Characterization of the Nuclear Matrix Proteins in a Transgenic Mouse Model for Prostate Cancer

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Abstract The nuclear matrix (NM) contains a number of proteins that have been found to be associated with transformation. We have previously identified changes in the NM associated with prostate cancer. In this study, we examine the molecular changes that are associated with prostate cancer development in transgenic adenocarcinoma of mouse prostate (TRAMP) model by studying the differences in the NM proteins (NMPs). We collected prostates from the TRAMP males at six critical time points: 6 weeks (puberty), 11 and 19 weeks (development of mild hyperplasia), 25 weeks (development of severe hyperplasia), 31 and 37 weeks (development of neoplasia). The nuclear matrices from the prostates collected at these time points were then isolated and the NMPs were characterized by high-resolution two-dimensional gel electrophoresis. We found three NMPs (E1A, E1B, and E1C) that were present in the 6-week-old prostate and two NMPs (E2A and E2B) that were present in the 11-week-old prostate. These NMPs were absent in the 31- and 37-week-old prostate. We also found five NMPs (E3A–E3E) that were present in the 31-week-old prostate, but absent in the earlier time points. In addition, three NMPs (Le1, Le2, Le3) were present at higher expression in the 6-, 11-, 19-, and 25-weeks old TRAMP prostates, but they were expressed lower during the development of neoplasia at 31- and 37-weeks old. Identification of these NMPs permits the development of novel markers that can characterize various stages of prostate cancer development as well as potentially therapeutic targets. J. Cell. Biochem. 86: 203–212, 2002.

Key words: TRAMP; nuclear matrix protein; prostate cancer

The nuclear matrix (NM) is the residual framework scaffolding of the nucleus, which consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network and residual nucleoli [Berezney and Coffey, 1974]. After a series of extractions of the nucleus with detergent, salt, DNase and RNase, the NM consists of ~10% of the 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. 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 [Getzenberg, 1994]. The variation in the NM composition between normal and transformed cells may play a role in the differences in gene expression observed during transformation. Since the nuclear shape and architecture are at least, in part, determined by the NM composition, NM proteins (NMPs) may be involved in transformation for different types of cancer. The NM has been reported to be a cellular target for transformation proteins and some retrovirus products such as large T-antigen and E1A protein, and that many of
the NMPs are phosphorylated at specific times in the cell cycle [Getzenberg et al., 1990]. The retinoblastoma (Rb) gene has been reported to interact with p84, a NMP [Durfee et al., 1994]. The SV40 large T-antigen has been demonstrated to target the NM of cells that are infected or transformed by SV40 [Staufenbiel and Deppert, 1983]. In addition, both the large T-antigen and p53 proteins have also been shown to associate and colocalize with the NM of xenopus egg extracts during both the S and G2 phases in the cell cycle [Vassetzky et al., 1999].

The NM contains proteins, most of which are common to all cell types and physiologic states, whereas some NMPs are tissue specific or altered with the state of the cell [Getzenberg, 1994]. 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]. 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 benign prostatic hyperplasia (BPH) and prostate cancer [Partin et al., 1993; Pienta and Lehr, 1993; Lakshmanan et al., 1998].

This year alone, prostate cancer is predicted to account for 30% (189,000) of new cancer cases in the United States and 30,200 men will die 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. 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].

A transgenic mouse model of prostate cancer, transgenic adenocarcinoma of mouse prostate (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]. In this study, we examined the molecular changes that are associated with prostate cancer development in the TRAMP model by studying the differences in the NMP composition during the progression from normal to neoplasia. We identified a series of NMPs that were either present or absent in the early development and later stage of the disease. Identification of these NMPs will potentially allow us to develop novel markers that can characterize the various stages of prostate cancer development as well as determine ways in which changes in NM composition can serve as potential therapeutic targets.

**MATERIALS AND METHODS**

**Transgenic Animals**

As previously described, both male and female TRAMP mice heterozygous for the PB-Tag transgene were maintained in a pure C57BL/6 background (Harlan Sprague–Dawley, Inc., Indianapolis, IN) [Greenberg et al., 1995]. Transgenic males for this study were routinely obtained as [TRAMP × C57BL/6] F1 offspring. Prostates from the TRAMP males were collected at six critical time points: 6, 11, 19, 25, 31, and 37 weeks of age. These time points correspond to puberty (6 weeks), development of mild hyperplasia (11 and 19 weeks), as well as progression to severe hyperplasia (25 weeks) and neoplasia (31 and 37 weeks) [Gingrich et al., 1996]. The prostate tissue from each animal was weighed; half of the tissue was sent for histopathological examination and the other half was used for analysis of NMP composition.

