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Nuclear Matrix Proteins as Cancer Markers

Tracy Davido and Robert H. Getzenberg*

Departments of Urology, Pathology and Pharmacology and the University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract The transformation of normal cells to a malignant state has long been detected by light microscopy as visible changes in nuclear morphology. These changes include abnormal nuclear shape, increased nuclear to cytoplasmic ratio, and the presence of additional and abnormal nucleoli. Metaplasia,dysplasia, carcinoma-in-situ, and gross malignant tumors are diagnosed and graded pathologically by this traditional method. The resulting relative increase in DNA concentration within these cells produces a greater affinity for Hematoxilyn and Eosin staining, and thus, the characteristic blue color of cancerous tissues. As understanding of the cell structure expanded, the nuclear matrix emerged as an integral component of genetic processing and therefore, became an important cellular entity for study of malignant transformation. Also, several types of cancer have revealed discreet alterations in their respective nuclear matrices. One potential application of these nuclear matrix changes is development of detection and monitoring tests that would reveal the presence of abnormal cells. These tests could be utilized at a number of points in the disease process including prior to gross physical symptoms, and thereby significantly reduce patient morbidity and mortality. A second potential application of the nuclear matrix is to utilize it as a tissue specific protein targeting system to address narrowly directed therapeutic treatments, and thereby avoid the systemic side effects from broad-spectrum therapies like radiation. This paper addresses the role of the nuclear matrix in both normal cells and transformed cells, and highlights several research efforts that have advanced the ability to detect, track, and potentially treat neoplasms at the molecular level. J. Cell. Biochem. Suppl. 35:136–141, 2000.

NUCLEAR MATRIX

Since its discovery in 1974 by Berezney and Coffey, the nuclear matrix has been a target of significant and varied research regarding its cellular function and role in tumorigenesis [Berezney and Coffey, 1974]. Comprising over 98% protein, 0.1% DNA, 0.5% phospholipid, and 1.2% RNA, the nuclear matrix is the three-dimensional support scaffold of the cell nucleus that performs several fundamental functions in an array of cellular activities [rev. in Reploglе-Schwab et al., 1996].Among these functions are: the determination of nuclear morphology, DNA organization throughout the cell cycle, stabilization and orientation of DNA during replication, the organization of gene regulatory complexes, and RNA synthesis.

Furthermore, the nuclear matrix is known to be intimately connected to the cytoskeleton and extracellular matrix (ECM) and can be structurally altered by its extracellular framework, thereby changing a cell's genetic expression and response to various stimuli such as growth factors and hormones, [Getzenberg et al., 1991]. The nuclear matrix also interacts with the ECM, and its protein composition is at least partially determined by these interactions.

Specifically, the nuclear matrix demonstrates deletions and additions of certain proteins when the ECM undergoes alterations such as those induced by nearby malignant tumors, [Getzenberg et al., 1991]. These changes may inappropriately amplify or delete a cell's active genes, and possibly induce the expression of oncogenes and/or turn off or decrease tumor suppressor genes. Given the multiple functions and external interactions of the nuclear matrix, it follows that disruption of this dynamic structural support system would have serious ramifications to cell morphology and cellular replication, transcription, and RNA splicing. Thus, the nuclear matrix has been recognized as an important potential determinant of the cancer process.

*Correspondence to: Robert H. Getzenberg, PhD, Department of Urology Research Laboratories, University of Pittsburgh, Suite G40, Shadyside Medical Center, 5200 Centre Avenue, Pittsburgh, PA 15232.
E-mail: getzenbergrh@msx.upmc.edu.

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The nuclear matrix consists of RNA-associated protein filaments in a discreet network, which includes the nuclear lamins, nucleoli, and pore complexes. Extending throughout the nucleus, this dynamic structure has been shown not only to provide the three-dimensional nuclear shape, but it also specifically orients and packages the approximately two meters of DNA throughout the cell cycle [Berezney and Coffey, 1977].

It has been shown that higher orders of DNA organization are intimately involved with the nuclear matrix. Specifically, the third level of organization or DNA loop domains are attached to the nuclear matrix at their bases at sites known as matrix attachment regions (MARs), or scaffold attachment regions (SARs) [Ludérus et al., 1992]. These loop domains are believed to be the sites of DNA replication, incorporating DNA replicases and topoisomerase II and other components into complexes which are bound to the nuclear matrix exclusively at the locations of a particular cell's active genes [Earnshaw et al., 1985]. Also, it has been proven that unexpressed DNA, which constitutes approximately 90%, is not associated with the nuclear matrix [Getzenberg et al., 1991]. Therefore, it appears that via the regions associated with the nuclear matrix, a particular cell's DNA is positioned in a manner which exclusively permits tissue-specific expression.

