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PROSPECT

Nuclear Matrix Proteins as Biomarkers in Prostate Cancer

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Abstract The nuclear matrix (NM) is the structural framework of the nucleus that consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli. The NM contains proteins that contribute to the preservation of nuclear shape and its organization. These protein components better known as the NM proteins have been demonstrated to be tissue specific, and are altered in many cancers, including prostate cancer. Alterations in nuclear morphology are hallmarks of cancer and are believed to be associated with changes in NM protein composition. Prostate cancer is the most frequently diagnosed cancer in American men and many investigators have identified unique NM proteins that appear to be specific for this disease. These NM protein changes are associated with the development of prostate cancer, as well as in some cases being indicative of cancer stage. Identification of these NM proteins specific for prostate cancer provides an insight to understanding the molecular changes associated with this disease. This article reviews the role of NM proteins as tumor biomarkers in prostate cancer and the potential application of these proteins as therapeutic targets in the treatment of this disease. J. Cell. Biochem. 86: 213–223, 2002.

Key words: tumor markers; nucleus; nuclear structure; nuclear skeleton; hormonal regulation

Prostate cancer is one of the most devastating cancers in men. It is predicted that in 2002, prostate cancer will account for 30% (189,000) of new cancer cases in men and that 30,200 men will die year from this disease [Jemal et al., 2002]. A boy born today has a 13% lifetime chance of developing prostate cancer, and there is a 3% risk that he will die from this disease [Walsh and Worthington, 1997]. The etiology of prostate cancer is not fully understood, but age and hormonal status appear to be central components. For example, men before age 40 who are castrated or have pituitary failure (in which the brain no longer able to stimulate the testes to function), rarely develop prostate cancer [Walsh and Worthington, 1997]. More than 80% of the men diagnosed with prostate cancer are over 65 years old, with 90% of the deaths being in the same age group [Walsh and Worthington, 1997]. Despite the advantage and usage of Prostate Specific Antigen (PSA) as well as increased awareness of this disease, the mortality rate from prostate cancer remains high (second only to lung cancer), although it appears to be improving.

Current diagnostic tools include early detection by combining the PSA assays and digital rectal examination (DRE). Today, standard treatment options for localized prostate cancer include prostatectomy, radiation therapy including external beam and brachytherapy, as well as watchful waiting. Although these standard treatments are effective for localized prostate cancer, treatment options for cancer advancing outside of the prostate and subsequently becoming androgen independent are more limited. Despite its widespread use, the PSA assay alone has many shortcomings, and thus is not considered to be an ideal tumor marker. PSA is a normal prostatic protein that is not typically expressed at higher levels in cancer, but it is inappropriately released into the blood serum with disease. PSA levels in the serum are also increased in benign conditions of the prostate, including benign prostatic hyperplasia (BPH) and prostatitis. In addition, abnormal serum PSA levels are detected in a number of individuals who may not have "clinically significant"

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prostate cancer, and therefore, may not require aggressive therapy. Finally, there are a number of men that have prostate cancer that do not have elevated PSA levels. The limitations in the PSA assay, along with the clinical questions that remain in prostate cancer support the discovery of new tumor markers that will have clinical impact as well as provide opportunities for exploration of more efficacious treatment options.

Typically, diagnosis of cancer by a pathologist is indicated by architectural alterations to cells and/or tissues [Nickerson, 1998]. One of the cellular hallmarks of the transformed phenotype is an abnormal nuclear shape and the presence of abnormal nuclei. Neoplastic transformation of a cell results in comprehensive changes in the nuclear structure of the cells and their resulting morphology. These changes include increased nuclear size, deformed nuclear shape, presence of more prominent nucleoli and larger clumps of heterochromatin, variations in patterns of lamin expression, and the alterations in the composition of nuclear matrix proteins (NMPs).

A majority of NM proteins are common to most cells, whereas some are both cell and tissue specific [Fey and Penman, 1989]. During neoplastic transformation in concordance with changes in nuclear shape are modifications in the composition of the NM proteins. Typically, cancer cells acquire certain features that may be associated with alterations in nuclear structure. These features include genetic instability, chromosomal rearrangements, and translocations, as well as changes in chromatin condensation. Since the NM is considered responsible for maintaining nuclear shape, alterations in the NMP composition could result in alterations in the nuclear shape, which as described above is hallmark of the cancer process. While understanding changes in the composition of the NM are central to understanding the role of nuclear structure in the cancer process, they also provide us with opportunities to develop diagnostic or prognostic markers for many cancers, including cancer of the prostate. In this article, we review the structure and function of the NM and its components, with respect to alterations observed in prostate cancer and how these specific changes can be used as biomarkers of the disease. We will also address the exploitation of the NM proteins as potential therapeutic targets in prostate cancer.

