Nuclear Matrix Localization of High Mobility Group Protein I(Y) in a Transgenic Mouse Model for Prostate Cancer

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Abstract   Nuclear shape and the underlying nuclear structure, the nuclear matrix in cancer cells. Since the NM composition is considered to maintain nuclear shape and architecture, nuclear matrix proteins (NMPs) may be involved in transformation. Our laboratory has recently characterized a subset of NMPs that are associated with prostate cancer development in the transgenic adenocarcinoma of mouse prostate (TRAMP) model. One of the identified NMPs, E3E, has a similar molecular weight (22 kDa) with a protein known as HMGI(Y). HMGI(Y) belongs to a group of non-histone and chromatin-associated proteins, high-mobility-group (HMG) proteins, and it has been shown to associate with the NM. HMGI(Y) has been reported to be elevated in different types of cancer including prostate cancer. In this study, we examined the expression of HMGI(Y) protein in the NMP composition of the TRAMP model during the progression from normal to neoplasia. The expression of HMGI(Y) in the NMP extracts of three prostatic epithelial cell lines derived from a 32-week TRAMP mouse: TRAMP-C1, TRAMP-C2, and TRAMP-C3 was also examined. Using both one-dimensional and high-resolution two-dimensional immunoblot analyses, we found that: (i) HMGI(Y) is a nuclear matrix protein expressed as two protein bands with MW of 22–24 kDa and (ii) HMGI(Y) expression is correlated with neoplastic and malignant properties in late stage TRAMP prostate tumors. Overall, these findings support the evidence that HMGI(Y) can be utilized as a marker and prognostic tool for prostate cancer.   J. Cell. Biochem. 88: 599–608, 2003.   © 2003 Wiley-Liss, Inc.

Key words: TRAMP; HMGI(Y); nuclear matrix; prostate cancer

One of the cellular hallmarks of the transformed phenotype is 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. The nuclear matrix (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]. As the dynamic scaffolding of the nucleus, composition of the NM is different between normal and transformed cells [Getzenberg, 1994]. Since the NM composition is considered to maintain the nuclear shape and architecture, nuclear matrix proteins (NMPs) may be involved in transformation for different types of cancer.

One central function of the NM is its role in DNA organization and transcription. 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 to the NM. These MARs or SARs are about 200 bp in length, and are typically, although not always, AT and TG rich regions. Several proteins have been demonstrated to associate with MARs [reviewed in Leman and Getzenberg, 2002]. A group of non-histone and chromatin-associated proteins, high-mobility-group (HMG) proteins, has been shown to associate with MARs in the nuclear matrix [Zhao et al., 1993; Saitoh and Laemmli, 1994; Martelli et al., 1998]. One of these HMG proteins, HMGI(Y) [MW 22 kDa], has been proposed as a candidate protein for its involvement in chromosomal rearrangements in prostate cancer cell lines [Takaha et al., 2002]. The HMGI(Y) protein has been reported to induce changes in DNA structure and architecture by binding to the AT rich regions in the minor groove of the DNA and therefore organizes the DNA regions associated with different genes as frameworks for transcription factors [Nissen and Reeves, 1995]. Over expression of HMGI(Y) has been shown in cells transformed by viral oncogenes, as well as in both chemically and virally induced tumors [reviewed in Wunderlich and Bottger, 1997]. Increased expression of HMGI(Y) has also been shown in different types of neoplasias including breast [Liu et al., 1999], colon [Abe et al., 1999], pancreatic duct cell carcinoma [Abe et al., 2000] and prostate [Takaha et al., 2002].

A transgenic mouse model for prostate cancer, transgenic adenocarcinoma of mouse prostate (TRAMP) was developed by Dr. Norman Greenberg in 1995. 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) of the epithelium of the mouse prostate [Greenberg et al., 1995]. The TRAMP model reproduces the spectrum of benign latent, aggressive and metastatic forms of prostate cancer. The TRAMP males 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 has recently characterized changes in NMPs that are associated with prostate cancer development in the TRAMP model [Leman et al., 2002]. One of the identified NMPs, E3E (MW 24 kDa, pI 5.7), was found to be present only in the TRAMP males that developed neoplasia in the prostate (31- and 37-weeks old) [Leman et al., 2002]. Since the E3E protein has similar molecular weight to HMGI(Y), we propose that this protein may indeed be HMGI(Y). Therefore, the major goal of this work was to test if the E3E protein is actually HMGI(Y). In this study, the expression of HMGI(Y) protein in the NMP composition of the TRAMP prostate tissues during the progression from normal to neoplasia was examined. HMGI(Y) was found to be expressed in the prostate tumor at 30 weeks of age and that its expression was increased in the 35–40 weeks old TRAMP prostates. The expression of HMGI(Y) in three prostatic epithelial cell lines (TRAMP-C1, TRAMP-C2, and TRAMP-C3) derived from a 32-week tumor of the TRAMP mouse was also examined. Immunoblot analyses revealed that HMGI(Y) was expressed in all cell lines at different levels, corresponding to their growth and tumorigenic properties. These findings suggest that increased expression of HMGI(Y) is correlated with neoplastic transformation and is found in the late stage tumor of prostate cancer, and therefore can be utilized as a marker and prognostic tool for prostate cancer.

