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Review

Electrophoretic analysis of nuclear matrix proteins and the potential clinical applications

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ABSTRACT

Nuclear matrix proteins form the skeleton of the nucleus and participate in the various cellular functions of the nucleus. These proteins have been demonstrated to be tissue-type specific and can potentially reflect changes in the state of differentiation of the cell. Elucidating nuclear matrix protein changes necessitates the use of high-resolution two-dimensional polyacrylamide gel electrophoresis. Separation of this complex mixture into its component parts resolves protein changes when comparing the normal state to a diseased state of a cell. Evidence has been reviewed which shows the potential use of nuclear matrix proteins and antibodies to nuclear matrix proteins as diagnostic tools for various cancers, autoimmune diseases, adenoviral infection, and other diseases. Consequently, the central functions of the nuclear matrix in the cell allow it to have significant potential as a diagnostic agent.

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LIST OF ABBREVIATIONS

bp	Base pair
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
hnRNA	Heterogeneous nuclear ribonucleic acid
IF	Intermediate filaments
kb	Kilobase
LIS	Lithium diiodosalycilate
M_r	Relative molecular mass
NM	Nuclear matrix
NP-40	Nonidet P-40
p*I*	Isoelectric point
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
SnRNP	Small nuclear ribonucleoprotein particle
SDS	Sodium dodecyl sulfate
TPA	O-Tetradecanoylphorbol 13-acetate

1. INTRODUCTION

The nuclear matrix is a filamentous network composed of protein and RNA which acts as the structural framework of the nucleus [1,2]. The function of the nuclear matrix does, however, go beyond this basic role. It has been shown to be associated with ongoing DNA and RNA processes occurring in the nucleus [3–6]. These processes are vital to the growth and maintenance of the cell whether it is in a normal state or has undergone a transformation to a diseased state.

The proteins comprising the nuclear matrix are numerous and varied [7,8]. Alterations in the nuclear matrix proteins by chemical modification, concentration, or induction of a new type of protein may be characteristic of certain diseases [7,9]. The elucidation of these changes has clinical relevance for the diagnosis of a disease and would lead to a better understanding of its cause. Although morphological changes in the nucleus can be visualized by microscopy they are not characteristic of a specific disease. A better approach would utilize two-dimensional polyacrylamide gel electrophoresis [7,10]. Resolution of nuclear matrix proteins into their individual entities is possible by this technique. It can be applied to detect the protein pattern differences in the nuclear matrix between normal and diseased states. Cancer is one of the major diseases in which early clinical diagnosis would be feasible through detection of unique nuclear matrix proteins which may be induced by the disease. The following review will probe into the clinical significance of the two-dimensional gel patterns of nuclear matrix proteins and their application in the development of diagnostic agents.

