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Review

Nuclear Matrix Protein Expression in Prostate Cancer: Possible Prognostic and Diagnostic Applications

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Abstract. *Different lines of evidence suggest that the nuclear matrix (NM), the protein scaffold of the nucleus, represents a functional unit playing a pivotal role in the spatial and temporal coordination of the events of gene activation. Any change in the gene expression pattern, which occurs during carcinogenesis, may partially depend on an impairment of the regulatory functions of the NM. Therefore, increasing interest has been addressed to the study of NM modifications associated with malignant transformations and to potential clinical applications. Here, recent results on the NM changes in prostate cancer are discussed. Tumor cells are characterized by a more complex NM protein pattern compared to normal tissue: the development of poorly-differentiated tumors is characterized by the expression of proteins that are not present in hyperplastic tissues or in more differentiated tumors. In addition, a few newly-expressed proteins are significantly correlated with the risk of biochemical progression. The potential application of these proteins at the diagnostic and prognostic levels calls for further studies.*

Nuclear matrix

The spatial organization of the nucleus is largely determined by a protein frame, termed the nuclear matrix (NM), first isolated by Berezney and Coffey in 1974 (1). The NM is composed of 2% DNA, 4% RNA and 94% proteins, which approximately corresponds to 10% of the nuclear proteins, and has been identified in a wide range of eucaryotes from yeast to man. The three main subdomains of the NM are: the peripheral lamins and pore complex, the residual nucleoli and an internal fibrogranular network called the internal nuclear matrix (INM). Since its isolation, it has been proposed that this subnuclear structure supports several fundamental functions: it determines nuclear morphology, directs the spatial organization of DNA transcription and replication, and organizes the regulatory mechanisms involved in gene expression and RNA synthesis. Specific transcription factors and some enzymes implicated in the modulation of the structure and transcriptional competence of chromatin (histone acetyltransferases, deacetylases and topoisomerases) have been co-isolated with the NM. The majority of its proteins are common components to all cell types, whereas some are tissue-specific and others are related to the stage of both differentiation and transformation (2-4).

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Nuclear matrix and chromatin

Inside the nucleus, DNA is organized in a hierarchy of structures, ranging from the double helix to the nucleosome, up to the condensed 30 nm chromatin fiber. The polynucleosomal chain is further folded into loop domains, with a size from 30 to 100 kb. A chromatin loop may contain one or several genes. At the base of the loop, there are sequences, called matrix or scaffold-attachment regions (M/SARs), that bind to proteins associated with the NM (5). The comparison of the DNA sequences of SARs shows that they do not share extensive homology, but are usually adenine/thymine-rich; therefore, SAR-DNA sequences have a high bending potential and may have a topological role. Chromatin loops containing expressed genes have an unfolded structure that is sensitive to DNase I digestion. The boundaries of the DNase I sensitive loop domain coincide with the position of SARs; moreover, these sequences, that in many cases contain recognition sequences for topoisomerases II, are placed close to the transcriptionally

active regions of chromatin so that transcription is initiated in the region of chromatin anchored to the NM (6). Thus, each chromosomal loop might represent an independent unit for the control of gene expression.

Using differential scanning calorimetry as a probe for the structure of chromatin, it has been shown that heterochromatin decondenses abruptly, according to an all-or-none mechanism, when two double-strand breaks are introduced into the loop (7). Therefore, chemical and structural changes in the NM might be directly involved in the modulation of the transition from "open" to "condensed" chromatin states, which in turn determines the passage from the transcriptionally competent to the incompetent state.

Cancer-associated nuclear matrix proteins

The stepwise development of cancer involves successive alterations of the nuclear morphology and of the amount and distribution of heterochromatin. For example, the most diagnostically important changes observable in all tumors are changes in the structure of the nucleus. The chromatin changes in carcinogenesis are related to alteration in the architecture of NM. Studies carried out in our and other laboratories have shown that: i) specific changes in NM proteins are observed during tumor progression (8-11) and ii) these changes are synchronous with large and definite rearrangements of the structure of heterochromatin, as directly shown by our previous investigation using the model of Solt and Farber for liver carcinogenesis in the rat (12).