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 various time points that correspond to puberty (6–8 weeks), mild hyperplasia (11–19 weeks), progression to severe hyperplasia (25 weeks), and development of neoplasia (31–40 weeks) [Gingrich et al., 1996].

Cell Culture

TRAMP-C1, TRAMP-C2, and TRAMP-C3 cell lines were grown in DMEM media (Life

Technologies, Grand Island, NY) containing 10% Fetal Calf Serum (Life Technologies), 5 mg/ml insulin (Sigma Chemical Co., St. Louis, MO), 25 U/ml of penicillin-streptomycin (Life Technologies), and 10 nM dihydrotestosterone (Sigma). LNCaP prostate cancer cells were grown in RPMI media (Mediatech) containing 10% FCS (Life Technologies) and 25 U/ml of penicillin-streptomycin (Life Technologies). The normal human prostate epithelial cell line 267B-1 was grown in tissue culture flasks coated with FNC coating mix (BRFF, Inc., Ijamsville, MD) and maintained in serum-free P4-8F cultured medium (BRFF, Inc.). All the cells were grown at $37°C$ in a humidified incubator with 5% CO_2.

Nuclear Matrix Protein Isolation

Nuclear matrix proteins (NMPs) were isolated from the harvested mice prostatic tissues and the cell lines according to the method described by Getzenberg et al. [1991]. Protein concentration was determined using the Coomassie Plus protein assay reagent kit (Pierce, Rockford, IL) with bovine serum albumin as standard. For one-dimensional gel electrophoresis, the NMPs were dissolved in $1 \times$ PBS. For two-dimensional gel electrophoresis, the 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.

One-Dimensional Immunoblot

Fifty microgram of NMPs were loaded and separated by 13% SDS/PAGE along with a separate lane containing 10 μl of Rainbow Markers (Amersham Life Sciences, Arlington Heights, IL). Proteins were then transferred to 0.22 μM nitrocellulose membranes (Bio-Rad, Richmond, CA) utilizing a semi-dry transfer apparatus (Bio-Rad). The membranes were incubated overnight in 5% non-fat dry milk in Phosphate Buffered Saline (PBS) with 0.2% Tween-20. The membranes were then incubated with 1:100 dilution of goat polyclonal anti-HMGI(Y) antibody (N19; St. Cruz Biotechnology, St. Cruz, CA) in PBS with 5% non-fat dry milk and 0.2% Tween-20 at room temperature for 1 h. The membranes were then washed for three times (10 min for each wash) with PBS and 0.2% Tween-20 and incubated for 1 h with horseradish peroxidase-conjugated donkey anti

goat IgG (St. Cruz Biotechnology) at a dilution of 1:10,000 at room temperature. The membranes were then washed again with PBS and 0.2% Tween-20 (three 10-min washes, followed by three quick washes and two 10-min washes). To control protein loading, all the membranes were also probed with mouse monoclonal anti-lamins A/C antibody (St. Cruz Biotechnology) at a dilution of 1:500 and then with anti-mouse antibody conjugated with horseradish peroxidase (Amersham Life Sciences, Arlington Heights, IL) at a dilution of 1:5,000 at room temperature. Proteins were detected by a chemiluminescence reaction using the ECL Plus chemiluminescence kit (Amersham Life Sciences). To quantitate the HMGI(Y) protein, the bands were analyzed densitometrically by FX-Phosphoimager Analysis (Quantity One) and normalized with the lamin bands. Statistical analysis were performed with two-way Anova an significant values were indicated with $P < 0.05$.

Two-Dimensional Immunoblot

A high-resolution two-dimensional electrophoresis was performed to separate the extracted NMPs using a 2-D gel system as described previously [Getzenberg et al., 1991; Leman et al., 2002]. One hundred micrograms of NMPs were loaded onto each tube gel ($1 mm \times 18$ 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 and then transferred to 0.22 μM nitrocellulose membranes (Bio-Rad). Thereafter, the same procedure described above for the one-dimensional immunoblot was performed for the two-dimensional immunoblot analysis.

Immunofluorescence

Briefly, the cells were plated into four-well chamber slides (Falcon) at 1,000 cells/well. The cells were grown overnight and then fixed with 4% parafolmadehyde (Sigma) and blocked with blocking solution ($1 \times$ PBS with 0.2% Tween-20, 5% FBS, and 2% BSA) for 1 h at room temperature. The cells were then incubated with either goat polyclonal anti-HMGI(Y) antibody (St. Cruz Biotechnology) at a dilution of 1:100 or with $1 \times$ PBS as negative controls for 1 h at room

temperature. The cells were then washed with $1 \times$ PBS and 0.2% Tween-20 for four times (5 min each wash), followed by incubation with fluorescein-conjugated donkey anti-goat IgG (St. Cruz) at a dilution of 1:200 for 1 h at room temperature. The cells were washed again with $1 \times$ PBS and 0.2% Tween-20 for six times (5 min each wash) and then counterstained with Hoechst for nuclei staining. The cells were then observed and photographed with a fluorescence microscope equipped with live camera (Zeiss) at $40 \times$ magnification.