The nuclear matrix also participates in the regulation of gene expression via its specific binding sites for steroid hormone receptors. Several hormone-responsive tissues have been shown to contain unique receptors within their nuclear matrices that facilitate activation of hormonally regulated genes by binding to DNA segments known as hormone response elements (HREs). The HREs in turn, are found in the promoter regions of hormonally regulated genes and thus affect transcription of these genes. Furthermore, these matrix-associated receptors are absent from non-steroid responsive tissues, and are known to be down-regulated during periods of decreased hormonal action [rev. in Getzenberg et al., 1990]. Many studies have demonstrated the phosphorylation of NMPs was also observed, which may indicate a linkage between gene expression regulation and the nuclear matrix via one of the most common cellular control mechanisms.

In concordance with being associated with the active genes of a cell, several investigators have shown that by providing a location for the merging of transcription factors with DNA, the nuclear matrix is also the site of RNA transcription [Jackson et al., 1981; Ciejek et al., 1982]. As stated previously, actively transcribed genes are associated with the nuclear matrix which also contains transcriptional complexes, newly synthesized hnRNA, (the precursor of mRNA), snRNA, and RNA-processing intermediates [rev. in Replogle-Schwab et al., 1996]. All of which suggest that the nuclear matrix may be the site of RNA transcription.

Furthermore, the nuclear matrix has been shown to be the binding site of various tumor-associated proteins, and therefore may play an actual role in the specific nuclear protein alterations that initiate and propagate cellular transformation. Examples include: the E1 A adenovirus transforming protein, [Sarnow et al., 1982], v-myc and c-myc proteins, [Eisenman et al., 1985], and the HPV oncoprotein E6, [Keesee et al., 1998], all of which are theorized to cause various types of DNA disruption.

Given the potential roles of the nuclear matrix in the transformation of cells, the application for cancer screening and detection of disease recurrence have been active research pursuits of a number of investigators. In particular, characterization of NMP profiles was recognized as a potentially more accurate method of cancer detection with profound implications not only to diagnostic sensitivity and disease monitoring, but also in precise direction of treatments. In this review, we will highlight a couple of areas of investigation which have revealed the potential role of the nuclear matrix in the cancer process.

Bladder Cancer

Currently, detection of most cancers relies upon patient complaint of presenting symptoms followed by clinical investigation and biopsy. For example, bladder cancer, which predominantly occurs in the sixth or seventh decade of life, is discovered most commonly after an individual presents with hematuria and/or other urinary symptoms and is diagnosed by cytologic examination of urine samples or cystoscope-directed biopsy. This method is effective for high-stage tumors, which are clearly visible with a cystoscope and shed large amounts of poorly-differentiated epithelial cells into the urine. However, low stage tumors with near normal appearing cytology are difficult to detect

by these traditional means and carcinoma in situ is often impossible to discern. Furthermore, patients that are diagnosed at earlier, lower stages have profoundly better morbidity and mortality rates, [American Cancer Soc., 2000]. Therefore, detection and management of bladder carcinoma would be greatly improved by an effective method for early detection.

Attempting to develop a specific and sensitive method to detect bladder cancer, we have identified six NMPs (BLCA-1 thru 6) unique to patients with transitional cell carcinoma and another three NMPs specific to normal bladder tissue that are absent in the cancer patients. These proteins were not identified in other cancer and normal tissue types studied. Utilizing high resolution two-dimensional gel electrophoresis, the NMPs were isolated from prepared bladder tissue samples. Of the six proteins that were identified, three were selected for sequencing based upon their abundance [Getzenberg et al., 1996]. The first of the proteins to be thoroughly characterized is BLCA-4. Using rabbits immunized with peptides encoding BLCA-4, antibodies were generated against the selected NMP. This permitted the development of a urine immunoassay that detects the presence of BLCA-4, and thus whether a patient has transitional cell carcinoma.

Our lab examined the expression of BLCA-4 in the bladders of individuals with bladder cancer and normal control individuals (organ donors). These studies revealed that BLCA-4 expression occurs throughout the bladder in individuals with bladder cancer. This positivity includes morphologically normal areas of the bladder. No BLCA-4 expression is detected in the bladders of individuals that do not have bladder cancer. This finding suggests that a "field effect" exists in the bladder epithelium [Konety et al., 2000a]. Recent data in animal models supports the concept that BLCA-4 is an early event in that expression is identified prior to the detection of grossly observable tumors. Therefore, such a marker would offer significantly earlier diagnostic information as compared with the current gold standard of urine cytology, and may also be appropriate as a screening test in high risk patients.

Furthering the investigation of the applicability of a BLCA-4 urine assay, our lab aimed at defining and evaluating possible confounding factors which may affect the accuracy of the test. A group of spinal cord injury patients (SCI) was selected due to their unusually high incidence of such factors as cystitis, tobacco use, and catheterization. This subset of patients is regarded as difficult to manage not only due to their significantly high rates of bladder cancer, but also due to their requirement for permanent or frequent catheterization and correspondingly high rates of cystitis. It was determined that the confounding factors of smoking, cystitis, and catheterization did not effect the results of the BLCA-4 assay [Konety et al., 2000b].