NUCLER MATRIX (NM)

The concept of a "cell/tissue matrix" system was first introduced by Bissel et al. [1982] and Isaacs et al. [1981]. The cell/tissue matrix system or the skeletal network in a tissue and/or cell is defined as an integrated three-dimensional skeletal network that organizes cellular structure and its functions from the peripheral network to the DNA [Getzenberg, 1994]. These matrix systems are interactive and dynamic and consist of linkages and interactions of the NM, the cytoskeleton, and the cell periphery, including the extracellular matrix. These interactive matrix systems organize and process spatial and temporal information to coordinate cell functions and gene expression [Getzenberg et al., 1990]. The dynamics and functions of the cell/tissue matrix in regulating nuclear organization, cell structure, and hormone action has been reviewed extensively in Getzenberg et al. [1990].

The NM is the residual framework scaffolding of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli [Berezney and Coffey, 1974]. According to Bosman [1996], the structure of the NM can be further divided into the nuclear membrane that comprises the nuclear lamina and pore proteins, the nucleolar proteins and the granular NM itself that extends from the nucleolus to the nuclear lamina. After a series of extractions of the nucleus with detergent, salt, DNase, and RNase, the NM consists of $\sim$10% of nuclear proteins and it is virtually devoid of lipids, histones, intermediate filaments, DNA, and most of the RNA [Fey et al., 1991]. The NM is believed to contribute to the maintenance of nuclear shape and its organization. Some of the NM proteins are common to all cell types and physiologic states, whereas some NM proteins are tissue specific or altered with the state of the cell [Getzenberg, 1994].

The NM has been demonstrated by many investigators to have a number of organizing functions within the nucleus [reviewed in Getzenberg et al., 1990; Replogle-Schwab et al., 1996]. One central function is its role in DNA organization. The DNA is organized into loop domains of approximately 60 kb in length. The bases of these DNA loops contain matrix-associated regions (MARs) or scaffold attachment regions (SARs) that facilitate attachment

of the NM. These MARs or SARs are about 200 bp in length, and are typically although not always AT and TG rich regions. The DNA loops attached to the nuclear structure are usually maintained during both interphase and metaphase of the cell cycle [Getzenberg et al., 1990]. While a number of MAR sequences have now been identified, little is known about the protein components of the NM in which they interact. Several proteins have been identified, which associate with MARs. These proteins include topoisomerase II [Berrios et al., 1985], nuclear mitotic apparatus protein (NuMA) [Luderus et al., 1994], the p114 protein in breast tumors [Yanagisawa et al., 1996], and lamins A and C [Hakes and Berezney, 1991; Luderus et al., 1992, 1994]. In addition to organizing DNA, the NM has fixed sites for DNA replication, which contains the replisome complex that includes polymerase along with other proteins and newly-replicated DNA [Replogle-Schwab et al., 1996]. Actively transcribed genes also associate with the NM, which contains transcriptional complexes, newly synthesized heterogeneous nuclear RNA, small nuclear RNA, and RNA processing intermediates [Replogle-Schwab et al., 1996]. The NM is also involved in nuclear regulation, where it serves as a cellular target for transformation proteins, some retroviruses products such as large T antigen, Rb, and E1A proteins [Getzenberg et al., 1990; Replogle-Schwab et al., 1996].

As with other components of transcriptional regulation, steroid receptors have also been demonstrated to interact with the NM. A variety of steroid receptors including androgen, estrogen, corticosteroid, progesterone, thyroid, and vitamin D receptors (VDRs) have been reported to interact with the nuclear matrices from different tissues and species [Getzenberg, 1994]. The binding of steroid receptors to the NM is both steroid and tissue specific, and requires the presence of an activated steroid receptor bound to its respective steroid [Barrack and Coffey, 1980]. For example, the NM of an estrogen-responsive tissue from chicken liver and of an androgen-responsive tissue from rat ventral prostate contain specific binding sites for estradiol and dihydrotestosterone (DHT), respectively [Barrack and Coffey, 1980]. In rat pituitary tumor cells (GC cells), the T3-nuclear receptor was reported to associate with the NM, suggesting that this interaction could regulate thyroid hormone action [Kumara-Siri et al., 1986]. Our laboratory has demonstrated that VDRs interact closely with the NM in both human and rat prostate tissues [Nangia et al., 1998]. Human prostate NM proteins contain VDRs of 52 kDa compared to rat ventral prostate NM proteins (57 and 37 kDa) and rat dorsal prostate NM proteins (52 and 26 kDa). These varying molecular weight proteins reactive with the anti-VDR antibody within these tissues may represent different isoforms, proteolytic cleavage of VDR, or post-translational modification.