2. STRUCTURE AND FUNCTION OF THE NUCLEAR MATRIX

The nuclear matrix is the non-chromatin structure of the cell nucleus. It is a

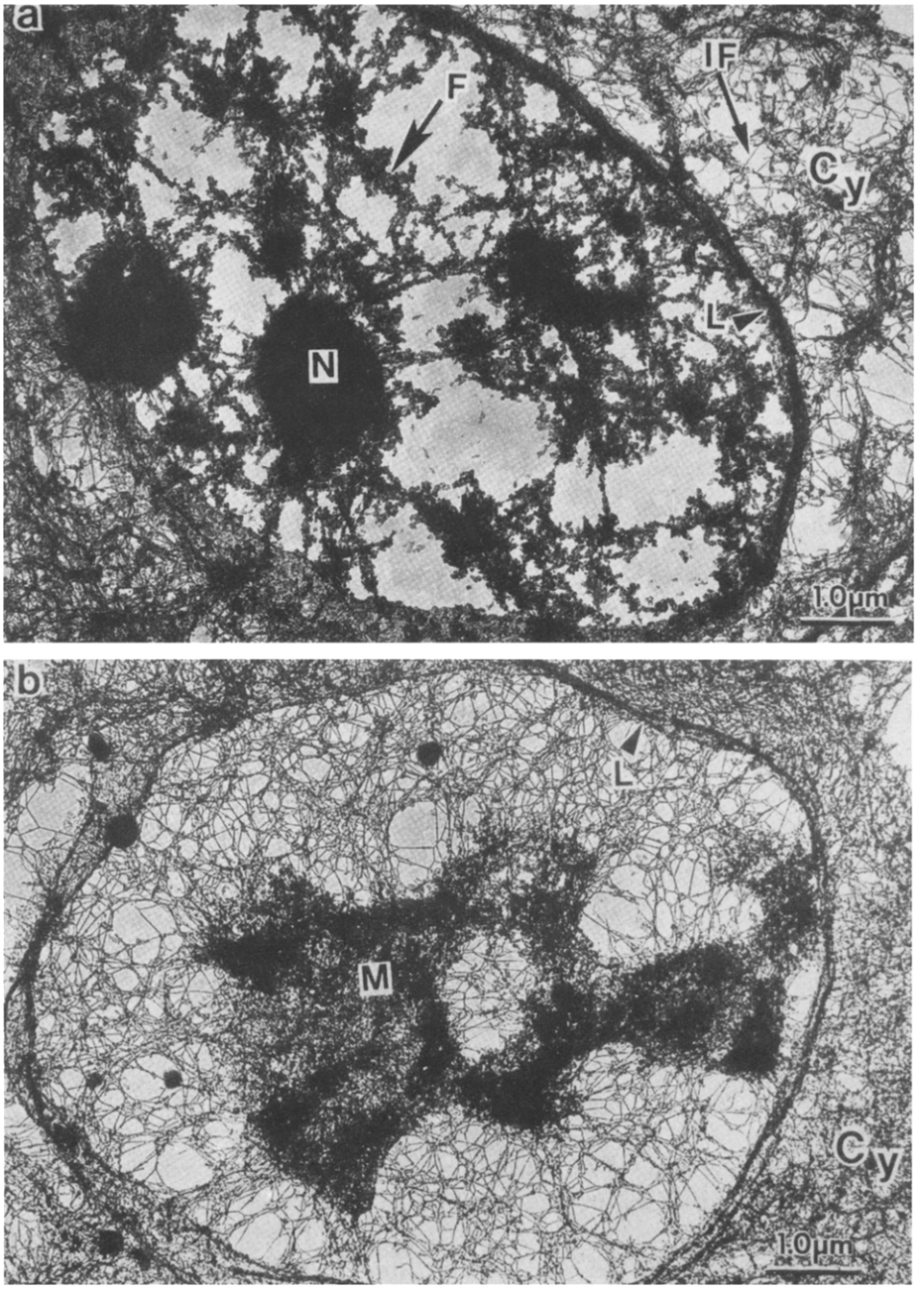

Fig. 1.

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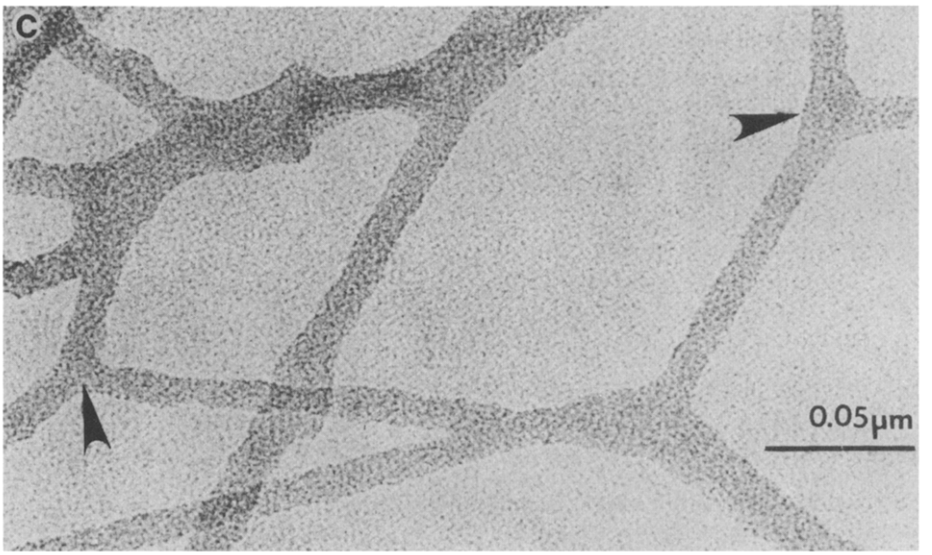

Fig. 1. Core filaments of the HeLa nuclear matrix. (a) Nuclear matrix. Cells grown in suspension were detergent-extracted, digested with DNase I, and treated with 0.25 *M* ammonium sulfate revealing the nuclear matrix. The nuclear interior contained thick nuclear fibers (F) together with dark granular nucleoli (N), bounded by the nuclear lamina (L) which also anchored the intermediate filaments extending from the lamina into the cytoplasmic space. This structure is the matrix-intermediate filament complex. (b) Core filaments of the nuclear matrix. The nuclear matrix-intermediate filament complex was further extracted with 2 *M* NaCl. This treatment removed most of the nuclear matrix proteins and revealed an underlying network of slender filaments. These HeLa core filaments were much thinner and more uniform than the original matrix fibers. (c) High magnification view of HeLa core filaments. In this view core filaments, marked by arrowheads, could be clearly seen. The junctions were smooth, lacking evidence for a separate junction structure. (Reprinted from ref. 1 with permission.)

complex network composed of both protein and RNA. The nuclear matrix acts as the skeleton of the nucleus specifying its shape. The property of shape may be involved in determining cellular functions, for example differentiation and proliferation [11].