**Isolation of NMP Composition**

NMPs were isolated from the harvested mouse prostatic tissues according to the method described by Getzenberg et al. [1991]. Briefly, the tissue was minced into small pieces and homogenized using a polytetrafluoroethylene pestle on ice in a 0.5% solution of Triton X-100 containing 2 mM of the ribonuclease inhibitor vanadyl ribonucleoside to release proteins and
lipids. The extracts were filtered through a 350 μM nylon mesh and extracted with 0.25 M ammonium sulfate to release the cytoskeletal elements.

Soluble chromatin was removed using deoxyribonuclease treatment at room temperature, and the remaining fraction that contained intermediate filaments and NMPs was disassembled with 8 mM urea. The insoluble components, which mainly consisted of carbohydrates and extracellular matrix components, were pelleted by ultracentrifugation. The urea was dialyzed out and the intermediate filaments were allowed to reassemble and were subsequently removed by ultracentrifugation. All solutions contained freshly prepared 1 mM PMSF, 0.3 mM aprotinin, 1 mM leupeptin, and 1 mM pepstatin. The NMPs were then ethanol precipitated. Protein concentration was determined by resuspending the proteins in PBS and using the Coomassie Plus protein assay reagent kit (Pierce, Rockford, IL) with bovine serum albumin as standard. For gel electrophoresis, the ethanol precipitated NMPs were dissolved in sample buffer consisting of 9 M urea, 65 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 2.2% ampholytes, and 140 mM dithiorthreitol. The final pellet containing NMPs represented less than 1% of the total cellular proteins.

Analysis of NMP Composition

High-resolution two-dimensional electrophoresis was performed to separate the extracted NMPs as described previously [Getzenberg et al., 1991]. Fifty micrograms of NMPs were loaded onto each tube gel (1 mm × 18 inch). One-dimensional isoelectric focusing was carried out for 18,000 V-h after 1.5 h of prefocusing. The tube gels were then extruded and placed on top of 1 mm 10% SDS–Duracyl (Genomic Solution, Ann Arbor, MI) high tensile strength PAGE slab gels. The slab gels were electrophoresed at 12°C constantly for 5–5.5 h. The gels were then fixed with 50% methanol and 10% acetic acid. After thorough rinsing and rehydration, the gels were treated with 5% glutaraldehyde and 5 mM dithiorthreitol after buffering with 50 mM phosphate (pH 7.2). The gels were stained with silver stain according to the methods described by Wray et al. [1981]. Only spots clearly reproducibly observed in the gels were counted as representing NM components. The gels were analyzed on the BioImage 2D Electrophoresis Analysis System (BioImage, Ann Arbor, MI). Multiple gels were run for each sample and multiple samples were run at different times. The gels shown in this article are representative of at least three gels produced for each sample.

RESULTS

In this study, prostates were collected from the TRAMP males at six different time points. These time points correspond to puberty (6-weeks old), development of mild hyperplasia (11- and 19-weeks old), progression to severe hyperplasia (25-weeks old), and development of neoplasia (31 and 37 weeks) [Gingrich et al., 1996]. The mean prostatic weight at each time point is shown in Figure 1. As shown in this figure, the average size of the prostate varies significantly from 0.046 g, when the mice are at puberty (6-week old) to 1.58 g, when these mice develop neoplasia (37-weeks old).