RESULTS

Previously, we identified a subset of NMPs associated with prostate cancer development in the TRAMP model [Leman et al., 2002]. One of the identified NMPs, E3E (MW 24 kDa, pI 5.7), was expressed only in the 31- and 37-weeks old TRAMP prostates (during development of neoplasia). Since the E3E protein appears to have similar molecular weight to HMGI(Y), we propose that this protein may indeed be HMGI(Y). In this study, the expression of HMGI(Y) protein in the NMPs isolated from TRAMP prostate tissues at various time points that correspond to the progression from normal state to neoplasia was examined. These time points correspond to puberty (6–8 weeks), mild

hyperplasia (11 and 19 weeks), progression to severe hyperplasia (25 weeks) and development of neoplasia (31–40 weeks) [Gingrich et al., 1996]. Immunoblot analyses of the NMPs from the TRAMP prostates (Fig. 1) show that HMGI(Y) is expressed during the development of neoplasia (30–40 weeks). HMGI(Y) is detected as two protein bands with MW of 22–24 kDa in the nuclear matrix. The presence of HMGI(Y) indicated by these bands at 22–24 kDa was not detected in the TRAMP prostate tissues at earlier time points (6–25 weeks), which represent a normal state to severe hyperplasia. Similarly, the HMGI(Y) bands at 22–24 kDa were not detected in the control non-transgenic mouse prostates from 6 to 49 weeks of age. However, a protein band was present at a lower molecular weight (~ 20 kDa) in the TRAMP prostate tissues at earlier time points (6–25 weeks), and this band was also detected in the control non-transgenic mouse prostates from 6 to 49 weeks old.