2.1. Nuclear matrix structure

Some of the clearest and probably most accurate *in vitro* views of the matrix are seen in resinless electron microscopy sections [1,2,12,13]. Using a temporary embedment medium, diethylene glycol distearate, ultrathin sections can be produced which are rigid and self-supporting. Removal of the resin prior to electron microscopy allows visualization of the cell structures which form high-contrast, three-dimensional images without metal stains. An illustration of the nuclear matrix after digestion with DNase I and treatment with 0.25 *M* $(NH_4)_2SO_4$ is seen in Fig. 1a. Most of the histones and more than 97% of nuclear DNA were

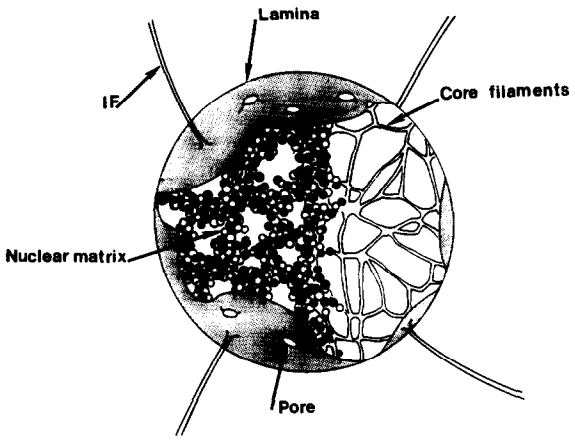

Fig. 2. Model of the nuclear matrix structure. The nuclear lamina with its nuclear pores forms the outer boundary of the nuclear matrix. Intermediate filaments (IF) radiate out from the lamina into the cytoplasmic space. The interior nuclear matrix is composed of thick, polymorphic fibers. The protein composition of these thick fibers is complex and specific to cell type [7]. Further extraction with 2 *M* NaCl removes many of these matrix proteins and reveals the much thinner core filaments. (Reprinted from ref. 1 with permission.)

removed. Further treatment of the nuclear matrix with 2 *M* NaCl removes many nuclear matrix proteins leaving behind a filamentous network of the remaining nuclear matrix proteins (Fig. 1b and 1c). This network of filaments can be fragmented by RNase digestion. The resulting model of nuclear matrix structure suggested by the above researchers [1] is summarized in Fig. 2. The highly branched core filament network appears to be held together by RNA. Numerous other proteins are associated with this network, lining the filaments and enlarging their overall diameter. The thick fibers contain the proteins specific to cell type [7].

2.2. Nuclear matrix and DNA packaging

Various cellular functions have been associated with the nuclear matrix. A mammalian nucleus contains a total length of approximately 50 cm of DNA which is packaged in a nucleus with a diameter of about 10 μm. The total length of DNA must be reduced 50 000-fold to fit in the nucleus [14]. Histone proteins only allow for approximately a 40-fold reduction in the DNA. Evidence has been shown that eukaryotic DNA is attached to the nuclear matrix and that this association is responsible to a large extent for the remaining packaging of the DNA [15–18]. In these studies, isolation of the nuclear matrix was performed under non-physiological conditions (high salt). To control for any potential artifacts produced by high salt, a re-investigation of DNA binding to the nuclear matrix under more physiological conditions was undertaken. This study still

showed a stable attachment of DNA fragments to the nuclear matrix [19]. HeLa cells were encapsulated in agarose microbeads and lysed using Triton X-100 in a physiological buffer. Packaging of the residual DNA fragments protected about 1 kb (kilobase) from nuclease attack which is a greater amount than a typical protein-binding site of 10–20 bp (base pairs). The maintenance of chromatin organization in the nucleus has also been shown to involve nuclear RNA which is associated with nuclear matrix proteins [12]. The disruption of nuclear RNA *in situ* with RNase or *in vivo* with actinomycin leads to the collapse of the nuclear matrix structure and that of the chromatin.

2.3. Role of the nuclear matrix in replication and transcription

In addition to packaging the DNA, the nuclear matrix contains the potential to unwind DNA. Topoisomerase II has been shown to be a major component of the nuclear matrix fraction [20,21]. This function is consistent with the proposed role of the nuclear matrix in replication and transcription. Replication origins and newly synthesized DNA have been shown to be attached to the nuclear matrix and this DNA attachment is not induced by conditions of high ionic strength [3,4,22]. In addition, DNA polymerase α activity is found associated with the nuclear matrix fraction in HeLa cells when the nuclei are incubated for 30 min at 37°C prior to 2 *M* NaCl extraction [23]. This association is not induced by high ionic strength. Upon incubation of the nuclei at 0°C prior to 2 *M* NaCl extraction less than 5% of the activity was found associated with the nuclear matrix. Heterogeneous nuclear RNA (hnRNA) synthesis is also associated with the nuclear matrix [24]. Small nuclear ribonucleoprotein particles (snRNPs) along with hnRNA are required for the assembly of functionally active spliceosomes [25]. These two RNA components are associated with the nuclear matrix and appear to function in the process of premessenger RNA splicing [5,6,26]. Actin-containing filaments have been suggested to be involved in the binding of prespliced (immature) messages to the cell nucleus [27]. RNA polymerases I and II were also reported to be associated with the nuclear matrix [28]. Thus, the nuclear matrix appears to play an important role in many of the functions which are ongoing in the nucleus and are necessary for cell growth and maintenance. Furthermore, the nuclear matrix can bind steroid hormones [29]. The interaction of these hormones may be an essential occurrence in the control or modulation of the nuclear events described above.