As chromatin decondensation is believed to represent an early event in gene activation, alterations in the composition and organization of the NM could directly determine the onset of the changes in the gene expression pattern associated with the initiation of carcinogenesis. Thus, the study of the malignant alterations of the nuclear architecture could disclose key mechanistic aspects of cancer development.

Several reports from different laboratories have identified NM proteins whose expression is significantly related to the occurrence of various tumors. In every case, cancer development has been associated both with the expression of several new proteins and the cessation of the expression of others, as reported in Table I.

Some of these tumor-associated proteins are employed in clinical diagnosis or in preclinical studies. For instance, the use of the NMP22 protein, a fragment of the Nuclear Mitotic Apparatus protein (NuMA), has been approved by the Food and Drug Administration for monitoring the recurrence of transitional-cell carcinoma of the urinary tract (22, 23). Recently, it has been shown that urinary NMP22 is also correlated with the presence of renal cell carcinoma (24, 25). BLCA-4, a NM protein present only in bladder cancer, is a very sensitive and specific marker for this tumor (26) and it has been recently demonstrated that this protein is a member of the EST transcription factor family; in an animal model, its expression is one of the earliest changes related to bladder cancer (27). Finally, a preclinical feasibility study for the early detection of high- and low-grade cervical intraepithelial neoplasia has demonstrated that the NM protein identified by monoclonal antibody NM 179 may be a useful additional tool for the early detection of this lesion (28).

Prostate cancer-associated nuclear matrix proteins

In 1993, Partin *et al*. (20), for the first time, identified by molecular weight (Mw) and isoelectric point (pI) 14 different NM proteins, that were consistently present or absent among normal prostate (NP), benign prostatic hyperplasia (BPH) and prostatic cancer (PCa). One protein, called PC-1, with a Mw of 56,000 and pI 6.58, appeared in all NM preparations from PCa but was not detected either in NP or in BPH. The same researchers, some years later, developed a monoclonal antibody, PRO:4-216, against PC-1. Immunohistochemical analysis showed that the antibody was able to bind to a unique protein in 85% of the cancerous, 5% of the benign hyperplastic and 9% of the normal prostate tissues, respectively (29). Further work by Partin's group showed that the PRO:4-216 antibody actually recognized the B23/nucleophosmin protein, a nucleolar phosphoprotein, that has been found to be more abundant in tumor and proliferating cells than in normal resting cells (30). Lekshmanan *et al*. (31) further described the differential expression of NM protein in human prostate cancer tissues with different levels of aggressiveness. A specific protein (YL-1) appeared to be related to the pathological stage, suggesting that this species could represent a potential marker of poor prognosis for clinically localized prostate cancer.

In 1996, our group presented a preliminary characterization of the NM-IF (intermediate filaments) complex isolated from PCa from 10 patients undergoing radical retropubic prostectomy (32). More recently, this study was extended to 29 PCas with a different Gleason score (9). Extensive changes in the expression of both the NM and IF proteins were identified; they were, however, related in a different way to tumor progression. Poorly-differentiated PCa (Gleason score 8-9) showed a strong down-regulation of several constitutive cytokeratins (CKs 8, 18 and 19); their expression significantly decreased with respect to both NP and BPH and, more interestingly, also with respect to moderately- and well-differentiated tumors (Gleason score 6-7, Gleason score 4-5, respectively). At variance with these continuing alterations in expression, the NM proteins underwent stepwise changes related to the level of differentiation, as already observed during the

Table I. *Cancer-associated nuclear matrix proteins.*

Tissue type	NM proteins		References
	Normal[1]	Cancer[2]	
Bladder	BLNL 1-3	BLCA 1-6	(13)
	-	NMP22	(14)
Breast	NMNB A-B	NMBW-Z	(15)
	-	p114[3]	(16)
Cervix	-	CvC 1-5	(17)
Colon	NC 1-4	CC 1-6	(18)
	NC1-6	CC1-CC6 (a, b)	(11)
Head and Neck	N 12-15	C 1-11	(19)
Prostate	NP1-3	PC-1	(20)
	-	NM 6-7	(9)
Renal	RCNL-1	RCCA1-5	(21)