The positioning of steroid receptors as transcription factors on the NM may play a crucial role in regulating gene expression. The activated steroid receptor complex with bound ligand is believed to interact with the NM through acceptor sites [Getzenberg, 1994]. One well-characterized acceptor protein is the acceptor protein for the progesterone receptor, RBF-1 [Schuchard et al., 1991]. RBF-1 is a NM protein that has a high affinity for the progesterone receptor. Landers and Spelsberg [1992] proposed that steroid receptors interact with the NM and the DNA through these acceptor proteins, and through them the steroid receptors may position themselves in the appropriate location for the activation and/or repression of gene transcription. The androgen receptor (AR) may interact with the NM by association with acceptor protein(s) that may be similar to the RBF-1 protein [Getzenberg, 1994]. Furthermore, the NM can also arrange the DNA loop domain in a specific orientation that allows the activated AR–DHT complex to interact with both the NM and the androgen responsive element, resulting in the regulation of gene expression.

The receptor-binding factor (RBF) for the avian oviduct progesterone receptor has also been demonstrated to bind to a 54-bp element in the 5′-flanking region of the progesterone-regulated avian *c-myc* gene and NM-like attachment sites flank the RBF element in the *c-myc* promoter [Lauber et al., 1997]. GRIP120, one of the co-factors that interacts with glucocorticoid receptors, has been shown to be identical to the heterogeneous nuclear ribonucleoprotein U (hnRNP U), a NM protein binding to RNA as well as to SARs [Eggert et al., 1997]. The DNA Binding Domain (DBD) of glucocorticoid receptors has been implicated to possess a NM-targeting signal [van Steensel et al., 1995; Tang and DeFranco, 1996]. Further studies by Tang

et al. [1998] demonstrated that while necessary, the rat glucocorticoid receptor DBD is not sufficient for NM targeting. In this study, Tang et al. [1998] showed that a minimal NM-targeting signal could be generated by linking the rat glucocorticoid receptor DBD to either the τ2 transactivation domain in its natural context, or a heterologous transactivation domain. Furthermore, they also identified at least one NM protein, hnRNP U, could play a role in directing the receptor to the NM either through direct or indirect interactions with the glucocorticoid receptor NM-targeting signal [Tang et al., 1998].

HORMONAL REGULATION OF THE NMPS IN NORMAL PROSTATE

Steroid receptors bind to the NM and act as transcription factors to activate and/or repress gene transcription. Interactions between steroid receptors and the NM are steroid and tissue specific, and this interaction can be regulated by appropriate hormonal stimuli. The NM and its protein composition can in turn regulate the tissue specificity of steroid hormone regulation. A series of experiments by Getzenberg and Coffey [1990] demonstrated that tissue specificity of the hormonal response in sex accessory tissues such as the prostate and seminal vesicle is in turn associated with the NM protein pattern. In this study, the NM patterns were compared in intact rat ventral prostate and seminal vesicle vs. castrated rat ventral prostate and seminal vesicle [Getzenberg and Coffey, 1990]. This comparison revealed three sets of NM proteins. The first set showed seven and eight NM proteins that were specific for rat ventral prostate and seminal vesicle, respectively. The second set showed nine NM proteins that either appeared or disappeared upon androgen withdrawal (castration). The last set contained NM proteins that were common for both tissues and did not change with the hormonal state; these proteins represented a large majority of the NMPs. The discovery of the tissue-specific protein composition of the NM in this study further supports the hypothesis that NM proteins could be essential in regulation of gene expression. Since the NM plays an important role in DNA organization, different NM protein composition may organize the DNA differently between the prostate and seminal vesicle, and thus resulting in different gene expression.