Since HMGI(Y) has been reported as a nuclear matrix protein in human prostate cancer cell lines (LNCaP, DU145, and PC3) [Takaha et al., 2002], we also checked the presence of this protein by performing immunoblots on the extracted NMPs that were separated on high-resolution two-dimensional electrophoresis. As shown in Figure 2, immunoblot analyses of the
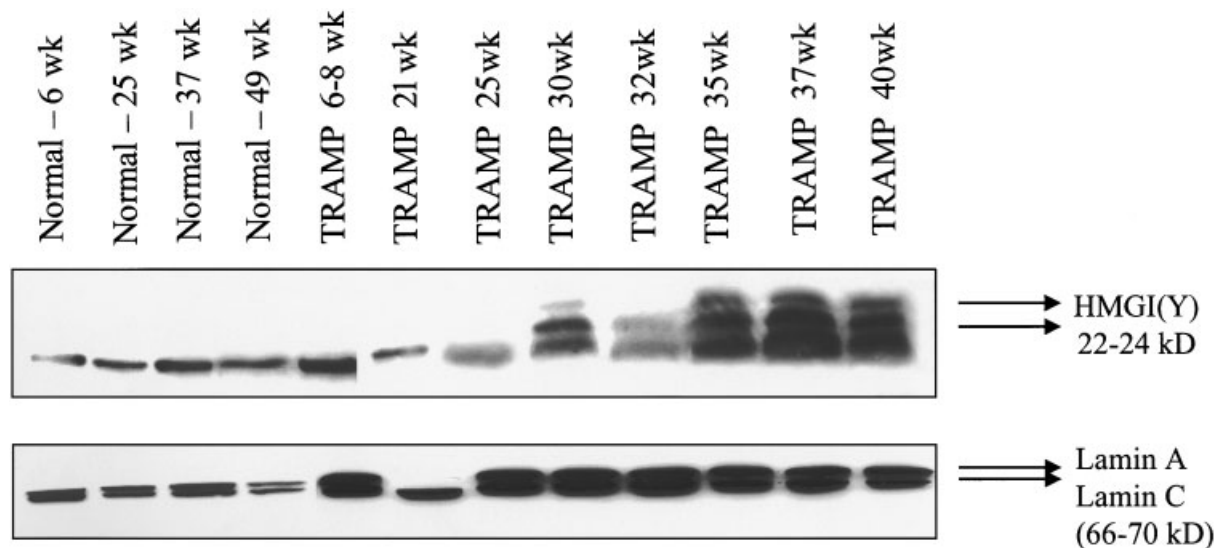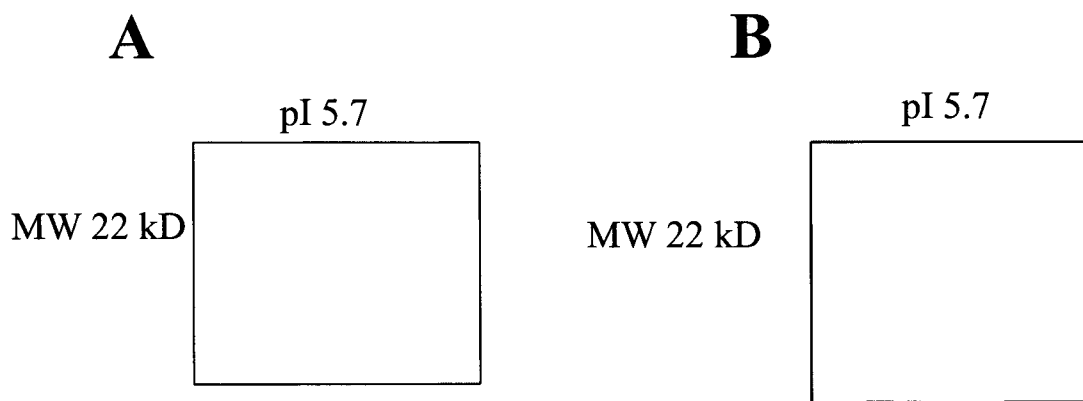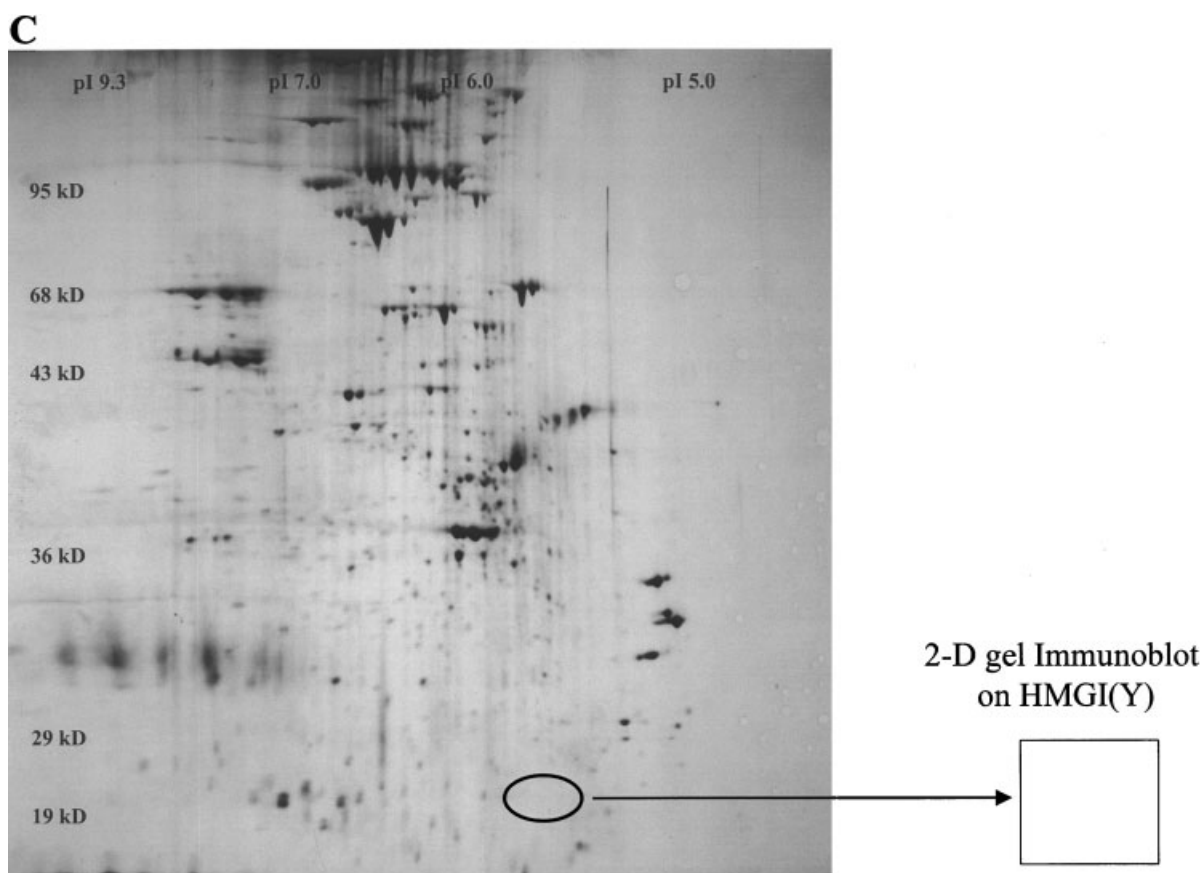

Fig. 1. One-dimensional immunoblot analysis for HMGI(Y) from the nuclear matrix extracts of TRAMP prostate tissues at different time points (6–40 weeks of age). These time points correspond to progression of prostate in the normal state to development of neoplasia in the TRAMP model. The presence of HMGI(Y) was detected by the goat polyclonal anti-HMGI(Y) antibody at 22–24 kDa. As a comparison, prostate tissues from normal non-transgenic mice at various time points (6–37-weeks old) were used as control groups.

A

pI 5.7

MW 22 kD

B

pI 5.7

MW 22 kD

2-D gel Immunoblot
on HMGI(Y)

C

pI 9.3   pI 7.0   pI 6.0   pI 5.0

95 kD

68 kD

43 kD

36 kD

29 kD

19 kD

2-D gel Immunoblot
on HMGI(Y)

Silver stain 2-D gel on TRAMP prostate NMP at 19-week

Fig. 2.   Two-dimensional immunoblot analysis for HMGI(Y) from the nuclear matrix extracts of TRAMP prostate tissues at: (**A**) 11-weeks old (mild hyperplasia), (**B**) 19-weeks old (mild hyperplasia), (**C**) 25-weeks old (severe hyperplasia), and (**D**) 31-weeks old (neoplasia). The presence of HMGI(Y) was only detected in the 31-weeks old TRAMP prostate (MW 22 kDa, pI 5.7).