3. ELUCIDATION OF THE COMPLEX MIXTURE OF NUCLEAR MATRIX PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

Two-dimensional gel electrophoresis is a powerful technique for the resolution of complex mixtures of proteins. Although the nuclear matrix proteins only comprise approximately 1% of the total cellular protein, there is a myriad of dis-

tinctive proteins. In addition, a large number of these proteins only differ to a slight degree due to chemical modifications such as phosphorylation or glycosylation. Resolution of these proteins can be elucidated by the two-dimensional gel technique originally developed by O'Farrell [30]. However, a major drawback has been the reproducibility of protein patterns on the two-dimensional gels among different labs.

Two-dimensional gel electrophoresis of isolated nuclear matrix proteins resolve many protein spots most of which are yet unidentified. An example of a high-resolution two-dimensional gel of a nuclear matrix sample is shown in Fig. 3. The lamins (designated as A, B, and C) which form the outer boundary of the nuclear matrix are around $70 \cdot 10^3$ relative molecular mass (M_r) and are represented by lines of protein spots in the pattern. Ribonuclear proteins between $30 \cdot 10^3$ and $50 \cdot 10^3$ M_r in the basic region of the gel also appear to be common

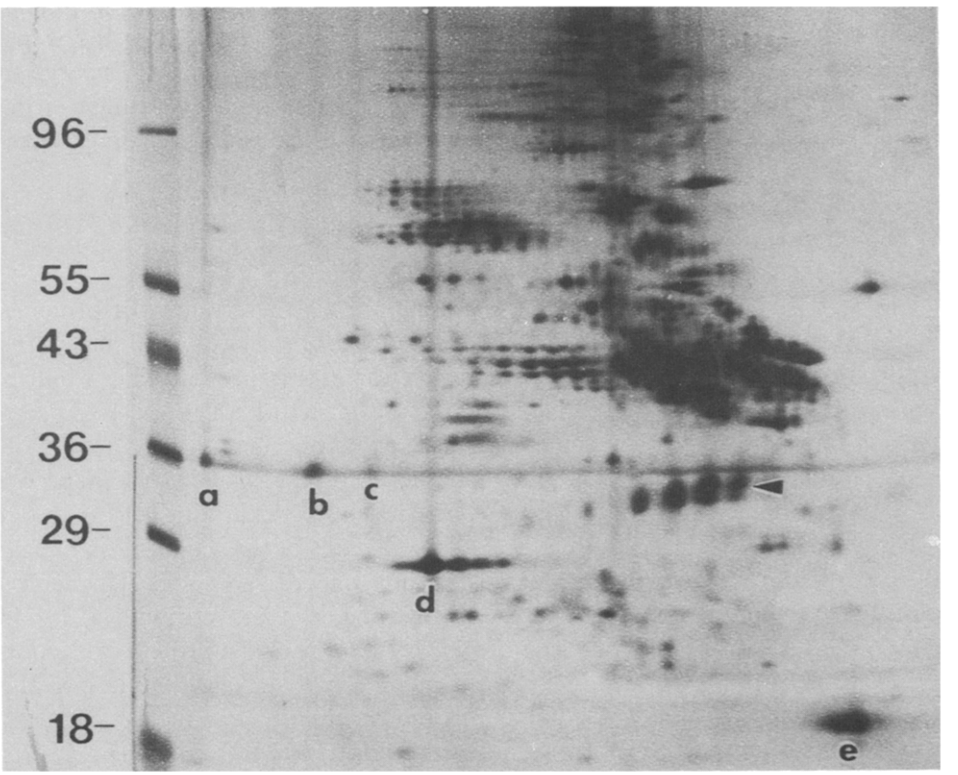

Fig. 3. Two-dimensional gel of nuclear matrix proteins from CaSki, a human cervical carcinoma cell line. Approximately 15 μg of protein were loaded on the gel. CHAPS was the detergent used in the first dimension. The p*I* of the protein standards in this dimension were lactate dehydrogenase, (a) 8.5, (b) 8.4 and (c) 8.3, carbonic anhydrase, (d) 5.95, and trypsin inhibitor protein, (e) 4.55 at approximately 0.5 μg of each protein. The arrow points to DNase I contaminant protein spots added to the nuclear matrix sample during its preparation. (Reprinted from ref. 10 with permission.)

[1,7,8,10]. Intermediate filament proteins, cytokeratins ($40 \cdot 10^3$–$68 \cdot 10^3$ M_r) and vimentin ($57 \cdot 10^3$ M_r), can be resolved in the acidic region of the gel when present as contaminants of the nuclear matrix protein preparation. Evidence has been shown *in vivo* that intermediate filaments can associate cooperatively with lamin B [31]. The elucidating power of two-dimensional gels can also resolve recognizable patterns. For instance, phosphoproteins are visualized as several spots in a horizontal line. They have the same molecular mass but possess a slightly different charge which is separated by the first dimension. Glycoproteins, on the other hand, have small changes in molecular masses and similar charge. Their pattern shows protein spots which line up in a diagonal. Transformed cell lines appear to contain more of these two types of protein modifications in the nuclear matrix. A dramatic increase in non-histone nuclear protein phosphorylation was detected when a carcinogenic substance was administered to a rat [32]. Similarly, the oligosaccharide pattern of glutamyltranspeptidase from rat hepatoma cells was significantly more complex than from normal rat liver [33]. Other side-chain modifications of the nuclear matrix proteins which are enhanced in the transformed state are acetylation, methylation and ADP-ribosylation [34]. Separation of these small modifications of proteins allows the detection of changes in the state of the cell from the normal condition and would be useful for clinical diagnosis.