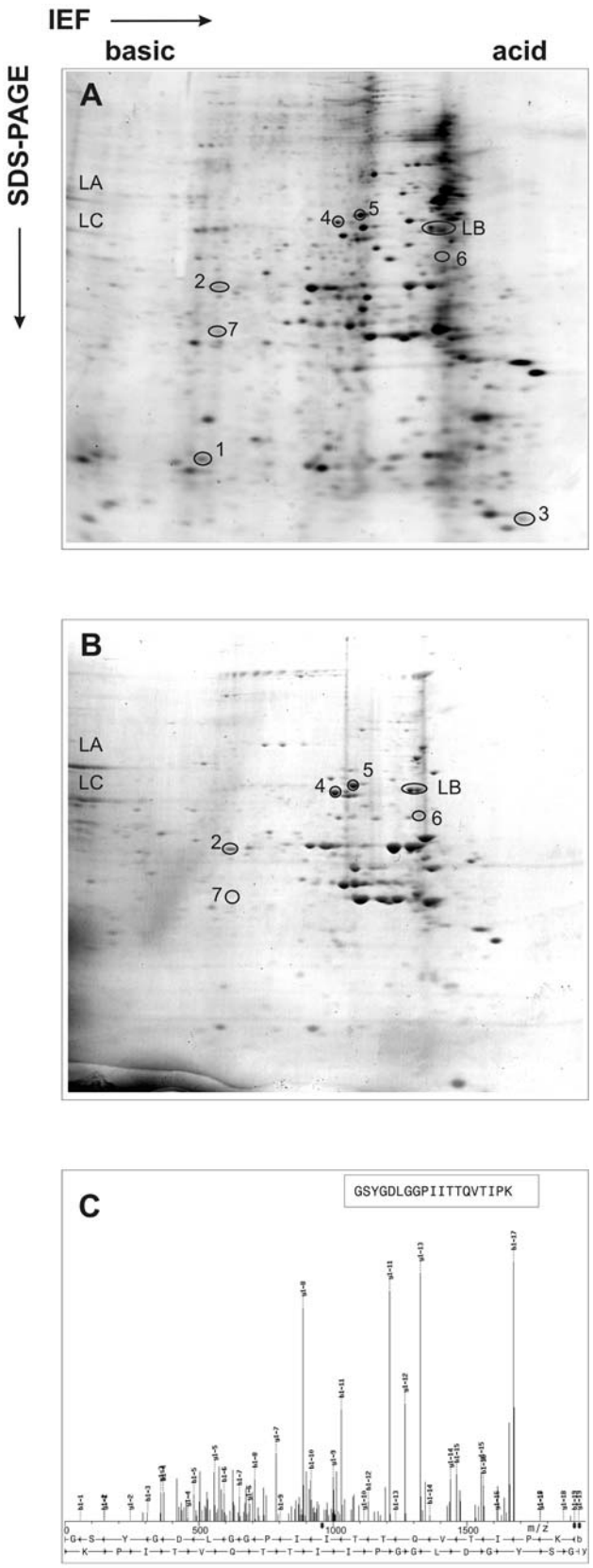
[1]NM proteins found in normal tissue only
[2]NM proteins found in cancer tissue only
[3]MAR-binding protein

transformation of hepatocytes in the resistant hepatocyte model of Solt and Farber (8). The development of less differentiated tumors is characterized by a decrease in the expression of a few proteins and by the stabilization of the expression of several components, some of which were already present in BPH or in tumors having a lower Gleason score. Our data, therefore, confirm previous results by Partin's group (20), in support of a model of tumor progression in which BPH shares some of the changes in the NM proteins which occur in PCa. We have extended this study to 75 patients, focusing only on the expression of 8 tumor-associated NM proteins (NM 1-8). NM proteins 6-8 were found to be strongly correlated with the Gleason score and nodal involvement. After a median follow-up time of 54 months, the best prognoses were for patients whose tumors expressed none or just one of proteins 6, 7 or 8, while the worst prognosis was associated with patients whose tumors expressed all 3 proteins together (33).