Using high-resolution two-dimensional gel analysis, the NM composition from the TRAMP prostates at each of these time points were compared. A total of 13 differentially expressed NMPs were identified by comparison of the NM composition in the six time points. NMPs from the 6-week-old TRAMP prostates (puberty state) showed three proteins (E1A, E1B, and E1C) that were absent in the neoplastic prostate tissues (31- and 37-week-old TRAMP) (Figs. 2B and 3A). Some of these NMPs were still present in the 11- (E1A and E1C), 19- (E1A and E1B), and the 25-week time point (E1B) prostates. The 11-week time point TRAMP prostates showed two unique NMPs (E2A and E2B) that were also absent in the TRAMP prostates at the
neoplastic state (31 and 37 weeks) (Figs. 2B and 3A). The two NMPs from the 11-week TRAMP prostates were still present in the 19-week time point (E2A and E2B) and the 25-week time point TRAMP prostates (E2B only). No unique NMPs were identified in the 19- and 37-week TRAMP prostate tissues. The prostates from the 31-week-old TRAMP showed five NMPs (E3A–E3E) that were only specific for this time point when compared to the prostates from the earlier time points (6, 11, 19, and 25 weeks). Four of the NMPs identified from the 31-week-old TRAMP prostates (E3A, E3B, E3C, and E3E) were still present at lower expression in the 37-week-old TRAMP prostates (Figs. 2E,F and 3B). However, one NMP, E3D from the 31-week-old TRAMP was absent in the 37-week-old TRAMP (Fig. 2F).

As shown in Figure 2, three NMPs (Le1, Le2, and Le3) were also identified that were expressed at higher levels in the 6-, 11-, 19-, and 25-week-old TRAMP prostates, but these

![Fig. 2. Representative 2D gels of NMP composition of the TRAMP prostate. High-resolution two-dimensional gel electrophoresis of NM preparations isolated from: (A) 6-week, (B) 11-week, (C) 19-week, (D) 25-week, (E) 31-week, (F) 37-week TRAMP prostate tissues, and (G) 25-week prostate tissue from control non-transgenic mice. Circles and arrows in the figures represent presence and absence of NMPs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]](image-url)
proteins were found at lower levels when these mice developed neoplasia in the prostate (31- and 37-weeks old). In addition, we also compared the prostates from the six time points with prostates from 25-week-old non-transgenic mice as controls. The 25-week-old prostate from the non-transgenic mice represents normal prostatic state in adult mice. Interestingly, the 6-week-old TRAMP prostate, which is still at normal state, has a very similar NMP composition in comparison to the 25-week-old prostate from the non-transgenic mice. Both the 6-week-old TRAMP and 25-week non-transgenic mice contain the E1A, E1B, E1C, Le1, Le2, and Le3 proteins at similar levels of expression.

Figure 2 is an illustration of the typical high-resolution two-dimensional gel electrophoresis patterns for NMPs isolated from 6-week-old (Fig. 2A), 11-week-old (Fig. 2B), 19-week-old (Fig. 2C), 25-week-old (Fig. 2D), 31-week-old (Fig. 2E), and 37-week-old (Fig. 2F) TRAMP prostates, as well as prostate from the 25-week non-transgenic mice (Fig. 2G). Table I summarizes the molecular weight and isoelectric points of the 13 identified NMPs from prostate.
tissues of the TRAMP mice collected at different time points. Gel spots representing NMPs that are described here have been marked with circles and arrows and identified with labels corresponding to those in Table I. Figure 3 summarizes the location of the protein spots found to be consistently present or absent when comparing NMPs from the TRAMP prostates.

Histologies of the prostate tissues collected from various time points of the TRAMP mice are described in Figure 4. A random sample of four prostates from each group was analyzed. In the 9-week-old TRAMP prostate, microscopic morphology demonstrates normal and well-differentiated prostatic glands (Fig. 4A). In 13-week-old TRAMP prostate, microscopic morphology shows well-differentiated glands with some PIN (Fig. 4B). In both 19- and 25-week-old TRAMP prostate, prostatic glands are moderately differentiated (Fig. 4C,D). The 31-week-old TRAMP prostate demonstrates moderately-differentiated glands (Fig. 4E), whereas in 37-week-old TRAMP prostate (Fig. 4F), the prostatic glands are characterized as moderately to poorly differentiated.

**DISCUSSION**

We have previously examined changes in the NMP composition associated with rat and human prostate cancer [Getzenberg et al., 1991; Partin et al., 1993]. In this study, we examined a subset of the molecular changes that are associated with prostate cancer

<table>
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<th>TABLE I. NMP Composition From the TRAMP Prostate Tissues</th>
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+, presence of protein; −, absence of protein; |, decreased protein expression.
Fig. 4. Histologies of prostates from various time points of TRAMP. In the 9-week-old TRAMP prostate (A), microscopic morphology demonstrates normal and well-differentiated prostatic glands. In the 13-week-old TRAMP prostate (B), microscopic morphology shows well-differentiated glands with some PIN. In both the 19-week time point (C) and 25-week time point (D), prostatic glands are moderately differentiated. The 31-week-old TRAMP prostate (E) demonstrates moderately-differentiated glands, whereas in 37-week-old TRAMP prostate (F), the prostatic glands characterized as moderately to poorly differentiated.
development in the TRAMP model by identifying differences in the NMP composition. The TRAMP model was previously developed by Greenberg et al. [1995], which mimics the progression of prostate cancer in humans. The TRAMP mice develop histological PIN by 8–12 weeks of age that progress to adenocarcinoma by 24–30 weeks of age [Gingrich et al., 1996].