3.1. Isolation of nuclear matrix proteins

The term nuclear matrix proteins encompasses a large diversity of proteins. Thus, different methods of isolation of these proteins have been used depending on the specific need. One characteristic of nuclear matrix proteins as a whole is their insoluble nature, a common feature of many structural proteins. This property has been utilized in the isolation of these proteins.

There are basically three ways in which nuclear matrix proteins are prepared. The initial procedures are basically the same. Generally, eukaryotic cells are lysed with 0.5% Triton X-100 removing the soluble proteins of the cell (approximately 60–70% of the total protein content depending on cell type [35]). This leaves behind the cytoskeletal framework which can be removed with 0.25 *M* $(NH_4)_2SO_4$. At this point nuclei can be pelleted [36,37] or the protein extraction process can continue without this extra step [35,38,39]. DNase digestion in the presence of 0.25 M $(NH_4)_2SO_4$ eliminates the chromatin and nuclear proteins, for instance, histones, polymerases, and transcription factors. Nuclear matrix proteins remain associated with the hnRNA and intermediate filaments. Treatment with RNase leaves only the protein. The final step separates the nuclear matrix proteins from the intermediate filaments (NM-IF fraction). At this point the procedures for the solubilization of the nuclear matrix proteins diverge. The most commonly used method involves a high salt concentration (2 *M* NaCl) [36,37,40]. The other approaches use 8 *M* urea [35,38,39], or the detergent, lithi-

um diiodosalycilate [41]. Using 2 *M* NaCl nuclear matrix proteins can be solubilized containing a minimal amount of intermediate filament proteins which can be removed by centrifugation. The stepwise addition of salt is necessary in the above isolation. If 2 *M* NaCl was added directly to the DNase-digested nucleus, a different morphological pattern results. The core filaments become obscure when viewed by resinless sections and the condition of isolated matrix proteins is altered. Utilizing 8 *M* urea, a more complete assemblage of nuclear matrix proteins can be isolated based on two-dimensional gels [1]. Disassembly of the NM-IF fraction with 8 *M* urea leaves behind mainly insoluble carbohydrates and extracellular matrix components. Dialysis of this solubilized fraction allows reassembly of the IF which are pelleted. The resulting nuclear matrix proteins contain minimal IF proteins. Detergent extraction of isolated nuclei with lithium diiodosalycilate (LIS) allow solubilization of the DNA [41]. Centrifugation pellets the nuclear matrix proteins. However, neither the morphology or the resultant proteins were well characterized as in the other two methods. Concentration of the isolated proteins from solution can be accomplished by ethanol precipitation [10]. This simplifies sample preparation for electrophoresis on one- and two-dimensional sodium dodecyl sulfate (SDS) polyacrylamide gels or monoclonal antibody production.

3.2. High-resolution two-dimensional gel electrophoresis

Improvements have been made which have dramatically increased reproducibility, resolution, and sensitivity of the gels. Substitution of Nonidet P-40 (NP-40) with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) in the isoelectric focusing gels increased the solubility of the sample during migration [42]. Application of higher electrophoresing currents with maintenance of constant gel temperature by cooling and use of dithiothreitol (DTT) instead of mercaptoethanol were also implemented. Introduction of an enhanced silver staining, replacement of the agarose overlay of the first-dimension tube gel on the second dimension with rapidly polymerizing acrylamide, plus several other modifications allowed the resolution of over 300 nuclear matrix proteins on a two-dimensional gel from a 15-μg sample [10]. An example of this gel is illustrated in Fig. 3. Further improvements have overcome the problems of cathodic drift where the entire protein pattern slowly migrates toward the cathode and variable ampholyte properties which cause discontinuities in the pH gradient by replacing the first-dimension carrier ampholytes with immobilines [43,44]. Recently, Millipore has produced a 0.08-mm thread which is incorporated in the first-dimension tube gel prior to polymerization of the acrylamide. Stretching or breaking of the gel prior to loading the second dimension is reduced enhancing reproducibility of the gels. Thus, the potential of this technique is obvious with increased usage producing further improvements.

3.3. Computer-assisted analysis of two-dimensional gel patterns

Analysis of the complicated protein patterns which are becoming more complex with increased two-dimensional gel resolution and sensitivity has become feasible with commercially available computer software. Several different kinds of software packages are based on alternative approaches to the gel analysis [45–48]. These programs can reduce analysis time and tedium while allocating statistically significant parameters to the protein spots. For instance, they can determine relative and absolute protein concentrations on the gel, isoelectric point (p*I*), and molecular mass all based on electrophoresed standards. Pinpointing the presence of unique and common proteins when comparing gels is another feature. Furthermore, a composite gel can be generated from a group of gels reducing the differences which may occur from day to day when running the same sample on polyacrylamide gels. These features along with others have added a new dimension to two-dimensional gel analysis expanding the usefulness and applicability of two-dimensional gel electrophoresis.