The search for prostate tumor markers with improved diagnostic and prognostic features has recently taken advantage of the development of more accurate and sensitive techniques for two-dimensional electrophoretic analysis, using immobilized pH gradients in combination with mass spectrometric sequencing, which often leads to the identification of protein samples directly excised from the gels (34). An example of the application of the latter procedure is given in Figure 1. In the two-dimensional electrophoretic pattern shown in (A), numbers mark 7 tumor-specific proteins, as already reported in Alberti *et al*. (9); the NM proteins were detected by the silver staining procedure of Henkestaven and Dernick (35). Another gel (B) of proteins extracted from the same tumor sample was run under comparable conditions and stained utilizing a very sensitive colloidal Coomassie G-250 staining compatible with mass spectrometry analysis (36). Spot number 6, after excision and trypsin digestion, was analyzed using an automated LCQ-DECA MS/MS ion trap mass spectrometer coupled to a HPLC Surveyor (Thermo Finnigan) equipped with a Hypersil BDS C_{18} column 1 X 100 mm (36). A representative fragmentation spectrum of 1 of the 4 peptides obtained is shown in (C). This result identifies NM-6 as hnRNP K. This is an interesting finding, since in breast cancer cells hnRNP K enhances cell proliferation and anchorage-independent growth (37). Moreover, it is active both at the chromatin level, binding directly to the promoter region of the human c-*myc* gene, and in cytoplasm inhibiting translation of specific RNAs (38). As a rule, hnRNPs have been found to be associated with actively proliferating cells (39) and it remains to be established whether this represents an intrinsic feature of transformation *per se*, or merely reflects the accelerated RNA metabolism.

Nuclear matrix and tumor biology: future perspectives

Although the NM was isolated more than 30 years ago (1) and its manifold functions in the regulation of nucleic acid metabolism and spatial organization of nucleic acid recognized early, the way in which different activities are bound to a scaffold structure in the native nucleus still remains a controversial point. For example, gene activation requires the unfolding of condensed chromatin domains (heterochromatin), which in turn depend on both post-translational modification of histones and rearrangements of the chromatin loops with respect to the scaffolding structure. In an extreme view, the existence of an internal NM has been disputed (40); alternatively, it has been referred to as an operationally-defined structure, that is confined to the realm of artifacts. The visualization of an intranuclear web of 10 nm fiber (core filaments), using resinless electron microscopy (41, 42), raised further biochemical and ultrastructural problems. Whether or not IF proteins were genuine components of the web, and the role of the hnRNA in the stabilization of the protein scaffold, have represented major controversial points. Although transcription factories (43) as well as hnRNP C1/2 and A (44) were found to be associated with core filaments, it cannot absolutely be ruled out that this finding depends on the occurrence of artifactual coprecipitation during the isolation of the NM.



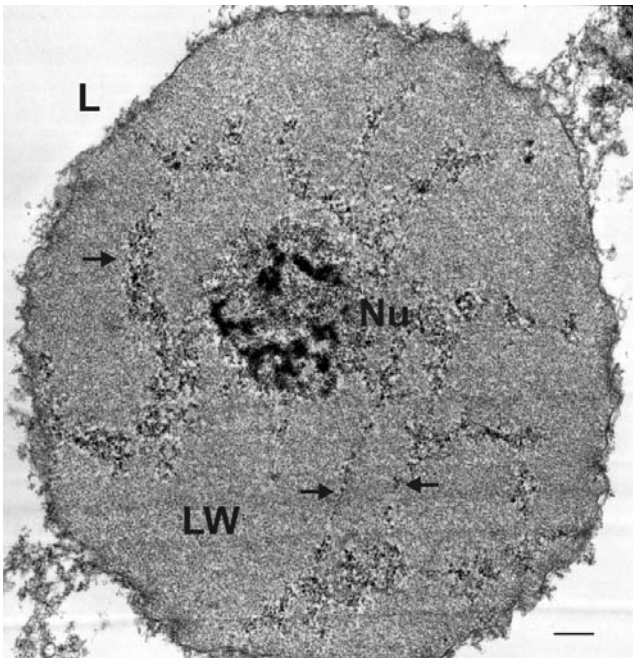


Figure 2. *A low magnification image of a well-preserved NM specimen isolated from rat hepatocytes in the presence of 2 mM vanadyl ribonucleoside complex. L, lamin; Nu, residual nucleolus; LW, lamin web. Several channels, connecting the residual nucleus with the lamin can be seen (arrows). The channels are filled with electron-dense material which has been found to be composed of NuMA and hnRNP by immunoelectron microscopic analysis. The bar is 0.4 μm.*