When comparing the NMP composition collected from six critical time points (6, 11, 19, 25, 31, and 37 weeks), 13 NMPs were identified, and they were either present/absent or had lower expression during the progression of prostate cancer in these mice. Five of these proteins (E1A–E1C, E2A–E2B) were present in the earlier time points that correspond to puberty (6-weeks old) and the development of mild hyperplasia in the prostate (11- and 19-weeks old). Two of these proteins (E1B and E2B) were still present until the development of severe hyperplasia in the 25-week-old TRAMP prostate. No unique proteins were found in the 19- and 25-week-old TRAMP prostates. At the time that the TRAMP mice developed neoplasia at 31 weeks, five new NMPs (E3A–E3E) were identified. These proteins were not found in the earlier time points and four of these proteins (E3A, E3B, E3C, and E3E) were still present at a later time point of neoplastic prostate (37 week). Normal non-transgenic mouse prostates from animals of 25 weeks of age were used as controls in this study. The prostates from both 25-week non-transgenic mice and 6-week-old TRAMP at puberty represent normal state of the prostatic tissues, and their NM composition looked almost identical. This demonstrates that the presence of the transgene alone did not cause changes in NMP composition.

Two of the NMPs (E1C and Le2) identified from this study appear similar in molecular weight and isoelectric point to two of the NMPs previously characterized by our group [Getzenberg et al., 1991] in the normal rat dorsal prostate and the Dunning rat prostate adenocarcinoma tissues. One of these proteins, Le2 (MW 32.5, pI 5.2–5.4) has similar molecular weight and isoelectric point as the protein NDP-10 (MW 32.5, pI 5.6). The NDP-10 protein was previously characterized to be specific to normal dorsal rat prostate and not present in the Dunning tumors [Getzenberg et al., 1991]. In this study, Le2 was expressed at higher levels in the TRAMP prostates from 6 to 25 weeks old; however, Le2 was expressed at lower levels when the TRAMP prostates developed neoplasia at 31 and 37 weeks of age. It is possible that higher levels of Le2 protein in the TRAMP mice at earlier time points may have similar characteristics and/or properties as the NDP-10 protein found in the normal rat dorsal prostate. Thus, Le2 may be responsible for maintaining the ‘normal’ differentiated phenotype of the prostatic cells and the absence of these proteins may contribute to neoplastic in the prostate.

On the other hand, the E1C protein (MW 40 kDa, pI 5.6) was present in the TRAMP prostate from 6 to 25 weeks of age. This E1C protein appears similar in molecular weight and isoelectric point to the D2 protein (MW 40 kDa, pI 5.91) found to be specific to all the Dunning tumors and not present in the normal dorsal prostate (Getzenberg et al., 1991). While they have similar characteristics, the relationship of these proteins to one another can only be established by protein sequencing. It is important to note that the E1C protein is absent in the TRAMP prostate from 11 to 37 weeks of age. Since the E1C protein is only present in the TRAMP prostate at 6 weeks of age (puberty), but absent when the prostate developed hyperplasia (11-, 19-, and 25-weeks old) as well as progression to neoplasia (31- and 37-weeks old), this protein may play a role for the ‘on set’ of neoplastic transformation from the hyperplastic state in the prostate. We speculate that the presence of E1C at its highest level during puberty at 6-weeks old is only sufficient to keep the prostatic cells in their differentiated forms before they eventually become moderately and poorly-differentiated as the mice develop prostate cancer at later time points.