D

2-D gel Immunoblot on HMGI(Y)
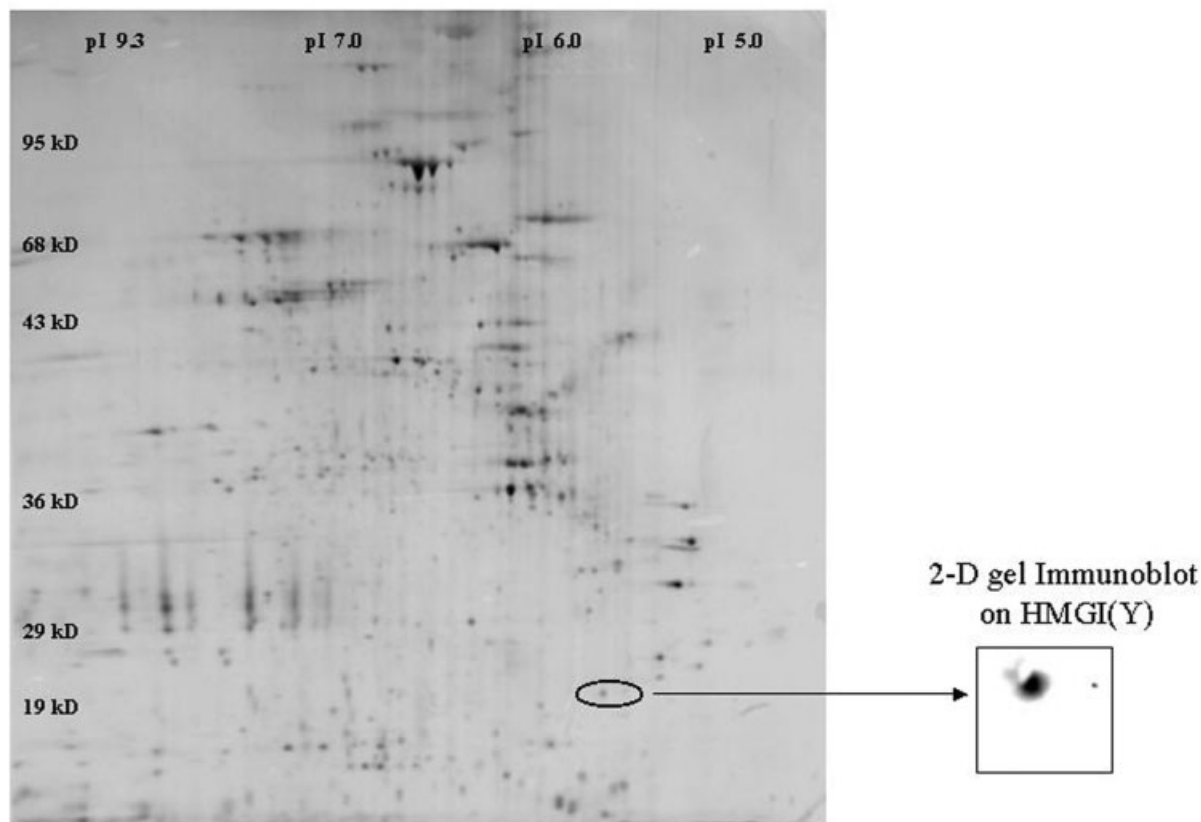
Silver stain 2-D gel on TRAMP prostate NMP at 31-week

Fig. 2. (*Continued*)

31-week TRAMP NMPs separated on two-dimensional gel electrophoresis confirmed the presence of HMGI(Y) protein at a MW of 22 kDa and isoelectric point of 5.7. The immunoblot analysis on the two-dimensional electrophoresis of 19-weeks old TRAMP prostate did not detect the presence of HMGI(Y) at this time point. Two-dimensional immunoblot analyses on TRAMP NMPs at 11- and 25-weeks old prostates were performed, and similarly, HMGI(Y) was not detected at these earlier time points. Therefore, the two-dimensional immunoblot analyses reveal that HMGI(Y) is indeed a nuclear matrix protein and they confirm the presence of HMGI(Y) in the TRAMP prostate tumor at a later time point during prostate cancer development.