Only recently it was demonstrated that, when the NM is isolated under conditions in which the integrity of RNA is carefully controlled, it is possible to observe, in a reproducible fashion, the different subdomains which constitute the INM (45, 46). An example of a morphologically well-preserved specimen of NM from rat liver hepatocytes is shown in Figure 2. A diffuse web of lamin microfibrils (marked by LW) represents the basic scaffolding structure of the INM; several channels connecting the residual nucleolus with the nuclear lamin are well evidenced. Intrachromatin granules and perichromatin fibrils are confined to the channels together with a high

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Figure 1. *High resolution two-dimensional gel electrophoresis of the NM-IF complex prepared from a PCa specimen with Gleason score 9. The gels were stained with the sensitive silver stain method (A) to detected minor components and with colloidal Coomassie Blue G-250, which is compatible with proteoma analysis (B). The tumor-associated NM proteins are marked by circles and identified with the same labels used in (9). Lamins A, B and C are designated by LA, LB and LC. NM-6 was selected and analyzed with LC MS/MS. A representative fragment spectrum of one of the four peptides obtained after trypsin digestion is reported in (C). The amino acid sequence obtained is shown in the inset.*

fraction of NuMA. This superstructure undergoes definite morphological changes and loss of NuMA and hnRNP as a consequence of RNA digestion (45). Thus, this improvement in the isolation of native NM samples should allow, for the first time, examination, both at the biochemical and ultrastructural level, of NM-dependent changes in the pattern of gene expression during transformation, so providing empirical studies on the NM in transformed cells with a mechanistic frame.

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References

1 Berezney R and Coffey DS: Identification of a nuclear protein matrix. Biochem Biophys Res Commun *60*: 1410-1417, 1974.

2 Fey EG and Penman S: Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci USA *85*: 121-125, 1988.

3 Doworetzky SI, Fey EG, Penman S, Lian JB, Stein JL and Stein GS: Progressive changes in the protein composition of the nuclear matrix during rat osteoblast differentiation. Proc Natl Acad Sci USA *87*: 4605-4609, 1990.

4 Davido T and Getzenberg RH: Nuclear matrix proteins as cancer markers. J Cell Biochem *Suppl. 35*: 136-141, 2000.

5 Bode J, Schlake T, Rios-Ramirez M, Mielke C, Stengert M, Kay V and Klehr-Wirth D: Scaffold/matrix-attached regions: structural properties creating transcriptionally active loci. Int Rev Cytol *162A*: 389-454, 1995.

6 Boulikas T: Chromatin domains and prediction of MAR sequences. Int Rev Cytol *162A*: 279-389, 1995.

7 Balbi C, Sanna P, Barboro P, Alberti I, Barbesino M and Patrone E: Chromatin condensation is confined to the loop and involves an all-or-none structural change. Biophys J *77*: 2725-2735, 1999

8 Barboro P, Alberti I, Sanna P, Parodi S, Balbi C, Allera C and Patrone E: Changes in the cytoskeletal and nuclear matrix proteins in rat hepatocyte neoplastic nodules in their relation to the process of transformation. Exp Cell Res *225*: 315-327, 1996.

9 Alberti I, Barboro P, Barbesino M, Sanna P, Pisciotta L, Parodi S, Nicolo G, Boccardo F, Galli S, Patrone E and Balbi C: Changes in the expression of cytokeratins and nuclear matrix proteins are correlated with the level of differentiation in human prostate cancer. J Cell Biochem *79*: 471-485, 2000.

10 Spencer VA, Samuel SK and Davie JR: Altered profiles in nuclear matrix proteins associated with DNA *in situ* during progression of breast cancer cells. Cancer Res *61*: 1362-1366, 2001.

11 Brünagel G, Vietmeier BN, Bauer AJ, Schoen RE and Getzenberg RH: Identification of nuclear matrix protein alterations associated with human colon cancer. Cancer Res *62*: 2437-2442, 2002.