The prostate is a complex organ composed mainly of epithelial and stromal cells, which is regulated by androgens, growth factors, and other components. 1,25 dihydroxyvitamin D_3 (1,25 D_3), a steroid hormone, has been shown to play a role in regulating the growth and differentiation of the normal prostate [Konety et al., 1996, 1999, 2000]. The presence of VDRs in normal prostatic tissues strongly suggests a role for this hormone in the normal gland. To ascertain the possible role of 1,25 D_3 in the normal prostate, we examined the interaction of 1,25 D_3 and androgens on the growth and development of the rat prostate [Konety et al., 1996]. In this study, all the rats were castrated and then administered with a super-physiological dose of 1,25 D_3 in the presence or absence of exogenous testosterone. We found that there was a greater degree of epithelial differentiation in the prostates of the rats treated with 1,25 D_3 and exogenous testosterone compared to the rat prostates that received testosterone supplementation alone. Analysis of the NM composition of the prostate tissues revealed five NM proteins that were present in the 1,25 D_3 and testosterone-treated animals, but absent in the animals that received testosterone alone. Since 1,25 D_3 is involved in transcriptional regulation, it is possible that 1,25 D_3 could alter the NM composition of the prostate by mediating specific NMPs to act as transcription factors in regulating gene transcription that is responsible for prostatic differentiation.

In a separate study, the effects of 1,25 D_3 administration on the normal prepubertal rat prostate were followed [Konety et al., 2000]. In this investigation, 1,25 D_3 at a superphysiological dose was administered to rats before puberty for 2 weeks, followed by supplementation with exogenous DHT when they became adult. Analysis of the NMP composition revealed that there were two NMPs present in the 1,25 D_3-treated prostate, but absent in the non-treated prostate. The analysis also showed the presence of ten NMPs in the 1,25 D_3- and DHT-treated prostate, but absent in the 1,25 D_3 alone treated prostate. In addition, there was one unique NMP that was present in the 1,25 D_3-treated prostate, but absent in the 1,25 D_3 and DHT treated prostate. Changes in these NMP compositions demonstrate that the NM can be modulated by hormonal status. Again, this further supports the concept that steroid hormones such as androgen and 1,25 D_3 could target the

NM by mediating a variety of transcription factors (as reflected by changes in the NMP composition) to regulate gene expression.

ROLE OF NMPS AS PROSTATE CANCER BIOMARKERS

One of the characteristics of a transformed phenotype is alteration in nuclear structure and architecture. As the dynamic scaffolding of the nucleus, composition of the NM is different between normal and transformed cells. The differences in the NM composition between normal and transformed cells may play a role in the differences in gene expression during transformation. Since the NM plays a critical role in DNA organization, alterations in NM architecture may result in altered DNA topology. Subsequently, this could lead to changes in interaction of various genes with the NM, which could play a role in modulating process like replication, transcription, and RNA splicing. For example, the retinoblastoma (*Rb*) gene has been reported to associate with p84, a NMP [Durfee et al., 1994]. The large T-antigen of the SV40 virus has been demonstrated to target the NM of cells that are infected or transformed by SV40 [Staufenbiel and Deppert, 1983]. Both the large T-antigen and p53 proteins have also been shown to associate with the NM of *Xenopus* egg extracts during the cell cycle, and that colocalization of these proteins within the NM occurs during both the S- and G2-phases [Vassetzky et al., 1999]. In addition, both Bcl-2 and Bax proteins, which are involved in regulation of apoptosis, have been reported to interact with the NM of the glioblastoma cell line U343 [Wang et al., 1999].

A number of investigators have identified NMPs that are associated with various neoplasms such as breast, prostate, colon, bladder, lung, ovarian, renal, as well as in squamous cell carcinoma of the neck and head [reviewed in Konety and Getzenberg, 1999]. These NMPs may have potential application as tumor markers and are being applied in clinical settings. An example of the clinical utility of NMPs can be found in our work of bladder cancer. We have previously identified six NMPs (BLCA1-6) that appear to be associated with bladder cancer [Getzenberg et al., 1996]. Recent studies in our laboratory have focused on of the bladder-specific NMP BLCA-4. Using antibodies raised against BLCA-4, we have shown that this protein is expressed early in the development of bladder cancer and throughout the bladder of individuals with the disease. A clinical urine-based assay for BLCA-4 has recently been developed, which has a sensitivity of 96.4% and a specificity of 100% in individuals with bladder cancer, and this protein is not elevated in patients with confounding conditions such as cystitis [Konety et al., 2000a]. Another bladder assay, NMP22 detects the nuclear mitotic apparatus (NuMA) protein, the urine of bladder cancer patients [Keesee et al., 1996]. The NMP22 assay has been reported to have a better accuracy than other standard diagnostic methods to identify patients who are at risk for imminent recurrence of cancer [Konety and Getzenberg, 1999]. Since the NuMA protein is present in all types of cells including non-cancerous cells, elevated levels of this protein in the urine does not necessarily indicate bladder cancer recurrence in all cases, especially in patients who have bladder inflammation [reviewed in Konety and Getzenberg, 1999]. Thus, utilization of cancer-specific NMPs, such as BLCA-4 may enhance the accuracy of bladder cancer diagnosis.