The presence of HMGI(Y) on prostate epithelial cell lines derived from the TRAMP model was also examined. Three prostate epithelial cell lines derived from a single 32-week-old TRAMP mouse: TRAMP-C1, TRAMP-C2, and TRAMP-C3 were compared. Both TRAMP-C1 and TRAMP-C2 cells are reported to be tumorigenic in vivo, whereas TRAMP-C3 is not [Foster et al., 1997]. TRAMP-C1 is also reported to have the fastest growth rate in vitro and in vivo, whereas TRAMP-C3 has the slowest growth rate in vitro [Foster et al., 1997]. Figure 3 shows the immunoblot analyses of HMGI(Y) in the NMPs extracted from the TRAMP-C1, TRAMP-C2, and TRAMP-C3 cells. The NMPs extracted from all TRAMP cell lines appear to express HMGI(Y), but the HMGI(Y) expression is slightly higher than that in TRAMP-C2 and TRAMP-C3 cell lines ($P < 0.05$). Since the human prostate cancer cell line LNCaP has been reported to express HMGI(Y) [Takaha et al., 2002], this cell line was used as a positive control for HMGI(Y). Our immunoblot analysis on the cell lines also showed that HMGI(Y) was not detected in the normal prostate cell line 267B1, thus confirming our immunoblot analysis on the TRAMP prostate tissues. Since HMGI(Y) is a nuclear protein, localization of this protein to the nucleus was demonstrated by
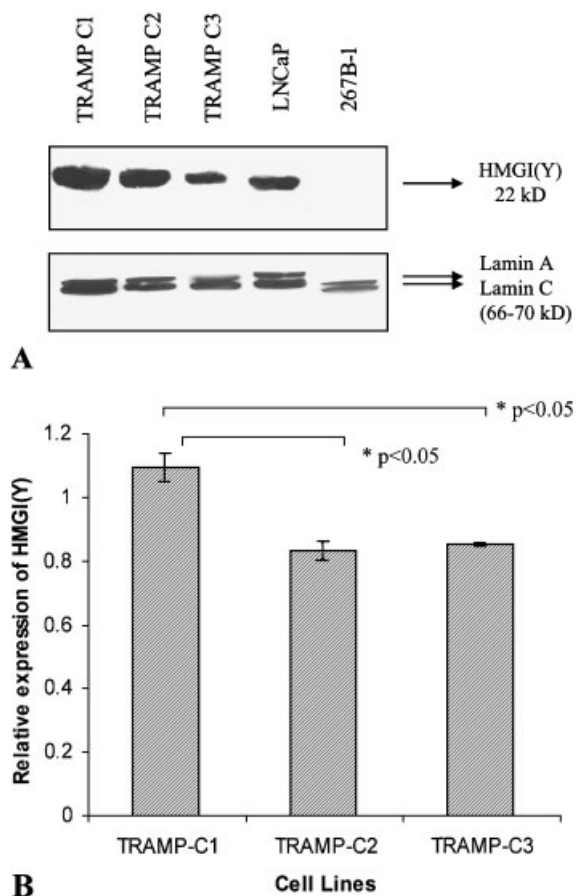
Fig. 3. A: Representative of one-dimensional immunoblot analysis for HMGI(Y) from the nuclear matrix extracts of the prostate epithelial cell lines (TRAMP-C1, TRAMP-C2 and TRAMP-C3) derived from a single 32-week TRAMP male. NMP extract from the human prostate cancer cell line LNCaP was used as a positive control, whereas NMP extract from the normal human prostate cell line 267B-1was used as a negative control. The presence of HMGI(Y) was detected by the goat polyclonal anti-HMGI(Y) antibody at 22 kDa. **B**: Densitometry analysis (n = 3) of the relative amount of HMGI(Y) protein normalized to lamins A and **C** in the TRAMP-C1, TRAMP-C2 and TRAMP-C3 cell lines. Error bars represent standard error mean.

immunofluorescence in these TRAMP cell lines (Fig. 4). However, there was no difference in the intensity of the nuclear staining among these cell lines. We reason that since the immunoblot analyses were performed on the extracted NMPs, the difference in the HMGI(Y) expression was more readily detected by immunoblot analysis rather than the immunofluorescence.

DISCUSSION

Changes in the NMP composition associated with prostate cancer development in the TRAMP model have recently been examined by

identifying differences in the NMP composition [Leman et al., 2002]. In the present study, we examined the expression of high mobility group protein I(Y) [HMGI(Y)] in the TRAMP prostate tissues at different stages during the development of the disease. The TRAMP model developed by Dr. Norman Greenberg in 1995 is genetically predisposed to prostate cancer and this model mimics the progression of prostate cancer in humans. It is well characterized that the TRAMP males develop histological PIN by 8–12 weeks of age that later progress to adenocarcinoma by 24–30 weeks of age [Gingrich et al., 1996].

High expression of the HMGI(Y) protein has been reported during embryonic development, whereas little or no detectable level of HMGI(Y) is reported in normal adult tissues or untransformed cells [reviewed in Wunderlich and Bottger, 1997]. However, increased expression of HMGI(Y) has been reported in transformed cells [Giancotti et al., 1985, 1987], as well as in different types of cancer including breast [Liu et al., 1999], colon [Abe et al., 1999], pancreatic [Abe et al., 2000], and prostate [Takaha et al., 2002]. We analyzed the expression of HMGI(Y) protein in the nuclear matrix extracts of the TRAMP prostates at various time points. These time points represent different states of the prostate during neoplastic transformation: puberty (6–8 weeks), mild hyperplasia (11–19 weeks), severe hyperplasia (25 weeks), and development of neoplasia (31 weeks and above). We found that HMGI(Y) was expressed during neoplastic state (30, 32, 35, 37, and 40 weeks), whereas HMGI(Y) was not detected during early stages of the disease (6–8 weeks, 21 and 25 weeks). HMGI(Y) was also not detected in the prostate tissues from the control non-transgenic mice at 6, 35, 37, and 49 weeks of age. These results support the concept reported by other laboratories [Bussemakers et al., 1991; Ram et al., 1993; Fedele et al., 1996], that the level of HMGI(Y) expression is correlated with the degree of neoplastic transformation in cancer development.