12 Barboro P, Pasini A, Parodi S, Balbi C, Cavazza B, Allera C, Lazzarini G and Patrone E: Chromatin changes in cell transformation: progressive unfolding of the higher-order structure during the evolution of rat hepatocyte nodules. A differential scanning calorimetry study. Biophys J *65*: 1690-1699, 1993.

13 Getzenberg RH, Konety BR, Oeler TA, Quigley MM, Hakam A, Becich MJ and Bahnson RR: Bladder cancer-associated nuclear matrix proteins. Cancer Res *156*: 1690-1694, 1996.

14 Zippe C, Pandrangi L and Agarwal A: NMP22 is a sensitive cost-effective test in patients at risk for bladder cancer. J Urol *161*: 62-65, 1999.

15 Khanuja PS, Lehr JE, Soule HD, Gehani SK, Noto AC, Choudhury S, Chen R and Pienta KJ: Nuclear matrix proteins in normal and breast cancer cells. Cancer Res *53*: 3394-3398, 1993.

16 Yanagisawa J, Ando J, Nakayama J, Kohwi Y and Kohwi-Shigematsu T: A matrix attachment region (MAR)-binding activity due to a p114 kilodalton protein is found only in human breast carcinomas and not in normal and benign breast disease tissues. Cancer Res *56*: 457-462, 1996.

17 Keesee SK, Marchese J, Meneses A, Potz D, Garcia-Cuellar C, Szaro RP, Solorza G, Osornio-Vargas A, Mohar A, de la Garza J G and Wu YJ: Human cervical cancer-associated nuclear matrix proteins. Exp Cell Res *244*: 14-25, 1998.

18 Keesee SK, Meneghini MD, Szaro RP and Wu YJ: Nuclear matrix proteins in human colon cancer. Proc Natl Acad Sci USA *91*: 1913-1916, 1994.

19 McCaffery JD, Gapany M, Faust RA, Davis AT, Adams GL and Ahmed K: Nuclear matrix proteins as malignant markers in squamous cell carcinoma of head and neck. Arch Otolaryngol Head Neck Surg *123*: 283-288, 1997.

20 Partin AW, Getzenberg RH, CarMichael MJ, Vindivich D, Yoo J, Epstein J and Coffey DS: Nuclear matrix protein patterns in human benign prostatic hyperplasia and prostate cancer. Cancer Res *53*: 744-746, 1993.

21 Konety BR, Nangia AK, Nguyen TT, Veitmeier BN, Dhir R, Acierno JS, Becich MJ, Hrebinko RL and Getzenberg RH: Identification of nuclear matrix protein alterations associated with renal cell carcinoma. J Urol *159*: 1359-1363, 1998.

22 Soloway MS, Briggman V, Carpinito GA, Chodak GW, Church PA, Lamm DL, Lange P, Messing E, Pasciak RM, Reservitz GB, Rukstalis DB, Sarosdy MF, Stadler WM, Thiel RP and Hayden CL: Use of a new tumor marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urol *156*: 363-367, 1996.

23 Miyanaga N, Akaza H, Ishikawa S, Ohtani M, Noguchi R, Kawai K, Koiso K, Kobayashi M, Koyama A and Takahashi T: Clinical evaluation of nuclear matrix protein 22 (NMP22) in urine as a novel marker for urothelial cancer. Eur Urol *31*: 163-168, 1997.

24 Huang S, Rhee E, Patel H, Park E and Kaswick J: Urinary NMP22 and renal cell carcinoma. Urology *55*: 227-230, 2000.

25 Ozer G, Altinel M, Kocak B, Yazicioglu A and Gonenc F: Value of urinary NMP22 in patient with renal cell carcinoma. Urology *60*: 593-597, 2002.

26 Konety BR, Nguyen TT, Dhir R, Day RS, Becich MJ, Stadler WM and Getzenberg RH: Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. Clin Cancer Res *6*: 2618-2625, 2000.