Identification of NMPs in Rat and Human Prostate Cancer

Differences in the NMPs have been demonstrated in the cancer and normal rat prostate [Getzenberg et al., 1991; Pienta and Lehr, 1993], as well as in human BPH and prostate cancer [Partin et al., 1993; Pienta and Lehr, 1993; Lakshmanan et al., 1998]. Using tumor cells derived from the Dunning rat prostatic adenocarcinoma, Getzenberg et al. [1991] identified a set of NMPs in the Dunning sub lines: G, H, AT2, AT6, and MLL. Utilizing a novel protocol for resolving NMPs, which involves high-resolution two-dimensional gel electrophoresis, two unique NMPs (G-1 and G-2) were identified that were specific for the androgen-sensitive and non-metastatic G and H tumor sub lines. Two unique NMPs: AM-1 and AM-2 were found in the highly metastatic androgen insensitive lines AT2, AT6, and MLL. In addition, ten NMPs (NDP1–NDP10) were in the normal dorsal prostate, but not present in any of the Dunning sub lines. Three proteins (D1–D3) were specific to all the Dunning tumors and were not found in the normal dorsal prostate. Our laboratory has been able to sequence the AM-1 and -2 proteins, as well as D1–D3 proteins. We have also raised antibodies against AM-1 and D2 proteins, and

these antibodies have been demonstrated to detect the presence of AM-1 and D2 in both rat and human prostate cancer tissues. These studies further demonstrate the conserved nature of at least some these NMPs.

Studies from Pienta and Lehr [1993] demonstrated that NM derived from the Dunning tumor sub line MLL and four human prostate cancer cell lines (LNCaP, TSU, PC-3, DU-145), as well as human prostate tumors shared several common proteins. Using high-resolution two-dimensional gel electrophoresis, ten NMPs (PROS1–PROS10) were identified in both the normal dorsal rat prostate and the Dunning sub line MLL. These ten NMPs were also found in both MLL cell line and all the human prostate cancer cell lines. In addition, all four human prostate cancer cell lines and the MLL cell line shared three NMPs (CANC1–CANC3), which were cancer cell-specific only. Using two human prostate tumors removed at radical prostatectomy, they also showed that these two tumor specimens contained similar NMPs (PROS1–PROS10 and CANC1–CANC3) as the cancer cell lines. These common NMPs suggest that there are common alterations in the NM phenotype that may accompany prostatic transformation.

Characterization of the NMP in the Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) Model

Although prostate cancer has been reported in some rodent and canine species, these animals have not provided a proper model to adequately study the molecular basis related to the early development and progression of human prostate cancer [Greenberg et al., 1995]. Studies to establish a transgenic animal model for prostate cancer by utilizing a prostate-specific transgene expression has been initiated by Greenberg's laboratory. A transgenic mouse model of prostate cancer, TRAMP was developed by Greenberg et al. [1995], which mimics the progression of prostate cancer in humans. This model was generated by constructing 426 bp of 5′ flanking sequence and 28 bp of 5′ untranslated sequence of the rat probasin promoter to target expression of SV40 large T-antigen (Tag) to the epithelium of the mouse prostate. This transgenic model reproduces the spectrum of benign, latent, aggressive, and metastatic forms of prostate cancer. The TRAMP males have been shown to develop histological prostatic intraepithelial neoplasia (PIN) by 8–12 weeks of age that progress to adenocarcinoma with distant metastases by 24–30 weeks of age [Gingrich et al., 1996].

Our laboratory is currently characterizing the NMP changes that are associated with prostate cancer development in the TRAMP model [Leman et al., 2002]. We have collected prostates from the TRAMP males at six critical time points: 6, 11, 19, 25, 31, and 37 weeks of age. These time points correspond with puberty (6 weeks), development of mild hyperplasia (11 and 19 weeks), as well as progression to severe hyperplasia (25 weeks), neoplasia (31 and 37weeks). The nuclear matrices from the prostates collected at these five different time points were then isolated and the NMPs were characterized by high-resolution two-dimensional electrophoresis.

