Fig. 1. Nested case-control study design for epidemiological validation of field effect tissue biomarkers.

TABLE I**Biomarkers Indicating Field Effects in the Human Prostate Gland**

Type of biomarker	Specific biomarker	References
Cytomorphology	Nuclear chromatin texture	Mairinger et al. ¹⁹
	Nuclear texture, size and shape	Veltri et al. ²⁰
	Architectural and cytological features	Montironi et al. ²¹
Gene expression	cDNA microarray (12,625 probes)	Chandran et al. ²²
	671-gene signature	Yu et al. ²³
	8-gene panel	Rizzi et al. ²⁴
	PCA3 (non-coding RNA)	Popa et al. ³⁹
Protein expression	TMPRSS2-ERG (gene fusion transcript)	Furusato et al. ⁵³
	Ki67, MCM-2	Ananthanarayanan et al. ²⁶
	AMACR	Ananthanarayanan et al. ²⁸
		Santinelli et al. ²⁷
	EPCA	Dhir et al. ³³
		Uetsuki et al. ³⁴
		Hansel et al. ³⁵
	PS2	Bonkhoff et al. ²⁹
	Akt-1	Ayala et al. ³⁰
	Ca++ channel receptor proteins	Slater et al. ³¹
	Androgen receptor	Olapade-Olaopa et al. ³²
	COX-2	Zha et al. ¹⁵
Epigenetic markers	GSTp1, RAR β 2 methylation	Hanson et al. ⁴¹
	GSTp1, RAR β 2, APC methylation	Henrique et al. ⁴²
	RAR β 2, APC, RASSF1A methylation	Mehrotra et al. ⁴³
	RASSF1A methylation	Aitchison et al. ⁴⁴
	HIN-1 methylation	Krop et al. ⁴⁵
DNA damage	Infrared spectroscopy profile	Malins et al. ⁴⁶
		Malins et al. ⁴⁸
		Malins et al. ⁴⁷
	Mitochondrial DNA mutation	Parr et al. ⁴⁹
Angiogenesis	Microvessel density	Siegal et al. ⁵⁰
		Montironi et al. ⁵¹

TABLE II**Strengths and Weaknesses of the Nested Case–Control Design for the Identification of Prostate Field Effects**

Strengths	Weaknesses
1. Uses biopsies, which are relevant to clinical prediction and many Phase II trial designs	1. Association of biomarker to PCa risk may depend on time between index negative biopsy and PCa diagnosis
2. Can compute Odds Ratios, that is, magnitude of risk associated with each biomarker level	2. True incidence density sampling permits cases to be selected as controls and controls to be selected as cases
3. Statistically efficient use of samples	3. Risk of PCa detection is influenced by many potential confounders (e.g., PSA, gland volume, age, urinary symptoms)
4. Allows for control of confounding by matching or multivariable modeling	
5. “Nested” controls reduce chances of selection bias in choosing controls	