One-dimensional immunoblot analyses show the presence of HMGI(Y) as two protein bands with MW of 22–24 kDa. Recently, investigations by Coffey's laboratory [Takaha et al., 2002] revealed that HMGI(Y) was identified in human prostate cancer cell lines LNCaP, DU145, and PC3 at a MW of 22 kDa. Since these results indicate the presence of HMGI(Y) as two bands,
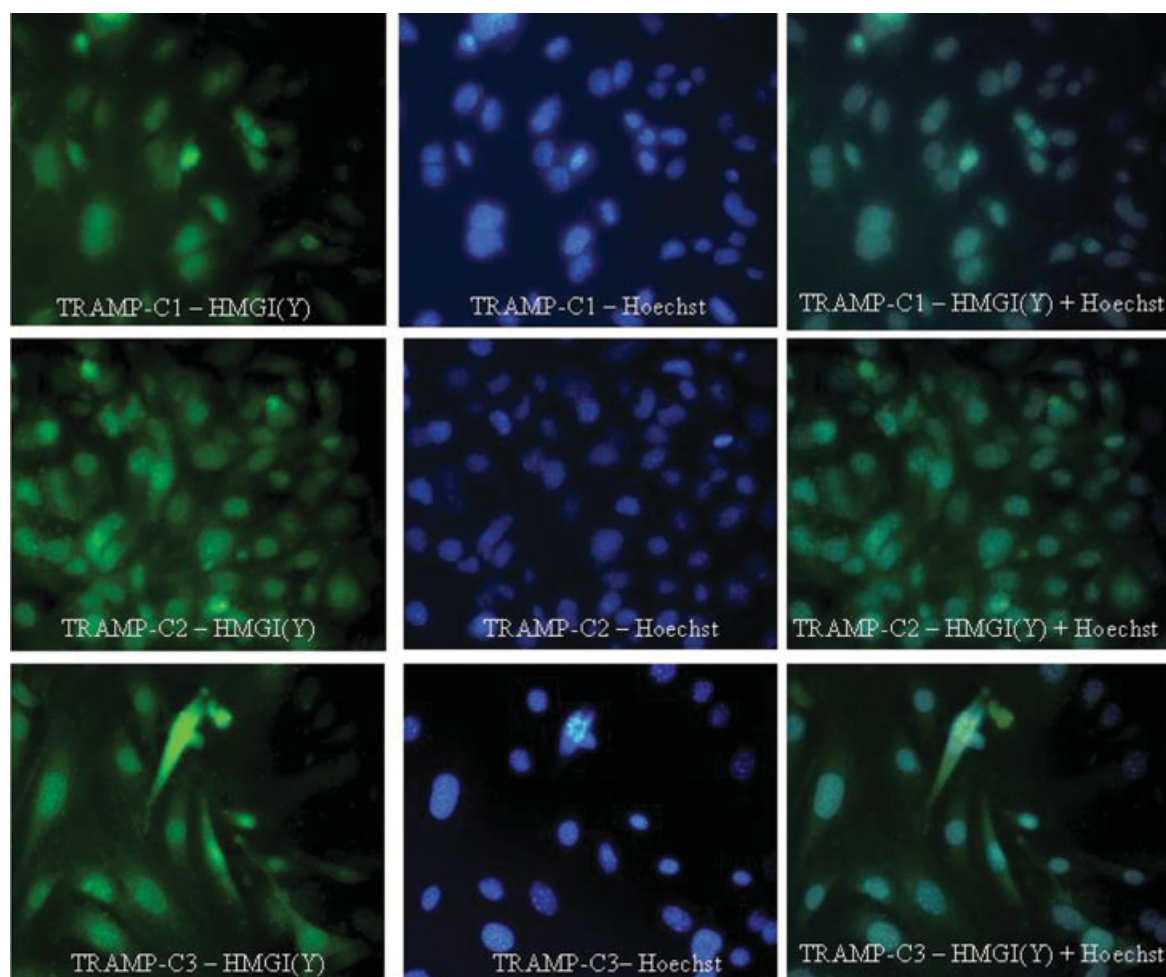
Fig. 4. Localization of HMGI(Y) in the nuclei of TRAMP-C1, TRAMP-C2, and TRAMP-C3 cell lines was examined by immunofluorescence analysis. The cells' nuclei were also counterstained with Hoechst and the HMGI(Y) staining was then superimposed with the Hoechst staining to further indicate the presence of HMGI(Y) in the nuclei.

we speculate that the presence of an additional band of 24 kDa observed in the TRAMP prostate tissues at 30–40 weeks could be a phosphorylated form of HMGI(Y) protein. Permanent phosphorylation of HMGI(Y) has been reported by Giancotti et al. [1993] and phosphorylation of this protein was found to be at higher levels in tumor tissues [Giancotti et al., 1993]. It is possible that the TRAMP prostate tumors at later time points also express higher phosphorylated forms of HMGI(Y) and this phosphorylated HMGI(Y) is maintained at the late stage tumor. Thus, phosphorylated HMGI(Y) may be used as potential marker or prognostic factor in prostate cancer. Further investigations on the role of phosphorylation on HMGI(Y) protein must still be established to further examine the potential role of this protein in prostate cancer development.