Our results show that the 6-week time point TRAMP prostate has three unique NMPs (E1A, E1B, and E1C). Some of these proteins are still present in the 11-week (E1C), 19-week (E1B), and 25-week time point (E1A) TRAMP prostates. However, these proteins are not present in both 31- and 37-week time points. The 11-week time point TRAMP prostate contains two NMPs (E2A and E2B) that are still found in the 19-week (E2A) and 25-week time point (E2B). Again, these two NMPs are not present in the 31- and 37-week TRAMP prostates. Prostates from the 31-week-old TRAMP showed five NMPs (E3A–E3E) that were specific for this time point when compared to prostates from the 6-, 11-, 19-, and 25-week time points. Four NMPs (E3A, E3B, E3C, and E3E) from the 31-week TRAMP prostate are found to have lower expression in the 37-week TRAMP prostate, whereas the E3D protein expression is lost in the 37-week TRAMP prostate. Our analysis examined alterations in NMP composition during the progression from normal to prostate cancer in the transgenic mouse model. For the first time, these results are allowing us to understand the temporal changes in the NM and its association with biological effects on the prostate. This has the potential to identify novel markers that can characterize the various stages of prostate cancer development as well as determine ways in which the NM changes can serve as potential therapeutic targets.

Human Prostate Cancer-Associated NMPs

Similar analysis in normal human prostate, BPH, and prostate cancer tissues also revealed

differences in the NMP composition [Partin et al., 1993]. Using fresh human normal prostate, BPH and prostate cancer tissues from 21 men undergoing surgery for localized prostate cancer, Partin et al. [1993] identified 14 NMPs that were consistently present or absent among the various tissues. Seven unique NMPs (NBP1–NBP7) were found in both normal prostate and BPH tissues, whereas three unique proteins (NP1–NP3) were found only in the normal prostate tissues. Three NMPs (BPC1–BPC3) were also identified in both BPH and prostate cancer tissues. Interestingly, one unique NMP PC-1 with a MW of 56 kDa and isoelectric point (pI) of 6.58 was identified only in the prostate cancer samples.

Following these findings, Partin et al. [1997] later developed a monoclonal antibody PRO: 4–216 to detect what was reported to be the PC-1 protein. This monoclonal antibody was used to analyze frozen tissues from 20 cancerous, 22 BPH, and 22 normal regions from fresh human prostate specimens [Partin et al., 1997]. Immunohistochemical analysis showed that the PRO: 4–216 antibody was able to detect the presence of a unique protein in 85% (17 of 20) of the cancerous, 5% (1 of 22) of BPH, and 9% (2 of 22) of the normal prostate tissues. However, the protein identified by this antibody has a somewhat smaller MW (~40 kDa) and lower isoelectric point (~pI 6.0) than the original PC-1 protein (MW 56 kDa, pI 6.58). As the authors later pointed out, although a weakly spot was identified in the region of PC-1 (MW 50–60 kDa) by two-dimensional immunoblotting, they did not believe that PRO: 4–216 reacted with PC-1 protein. However, they reported that the PRO: 4–216 monoclonal antibody demonstrated strong immunoreactivity to NMPs from the human bladder transitional cell carcinoma cell line J82 by one-dimensional gel electrophoresis and immunoblotting.

Further investigations by Partin's laboratory [Subong et al., 1999] revealed that PRO: 4–216 antibody was actually identifying the B23/nucleophosmin protein (MW 40 kDa, pI ~ 5.0) by Western blot analysis in five human prostate cancer cell lines (LNCaP, TSU, DU145, PC-3, and PPC-1), as well as in human prostate cancer NMPs by two-dimensional immunoblots. Nucleophosmin was originally described as B23 by Michalik et al. [1981] as a nuclear protein. B23/Nucleophosmin is an RNA-associated nucleolar phosphoprotein that has been found to be more abundant in malignant and growing cells than in normal non-dividing cells [Schmidt-Zachmann et al., 1987; Feuerstein et al., 1988; Chan et al., 1989; Derenzini et al., 1995]. Since NMPs are elevated in the serum of cancer proteins [Miller et al., 1992; Keesee et al., 1996], B23/nucleophosmin may have the potential to be utilized as a serum marker for detection of cancer, although its specificity for prostate is in question.

Lakshmanan et al. [1998] further characterized the differential NMP expression in human prostate cancer tissues with varying levels of aggressiveness. Analysis of the NMP composition in 39 human prostate cancer specimens with different prognoses (poor, intermediate, and good) was performed to elucidate differences in NMP patterns among these sample sets. In this analysis, poor prognosis was defined as specimens with seminal vesicle or lymph node involvement or extra capsular penetration (ECP) with a Gleason score of 7 or more; intermediate prognosis was defined as specimens with organ confined (OC) or focal capsular penetration (FCP) with Gleason score of 7 or ECP with Gleason score of 6. Good prognosis was defined as specimens with OC or FCP with Gleason score less than 7. A specific NMP, YL-1, (MW 76 kDa, pI 6.0–6.6) was found to be consistently present in 19 of 19 aggressive tumors (specimens with poor prognosis) and was present only in 1 of 10 in the group with good prognosis and weakly positive in 9 of 10 in the group with intermediate prognosis. These preliminary results suggest that YL-1 could be associated with aggressive prostate cancer and thus can be utilized as a potential marker to differentiate clinically-localized prostate cancer. Further characterization of the YL-1 protein has been reported to currently be in progress to further ascertain its clinical potential as prostate cancer tumor marker. A description of the NMPs that have been analyzed and specific proteins that have been identified from different prostate cancer cell lines and specimens are summarized in Table I.