Utilizing an entirely different approach, investigators at Matritech Inc. have developed an NMP detection test that measures urine levels of NMP22 or NuMA, a ubiquitous internal nuclear matrix protein present in the mitotic spindle of cells during mitosis. Hence, in rapidly dividing cells such as TCC, NMP22 is found in markedly elevated quantities, with the upper limit of normal being 7.5 U/mL [Soloway et al., 1996]. The protein is not specific to bladder cancer, however, and can be above normal levels in renal and prostate cancer as well as cystitis [Huang et al., 2000].

Matritech received FDA approval of the NMP immunoassay in 1996 for monitoring the recurrence of disease and in early 2000 for both management of previously diagnosed TCC and diagnostic application in conjunction with other standard diagnostic procedures.

Cervical Cancer

Cervical cancer is perhaps the most thoroughly understood of all malignant transformations, with the predominant causative agent, the human papillomavirus (HPV), being identified over two decades ago [Kirchener, 1991]. More than 90% of cervical carcinomas are the result of infection by HPV, particularly the aggressive strains 16, 18, 31, and 33 [Robbins, 1999]. Recent research shows promise in the development of discreet treatment of cervical cancer targeting the molecular level. Since the disease is typically confined to the cervix until late stages of distant metastasis, it is an excellent candidate for local drug treatment with minimal side effects.

A number of other promising developments have occurred recently, advancing the possibility of localized treatments, earlier detection, and more accurate monitoring. For example, Keesee et al have identified five unique NMPs of cervical carcinoma that may be utilized in new,

more accurate screening tests [Keesee et al., 1998]. And Yang et al demonstrated that the HPV-16 DNA specifically binds to different NMPs in normal and cervical carcinoma cells, and with higher affinity for the "newly expressed" acidic proteins of the transformed cells [Yang et al., 1997]. They concluded that monitoring of NMP alteration in cervical cells would be an earlier and highly accurate method of discerning cervical cancer transformation, applicable to both initial screening and post colposcopy follow-up [Yang et al., 1997].

Prostate Cancer

Prostate cancer is not only an important health problem with a recognizable early stage, but also early intervention yields markedly greater patient benefit. This is demonstrated by the epidemiology of the disease. By age 50, males have a 42% chance of developing prostate cancer, and after age 80, greater than 70% will have histologic evidence of prostate cancer. In addition, at inception, in theory all prostate cancers are confined to the gland, and prostatectomy is curative, while invasive tumors currently have no effective treatments. However, the marked discrepancy between high incidence and relatively low mortality rates indicates that a large proportion of the diagnosed tumors may not become clinically significant.

Both prostate tissue and prostate tumor specific NMPs have been found and are possible tumor markers. Getzenberg et al. [1991] showed that tumor cell lines of Dunning rat prostates not only displayed significant differences from the normal prostatic tissue line, but also exhibited alterations that correlated with the degree of phenotype transformation. More recently, our laboratory has characterized several Dunning NMPs, including D-1, D-2, D-3, AM-1, and AM-2, and successfully utilized them to develop antibodies capable of detecting human prostate cancer NMPs. In particular, we have developed an assay to the D-2 protein that is able to distinguish normal human prostate tissue from transformed tissue. In related research, Partin et al also identified NMPs unique to benign prostatic hypertrophy (BPH) and prostate cancer [Partin et al., 1991]. Specifically, protein PC-1 was identified in every prostate cancer specimen, but was absent in all BPH and normal tissues. Thus, it may be possible to use NMPs to diagnose, classify, and track the progression of prostate cancer in each patient.

Also, in their investigations of NMPs in prostate cancer, Partin et al have isolated a specific protein, YL-1, as a marker for the aggressive form of prostate cancer. YL-1 is consistently found in patients with intermediate to high-grade prostate cancer, while it is seldom isolated in patients determined by standard pathologic methods to be in the "good" prognostic group [Partin et al., 1991]. Therefore, NMPs may also be used as prognostic indicators in prostate cancer and to assist clinicians in determining the level of treatment aggressiveness for each patient.

Breast Cancer

Breast carcinoma remains the second leading cause of cancer death in women, and has an incidence of greater than one in nine despite improved diagnostic techniques and public awareness for early detection. And notwithstanding significant advances in early diagnostic techniques, the death rate has remained nearly constant for more than 30 years. Diagnostic screening via mammography has been the mainstay of breast cancer detection with conclusive biopsy performed upon detection of abnormality, but serious shortcomings in these techniques persist and the need for both improved methods of diagnosis and treatment remain.