The presence of HMGI(Y) as double bands in our studies also raises another speculation that HMGI(Y) protein represents two isoforms: HMG-I and HMG-Y isoforms. It has been reported that HMG-I and HMG-Y are splice variants of the same gene located on human chromosome 6p21 [Johnson et al., 1989], and together, these proteins are referred to as HMGI(Y) [Liu et al., 1999; Takaha et al., 2002]. The presence of HMGI(Y) as a doublet on SDS−PAGE has also been reported in highly metastatic breast carcinoma cell lines, but almost not detectable in non-metastatic breast cancer cell lines [Liu et al., 1999]. Taken together, it is possible that the double bands observed in the TRAMP prostate tumor at 31–40 weeks of age could be representative of two splice variants of HMGI(Y) protein and that HMGI(Y) doublets could be related to metastatic phenotype.

It is interesting to note that a protein band was present at a lower molecular weight (∼20 kDa) in the TRAMP prostate tissues at earlier time points (6–25 weeks), and this band was also detected in the control non-transgenic mouse prostates from 6 to 49 weeks old. However, this protein band was not detected in the TRAMP prostate tissues at later time points (30–40 weeks). Since this protein has a very close molecular weight to the reported molecular weight of HMGI(Y), we speculate that this protein could also be related to HMGI(Y). This protein could be another splice variant of HMGI(Y). Since microscopic morphology on the TRAMP prostate tissues demonstrated changes in the prostatic glands from normal well-differentiated (9–13-weeks old) to moderately and poorly differentiated (25–37-weeks old) [Leman et al., 2002], we speculate that this protein could be indicative or a marker of changes in the prostatie gland as the gland undergoes changes from normal to neoplastic state. Further investigations on this protein as another possible HMGI(Y) splice variants and whether it could be related to changes in the prostatic gland during neoplastic transformation must still be established in order to ascertain the possible involvement of this protein in prostate cancer progression in the TRAMP model.

Investigations by Coffey's laboratory [Takaha et al., 2002] also revealed that the HMGI(Y) protein is a nuclear matrix protein. Our one-dimensional immunoblot analyses showed that HMGI(Y) was detected in the NMP extracts from the TRAMP prostate tissues the age of at 31–40 weeks. To further confirm that HMGI(Y) protein was present in the NMP extracts from the TRAMP prostate tissues at later time points, we performed two-dimensional immunoblots on TRAMP NMPs at 11-, 19-, 25-, and 31-week time points. The two-dimensional immunoblot analyses demonstrated that HMGI(Y) was indeed a nuclear matrix protein (MW 24 kDa, pI 5.7) and that this protein was only detectable in the 31-week TRAMP prostate, but not in the earlier time points (11, 19, and 25 weeks). We have recently characterized a subset of NMPs associated with prostate cancer development in the TRAMP model [Leman et al., 2002]. One protein, E3E (MW 24 kDa, pI 5.7) was identified and this protein was only present in the 31- and 37-weeks old TRAMP prostate tissues. This E3E protein appears to have similar molecular weight and isoelectric point as HMGI(Y) and it is only expressed in the TRAMP prostates at later time points (30–40-weeks old). Therefore, it is possible that E3E protein could be the HMGI(Y) protein. Sequencing of the E3E protein is currently underway and the results will further test the similarity between E3E and HMGI(Y) proteins.

The expression of HMGI(Y) in three prostatic epithelial cell lines derived from the TRAMP model: TRAMP-C1, TRAMP-C2, and TRAMP-C3 was also examined. These cell lines were established from a prostate tumor of a single 32-weeks old TRAMP prostate [Foster et al., 1997]. We found that HMGI(Y) was expressed in all TRAMP cell lines. However, the TRAMP-C1 cell line, which was reported to be tumorigenic in vivo and grew the fastest in vitro and in vivo [Foster et al., 1997], expressed a higher level of HMGI(Y) compared to TRAMP-C2 cell line, which was also reported to be tumorigenic in vivo, and to TRAMP-C3 cell line, which was reported to be non-tumorigenic in vivo and grew the slowest in vitro [Foster et al., 1997]. In addition, HMGI(Y) was not detected in normal human prostate cell line 267B1. Again, these results confirm the findings by other investigators [Abe et al., 1999, 2000; Liu et al., 1999], that HMGI(Y) protein is associated with neoplastic transformation and malignant phenotype in cancer cells. It is also interesting to note that HMGI(Y) in the cell lines was detected as a single band at 22 kDa, and not as doublets as in the case of TRAMP prostate tissues. This is probably due to the protein not being able to resolve into the doublets, and this phenomenon has been reported in the human prostate cancer cell lines [Takaha et al., 2002]. In summary, expression of HMGI(Y) protein in the TRAMP prostate tissues at various time points during prostate cancer development has been studied. These time points represent different states of the prostate during progression of the disease. The results presented in this manuscript show that: (i) HMGI(Y) is a nuclear matrix protein, (ii) HMGI(Y) is expressed as two protein bands with MW of 22–24 kDa and (iii) HMGI(Y) expression is correlated with neoplastic and malignant properties in TRAMP prostate tumors. Further investigations that include examining the mRNA levels of HMGI(Y) as well as characterizing the potential role of HMGI(Y) in the TRAMP model are clearly warranted.

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