4. CLINICAL APPLICATIONS OF NUCLEAR MATRIX PROTEINS IN THE DEVELOPMENT OF DIAGNOSTIC AGENTS

Clinical diagnosis of cancer should encompass an early and reliable detection of the cellular transformation to neoplasia. The properties of a suitable marker must include tumor-type specificity, ready detectability at an early stage, indication of progression, and regression and prediction of recurrence [49]. Nuclear matrix proteins may just be these elusive markers.

4.1. Existence of unique nuclear matrix proteins between the normal and transformed state of the cell

The nuclear matrix has been shown to reflect cell type specificity [7]. Electrophoresis of nuclear matrix proteins from five different human cell lines on two-dimensional gels resolved sets of common and unique proteins among the different types. An extension of this study using mouse, human, and rat cells led to the observation that a high proportion of the murine common nuclear matrix proteins can also be found in rat and human cells [50]. The commonality of these "minimal matrix proteins" indicates evolutionary conservation with a proposed role in differentiation state-independent nuclear processes. Thus, detection of the unique nuclear matrix proteins will have clinical significance.

The feasibility of finding widespread unique nuclear matrix proteins among different tissue types may be possible when considering that the nuclear matrix may play a role in gene activation. Each cell contains the entire amount of DNA of the organism, yet only specific genes in each cell are active depending on the differentiation of that cell into its specialized function. The nuclear matrix is

involved in the nuclear processes of the DNA and RNA which was briefly explained in Section 2. Perhaps the localization of unique nuclear matrix proteins confers the specificity for gene regulation in each specific cell type. Certain evidence has been reported for this idea. An important regulatory element in the

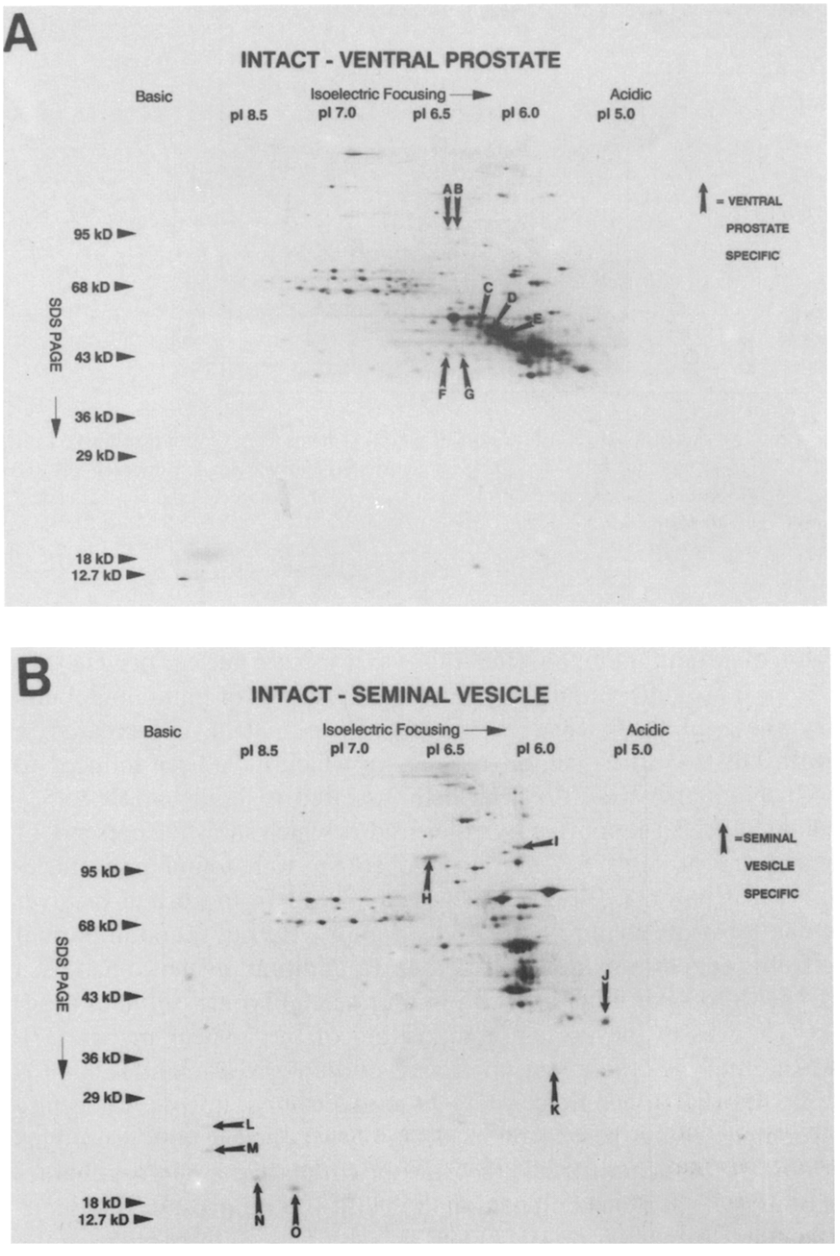

Fig. 4.