Although much of the discussion of NMPs associated with prostate cancer in this review have focused on the presence or absence of proteins, it is clear that many NMPs are post-translationally modified and that these modifications may be characteristic of the disease state. An example of this protein is protein kinase CK2. Protein kinase CK2 (formerly

TABLE I. Prostate Cancer-Associated NMPs

Cell lines/tissue type	Nuclear matrix protein			References
	Normal	BPH	Cancer	
Dunning rat prostate adenocarcinoma	NDP1–NDP10	—	D1–D3	Getzenberg et al. [1991]
Dunning rat prostate cancer cell lines: G, H, AT2, AT6, MLL	—	—	G1–G2 (for G and H cell lines) AM1–AM2 (for AT2, AT6, and MLL cell lines)	Getzenberg et al. [1991]
Human prostate cancer cell lines (LNCaP, TSU, DU-145, PC-3), Dunning cell line MLL, normal dorsal rat prostate and human prostate tumor tissues	PROS1–PROS10	—	PROS1–PROS10 CANC1–CANC3 (in all cell lines and tissues)	Pienta and Lehr [1993]
Human normal, BPH, and prostate cancer specimens	NBP1–NBP7 NP1–NP3	NBP1–NBP7 BPC1–BPC3	BPC1–BPC3 PC1	Partin et al. [1993, 1997]
Human prostate cancer specimens	—	—	YL-1	Lakshmanan et al. [1998]

known as casein kinase 2 or II) is a highly conserved, heterotetrameric, ubiquitous messenger-independent serine/threonine kinase that is involved in signal transduction in the NM in response to various stimuli [Guo et al., 1999]. Protein kinase CK2 consists of catalytic subunits (α, α') and a regulatory subunit (β) existing in the $\alpha 2\beta 2$, $\alpha\alpha'\beta 2$, or $\alpha' 2\beta 2$ configuration [Ahmed et al., 2000]. Protein kinase CK2 has been reported to play a significant role in nuclear signaling in neoplasia [reviewed in Ahmed et al., 2000]. CK2 has also been shown to be dysregulated in human prostatic neoplasia and that enhanced nuclear localization of CK2 has also been related to Gleason's tumor grade [Yenice et al., 1993].

Ahmed's group has reported that CK2 interacts closely with the NM in prostate cancer cells under different conditions and stimuli [Guo et al., 1999]. CK2 has been shown to interact with the NM in androgen sensitive LNCaP cells following stimulation by DHT and/or growth factors such as EGF, whereas the nuclear signaling of CK2 in androgen-insensitive PC3 cells occurs only in the presence of growth factors [Guo et al., 1999]. Overexpression of CK2 in BPH-1 cells revealed several fold enhancement of CK2 in the NM [Yu et al., 1999]. The transient overexpression of CK2-α, CK2-β alone or in combination or in a bicistronic construct CK2-$\alpha\beta$ in BPH-1 cells showed an increase in CK2 activity by 156, 8, 147, and 152%, respectively in the NM fraction [Yu et al., 1999]. Recently, studies by Guo et al. [2001] demonstrated that chemical-induced apoptosis in prostate cancer cell lines (LNCaP, PC-3, and ALVA-41), as well as other cell types by etoposide and diethylstilbestrol evokes an enhancement in CK2 association with the NM. The enhancement of CK2 in the NM appears to be a result of translocation of CK2 from the cytoplasmic to the nuclear compartment. This shuttling of CK2 to the NM may reflect a protective response to chemical mediated apoptosis. To further support this evidence, the authors also showed that transfections with CK2-α or CK2$\alpha\beta$ in PC-3 cells resulted in significant resistance to chemical-mediated apoptosis proportionate with the corresponding elevation of CK2 in the NM [Guo et al., 2001]. These results suggest that CK2 activity in the NM have a significant role in protecting cells against apoptosis.