Another NMP (E2A) identified from the TRAMP prostates is also similar to two of the NMPs identified in studies by Partin et al. [1993]. In analyzing specimens from 21 men undergoing surgery for clinically localized prostate cancer or BPH, they identified three NMPs (BPC1–BPC3) in both the BPH and prostate cancer tissues. We found that E2A protein (MW 42 kDa, pI 5.6) had similar molecular weight and isoelectric point as BPC1 (MW 42.5 kDa, pI 5.8) and BPC2 (MW 42 kDa, pI 5.7) proteins that were present in both the BPH and human prostate cancer specimens. The E2A protein was present only in the 11- and 19-week-old prostates from the TRAMP mice. Since these two time points correspond to the development
of mild hyperplasia [Gingrich et al., 1996], we speculate that E2A may be accountable for the development of PIN, which may lead to the development of prostate cancer. The development of prostate cancer involves a multi-step process [Carter et al., 1990]. Therefore, the E2A protein may play a critical role in the progression of PIN to prostate cancer. Our results demonstrate that when E2A is absent following the development of severe hyperplasia in the TRAMP prostate at 25 weeks of age, this could indeed trigger prostate cancer initiation in the TRAMP mice. Table II summarizes the NMPs identified in this study that have similar molecular weights and isoelectric points with the previously identified proteins [Getzenberg et al., 1991; Partin et al., 1993] in the rat and human prostate cancer.

The differences in NMPs among the different time points in these TRAMP prostates could be demonstrative of cell/tissue transformation, which may have occurred during tumor progression. Three NMPs (E1A, E1B, and E1C) were present at puberty (6-week time point); these proteins were still present until the development of severe hyperplasia at 25 weeks, but absent as the mice developed neoplasia at 31 and 37 weeks. It is possible that the absence of these proteins may account for transformation of the prostatic cells. The absence of these proteins at later time points may cause the prostatic glands to become poorly differentiated. Similarly, the presence of E3A–E3E proteins during neoplasia at 31 and 37 weeks may further maintain the transformed phenotype of the prostatic cells. The presence or absence of different NMPs at early and later time points may be representative of specific proteins that are altered with the physiologic state of the cells during transformation. Our findings, taken as a whole, suggest that protein composition of the TRAMP prostates at different time points may provide insight into the etiology of prostate cancer and may provide biological markers of growth and gene expression in the prostate. Future investigations that include sequencing of these NMPs must still be established to further examine the potential role of these proteins in prostate cancer progression.

Differences in NMP composition among these prostate tissues at different time points may also correlate with alteration in nuclear morphology/DNA organization during the development of this disease. In the TRAMP model, the rat probasin gene is used to target expression of SV40 large T antigen to the epithelium of the mouse prostate [Greenberg et al., 1995]. Since the NM plays an important role in DNA organization and it is a target for transformation protein such as large T antigen [Getzenberg et al., 1990], it is possible that cellular transformation by SV40 large T antigen results in alteration of nuclear morphology, which in turn causes the transformed cells to express different NMP composition.

In summary, this study examined and characterized alterations in NMP composition, which were found during the progression from normal to prostate cancer in the TRAMP model. We identified a total of 13 proteins from the comparison of NM composition in the TRAMP prostate at six different time points. Identification of these NMPs will further enhance our understanding of the molecular changes associated with prostate cancer in humans. For the first time, these findings will potentially allow the development of 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. Future studies including sequencing the NMP, as well as raising antibodies to detect the presence of these proteins in

**TABLE II. Comparison of the TRAMP NMPs With the Previously Identified NMPs From Rat and Human Prostate Cancer**

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<th>TRAMP NMPs</th>
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<td>E1C (MW 40 kDa, pI 5.6); present in 6-week time point</td>
<td>D2 (MW 40 kDa, pI 5.91); Dunning rat prostate adenocarcinoma NMP; Getzenberg et al. [1991]</td>
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<td>E2A (MW 42 kDa, pI 5.6); present in 11- and 19-week time points</td>
<td>BFC1 (MW 42.5 kDa, pI 5.8) and BPC2 (MW 42 kDa, pI 5.73); human BPH and prostate cancer NMPs; Partin et al. [1993]</td>
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<td>Le2 (MW 32 kDa, pI 5.2–5.4); highest expression in 6-, 11-, 19-, and 25-week time points</td>
<td>NDP-10 (MW 32.5 kDa, pI 5.46); normal dorsal rat protein NMP; Getzenberg et al. [1991]</td>
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prostate cancer tissues in cancer therapy are clearly warranted. This information will then be utilized to clone the potential target genes encoding these proteins and to further examine their role in prostate cancer.

REFERENCES