In the early 1990s several investigators identified NMPs unique to breast cancer cell lines and since then, researchers have made promising advances in diagnosis, prognostication, and cellular intervention treatments [Khanuja et al., 1993; Samuel et al., 1997]. For example, early immunoassays of breast carcinomas displayed differences in reactivity depending on the gross type of breast cancer i.e. medullary carcinoma expressed higher staining intensity than ductal carcinoma, suggesting that such analysis could be useful in diagnosis and selection of treatment [Wisecarver et al., 1993]. Samuel et al discovered NMPs (NMBC 1–5) exclusive to well-differentiated breast carcinoma and one (NMBC -6) distinctive to poorly differentiated cancer cells, suggesting that NMPs could be accurate prognostic indicators [Samuel et al., 1997]. Shortly thereafter, it was shown that certain NMPs of MCF-10 cell lines undergo discreet alterations in the phenotypic transformation from normal to cancerous

breast tissue. This demonstrated that NMPs could serve as biomarkers of tumor pathogenesis in breast cancer.

Additionally, Spencer et al have isolated NMPs that are exclusively found in estrogen receptor positive breast cancer cell lines as well as other NMPs unique to estrogen receptor negative cells [Spencer et al., 2000]. These NMPs changed when breast cancer cells became estrogen dependent. Specifically, they proved the intermediate protein filament vimentin is associated with DNA of the highly metastatic cell line MDA-MB-231. This research provides the gateway to more effective use of current chemotherapies as well as development of treatments targeted at the micro-cellular level for the deterrence of metastasis or aggressive tumor growth.

Renal Cancer

Renal cell carcinoma (RCC) is another neoplasm that shows promise in the development of pathologic markers based on nuclear matrix proteins. Traditionally, RCC is graded on the basis of nuclear size and shape. This is reflective of NMP abnormality and correlates with tumor aggressiveness [Carducci et al., 1999]. However, grade and stage of RCC are poor prognosticators for individual patients. The detection of RCC has been relatively haphazard, with the classic presenting symptoms of gross hematuria, flank pain, and abdominal mass or, more recently, as incidental findings on unrelated abdominal CT imaging. Also, the possible presence of adrenal carcinoma and metastatic tumors further complicates the diagnosis of RCC. Clearly, serum or urine NMP diagnostic markers would greatly improve clinicians' ability to detect RCC at an earlier, more manageable stage, provide more specific information of prognosis and differentiate it from metastatic sites or other nearby visceral tumors. In fact, the loss of capillary integrity associated with RCC tumors makes it particularly amenable to serum sample biomarker detection [Konety and Getzenberg, 1999].

In their comparison of NMPs from renal cell carcinoma and normal kidney tissue from the same patients, Konety et al, isolated five NMPs specific to RCC, and one exclusive to normal renal tissue [Konety et al., 1998]. Development of an antibody immunoassay to the unique RCC NMPs could provide a major improvement to diagnosis, prognosis, and recurrence follow-up of renal cancer, and initiate the ability to therapeutically target RCC tumors at the cellular level.

CONCLUSION

The nuclear matrix has been demonstrated to be a major factor in the unique spatial arrangements of chromatin within the nucleus and to include components singular to each tissue and its corresponding transformed state. Also, it is known that by incorporating fixed sites for DNA replication complexes and RNA transcription complexes, the nuclear matrix is partially responsible for genetic rearrangements and tissue specific gene expression. Therefore, due to their uniqueness and early involvement in the transformation process, NMPs have the characteristics desirable for malignant biomarkers.

The neoplasms highlighted above and several others indicate that NMP transformation occurs in unique and detectable forms that have important potential as biomarkers for cancer detection. Thus far, each malignancy studied has revealed NMPs specific to its cellular transformation. NMP assays would be applicable to an array of clinical purposes including diagnosis, disease management, prognosis, and disease recurrence monitoring. Furthermore, as exemplified by the recent developments in cervical cancer research, such as preliminary p53 pathway reactivation and lucidation of the differences of HPV-16 DNA binding in normal and transformed cells, it is apparent that nuclear matrices may facilitate precisely directed molecular treatments.

As demonstrated by the BLCA-4 bladder carcinoma biomarker, the specificity and sensitivity attributed to NMP assays can be significantly higher than current clinical and pathologic diagnostic methods, thereby providing a noninvasive yet extremely accurate screening test. And since NMP aberrations begin to occur prior to full malignant transformation, interventions will be possible at earlier stages of the disease process, contributing to improved treatment response and patient prognosis. And as indicated in some of the examples presented, the more accurate diagnostic information provided by such tests would greatly reduce patient morbidity associated with inappropriate and unnecessary treatments. NMP serum and urine assays represent a virtually

untapped area of possible screening tests, and promise to vastly change current pathologic and radiologic diagnostic methods.

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