In addition to studies examining cancer-associated changes in NMPs to elucidate the functional role of these proteins, as well as the potential role of these proteins as cancer biomarkers, the NM has been utilized as a target for prostate cancer chemotherapy. Since the NM plays an important role in DNA replication and gene expression, alkylating agents, ionizing radiation, anti-metabolites, as well as various drugs that function as topoisomerase and replication inhibitors have been employed to target the NM. Alkylating agents and ionizing radiation have been demonstrated to break DNA strands and bind to matrix-associated DNA and NMPs [Fernandes and Catapano, 1995]. This interaction in turn causes the disorganization of matrix-attached chromatin loops, and subsequently activates programmed cell death [reviewed in Muenchen and Pienta, 1999]. Anti-metabolites and anti-cancer drugs have been shown to inhibit NM-associated enzymes that are involved in replication [Muenchen and

Pienta, 1999]. Some of these anti-cancer drugs include estramustine (an estradiol-nitrogen mustard conjugate that interacts with replication complex), etoposide (VP-16), a topoisomerase II inhibitor and 9-aminocamptothecin (9-AC), a topoisomerase I inhibitor. Clinical trials have focused on these compounds to target the NM. In pre-clinical studies by Pienta and Lehr [1993a], estramustine and etoposide were shown to interact with the NM and selectively inhibit new DNA synthesis of both human and rat prostate cancer cell lines. They also showed that in vivo, estramustine and etoposide inhibited the growth of prostate adenocarcinoma in the Dunning rat model. These studies were further translated into phase I/II clinical trials to examine the effects of oral estramustine and etoposide in the treatment of patients with hormone-refractory prostate cancer [Pienta et al., 1994]. Estramustine alone had only 20% response rate in the patients, whereas estramustine combined with etoposide (topoisomerase II) inhibitor increased the response rate to 50%. Further studies by the same group [Naik et al., 1996] also showed estramustine combined with 9-AC (topoisomerase I inhibitor) interact at the level of the NM to inhibit the growth of prostate cancer cells in vitro. Combination of these two drugs also inhibited the growth of MLL cells injected in the Dunning model. These studies were later expanded by combining estramustine and etoposide with paclitaxel, a microtubule inhibitor, and the combinations of these three agents had a significant pre-clinical activity against androgen-independent prostate cancer cells [Pienta and Smith, 1997].

Based on these studies, a phase II clinical trial of estramustine and etoposide was conducted in 51 patients with hormone-refractory prostate cancer [Pienta et al., 2001]. The patients received two cycles of therapy by means of oral estramustine at 15 mg/kg/day and etoposide at 50 mg/m^2/day from day 1–21 at every 28 days over a period of 4 months. This trial resulted in 37% of the patients (19/51) experiencing various toxicities and two treatment-related deaths. Seven men in the remaining 32 patients who received two cycles of therapy showed partial response to this trial as demonstrated by 50% decrease in their PSA levels. As the authors pointed out, this trial demonstrated the toxicity of high-dose estramustine with only modest response rate, and therefore, further trials with low-dose estramustine are clearly warranted.

In a separate phase II trial of oral estramustine and etoposide, 18 men with early recurrent prostate cancer with increasing PSA levels (median 3.1 ng/ml) without any evidence of metastasis were given oral treatment of 50 mg/m^2/day etoposide and 15 mg/kg/day etoposide for 21 days followed by a 7-day rest period [Munshi et al., 2001]. Thirteen men had their serum PSA levels declined to undetectable levels, whereas two men had partial response to this therapy. However, the median duration of this response was only 8.5 months and most patients developed toxicities to this regimen. Again, the authors later point out that this trial was poorly tolerated due to significant toxicity, and thus, this regimen should not be employed as standard therapy for the treatment of early recurrent prostate cancer. New strategies that include development of new regiments, novel approaches, as well as better understanding of prostate cancer biology are currently being developed in order to minimize the toxicity and maximize the efficacy of the therapies [Munshi et al., 2001].

CONCLUDING REMARKS

Specific NMPs associated with the progression of prostate cancer have been identified. These NMPs permit investigators to further define molecular changes associated with prostate cancer. A common set of NMPs have also been identified in both rat and human prostate cancer cell lines as well is tumor specimens. These common NMPs suggest that there are universal alterations in the NM phenotype that may accompany prostatic transformation. The application of NMPs to clinical practice for the diagnosis/prognosis of tumors have been shown by developing antibodies against these proteins. These antibodies indeed have been able to detect the presence of specific NMPs found in different tumors. The unique NMPs that are specific for prostate cancer may serve as potential tumor biomarkers, which will in turn provide investigators/physicians with better tools for cancer screening and testing. Future directions that include utilizing the NMPs as 'predictors' for prostate cancer progression are clearly warranted for better treatment of this disease.

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