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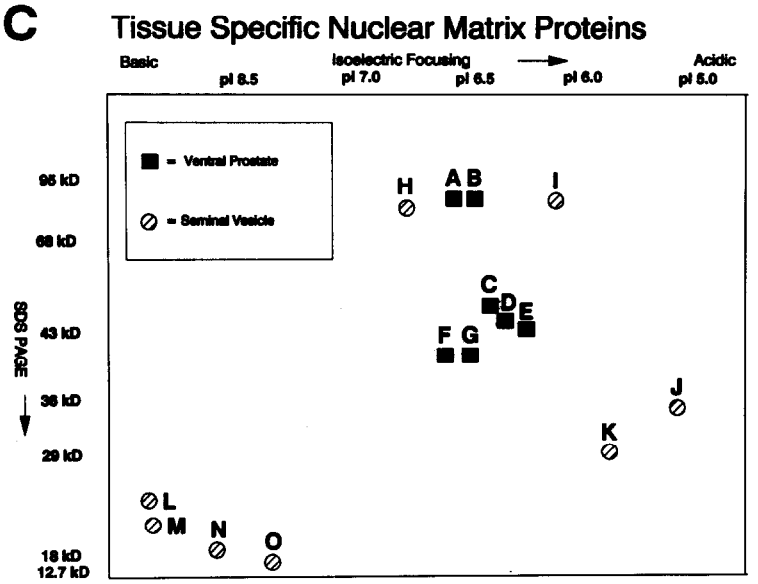

Fig. 4. High-resolution two-dimensional gels of rat nuclear matrix proteins from ventral prostate (A) and seminal vesicle (B). A 15-μg amount of isolated nuclear matrix protein was loaded on each gel. (C) Schematic of major tissue-specific nuclear matrix proteins of the ventral prostate and the seminal vesicle. (Reprinted from ref. 59 with permission.)

promoter region of immunoglobulin genes binds to a specific nuclear protein [51]. This protein appears to differentially regulate the expression of immunoglobulin genes. The synthesis of the nuclear matrix protein, numatrin, is increased in correlation with DNA synthesis in T lymphocytes which have been induced to proliferate [52]. Numatrin has also been demonstrated to bind to DNA [53]. These limited data are also supported by other studies which show the presence of unique nuclear matrix proteins. Several phosphoproteins were found in the nuclear matrix of a rat hepatoma cell line which were absent from normal rat liver [54,55]. Two nuclear proteins in primary breast tumor were not found in normal breast tissue from reduction mammoplasty [56]. In addition, evidence has been shown where the process of differentiation in murine embryonic cell lines is accompanied by changes in the protein composition of the nuclear matrix [57]. Chemical induction of the differentiation process of immortal human HL60 promyelocytes into mortal adherent cells by O-tetradecanoylphorbol 13-acetate (TPA) was accompanied by the phosphorylation of two nuclear matrix proteins [58]. Furthermore, it was demonstrated that the nuclear matrix undergoes characteristic alterations in its protein composition in the rat ventral prostate and seminal vesicle upon androgen withdrawal [59,60]. This can be seen by comparing the two-dimensional gels in Fig. 4A and B with those in Fig. 5A and B. Unique

nuclear matrix proteins were found to be tissue-specific and a set of nuclear matrix proteins appeared or disappeared upon androgen withdrawal. The schematic representation of these proteins is illustrated in Fig. 4C and Fig. 5C, respec-