27 Van Le TS, Myers J, Konety BR, Barder T and Getzenberg RH: Functional characterization of the bladder cancer marker, BLCA-4. Clin Cancer Res *10*: 1384-1391, 2004.
28 Keesee SK, Meyer JL, Hutchinson ML, Cibas ES, Sheets EE, Marchese J, Oreper A, Potz D and Wu YJ: Preclinical feasibility study of NMP179, a nuclear matrix protein marker for cervical dysplasia. Acta Cytol *43*: 1015-1022, 1999.
29 Partin AW, Briggman JV, Subong ENP, Szaro R, Oreper A, Wiesbrock S, Meyer J, Coffey DS and Epstein JI: Preliminary immunohistochemical characterization of monoclonal antibody (PRO:4-216) prepared from human prostate cancer nuclear matrix proteins. Urology *50*: 800-808, 1997.
30 Subong ENP, Shue MJ, Epstein JI, Briggman JV, Chan PK and Partin AW: Monoclonal antibody to prostate cancer nuclear matrix protein (PRO: 4-216) recognizes nucleophosmin/B23. Prostate *39*: 298-304, 1999.
31 Lakshmanan Y, Subong ENP and Partin AW: Differential nuclear matrix protein expression in prostate cancers: correlation with pathologic stage. J Urol *159*: 1354-1358, 1998.
32 Alberti I, Parodi S, Barboro P, Sanna P, Nicolo G, Allera C, Patrone E, Galli S and Balbi C: Differential nuclear matrix-intermediate filament expression in human prostate cancer in respect to benign prostatic hyperplasia. Cancer Lett *109*: 193-198, 1996.
33 Boccardo F, Rubagotti A, Carmignani G, Romagnoli A, Nicolo G, Barboro P, Parodi S, Patrone E and Balbi C: Nuclear matrix proteins changes in cancerous prostate tissues and their prognostic value in clinically localized prostate cancer. Prostate *55*: 259-264, 2003.
34 He QY and Chiu JF: Proteomics in biomarker discovery and drug development. J Cell Biochem *89*: 868-886, 2003.
35 Heukeshoven J and Dernick R: Improved silver staining procedure for fast staining in PhastSystem Development Unit. I. Staining of sodium dodecyl sulfate gels. Electrophoresis *9*: 28-32, 1988.
36 Candiano G, Bruschi M, Musante L, Santucci l, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L and Righetti PG: Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis *25*: 1327-1333, 2004.
37 Mandal M, Vadlamudi R, Nguyen D, Wang RA, Costa L, Bagheri-Yarmand R, Mendelsohn J and Kumar R: Growth factors regulate heterogeneous nuclear ribonucleoprotein K expression and function. J Biol Chem *276*: 9699-9704, 2001.
38 Michelotti EF, Michelotti GA, Aronsohn AI and Levens D: Heterogeneous nuclear ribonucleoprotein K is a transcription factor. Mol Cell Biol *16*: 2350-2360, 1996.
39 Pino I, Pio R, Toledo G, Zabalegui N, Vicent S, Rey N, Lozano MD, Torre W, Garcia-Foncillas J and Montuenga LM: Altered patterns of expression of members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family in lung cancer. Lung Cancer *41*: 131-143, 2003.
40 Pederson T: Half a century of "the nuclear matrix". Mol Biol Cell *11*: 799-805, 2000.
41 Jackson DA and Cook PR: Visualization of a filamentous nucleoskeleton with a 23 nm axial repeat. EMBO J *7*: 3667-3677, 1988.
42 He D, Nickerson JA and Penman S: Core filaments of nuclear matrix. J Cell Biol *110*: 569-580, 1990.
43 Hozak P, Hassan AB, Jackson DA and Cook PR: Visualization of replication factories attached to a nucleoskeleton. Cell *73*: 361-373, 1993.
44 Hozak P: The nucleoskeleton and attached activities. Exp Cell Res *229*: 267-271, 1996.
45 Barboro P, D'Arrigo C, Diaspro A, Mormino M, Alberti I, Parodi S, Patrone E and Balbi C: Unraveling the organization of the internal nuclear matrix: RNA-dependent anchoring of NuMA to lamin scaffold. Exp Cell Res *279*: 202-218, 2002.
46 Barboro P, D'Arrigo C, Mormino M, Coradeghini R, Parodi S, Patrone E and Balbi C: An intranuclear frame for chromatin compartmentalization and higher-order folding. J Cell Biochem *88*: 113-120, 2003.

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