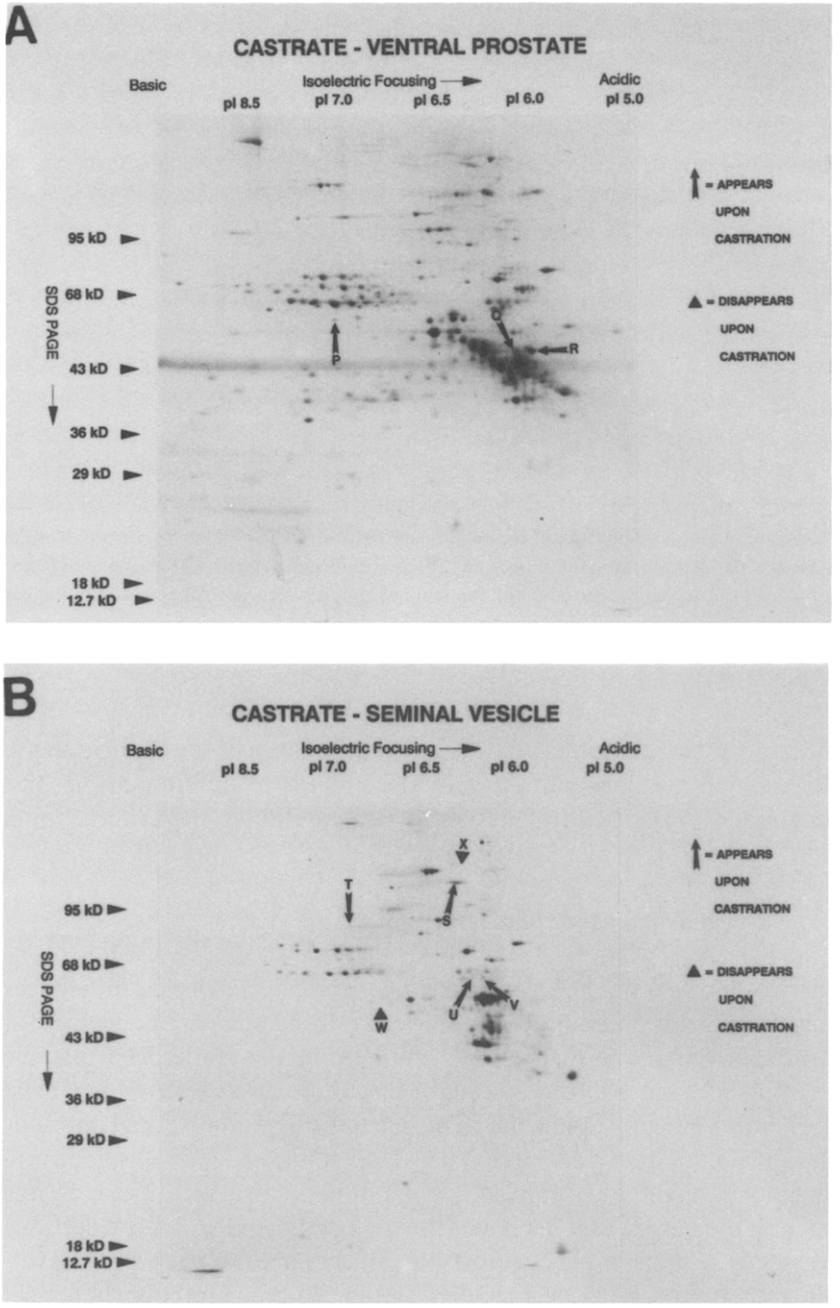

Fig. 5.

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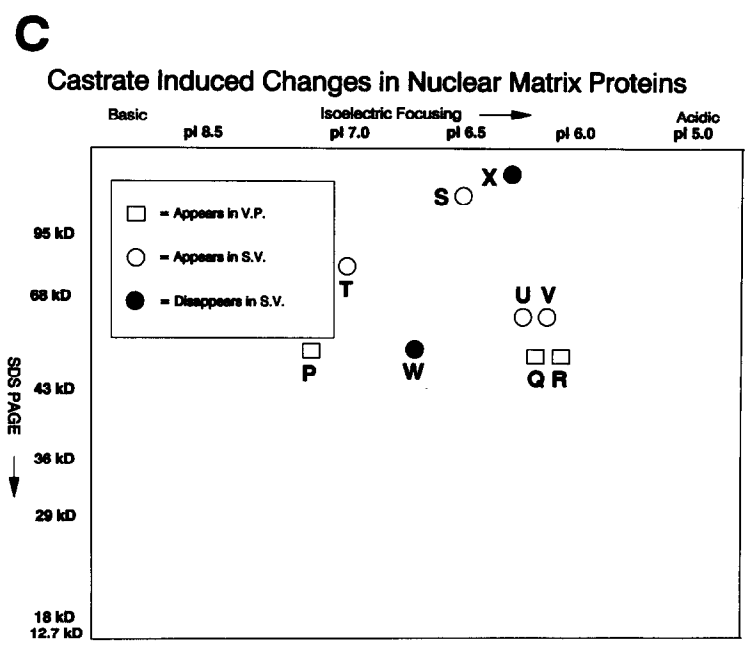

Fig. 5. High-resolution two-dimensional gel of rat nuclear matrix proteins from castrate ventral prostate (A) and seminal vesicle (B). A 15-μg amount of isolated protein was loaded per gel. (C) Schematic of major nuclear matrix proteins of the ventral prostate and the seminal vesicle which both appear and disappear after castration. (Reprinted from ref. 59 with permission.)

tively. Consequently, there is sufficient documented evidence of the changes in the nuclear matrix which are concomitant with the process of differentiation. The induction of unique proteins throughout this process fulfills the criteria for a tumor marker.

4.2. Development of monoclonal antibodies as diagnostic probes

Although two-dimensional gels can determine tissue-type specific differences in nuclear matrix proteins and differences between normal and carcinogenic cells, it is still a difficult technique to perform routinely in the clinic. The massive information attained from two-dimensional gels can be applied to a diagnostic agent which is more clinically applicable. The generation of monoclonal antibodies from relevant unique proteins would be a good solution.

Tumor diagnosis/staging determines the type of neoplastic cell (tissue origin) and subsequently the nature and degree of the malignancy. Histological sections of tissue specimens such as biopsies or cervical smears are the basis of the evaluation. Cellular morphology is used to identify the cell type and morphological changes indicate the degree of malignancy. These determinations are tedious and

extremely subjective. A method utilizing an antibody or mixture of several antibodies to screen the histological sections would eliminate many of the current problems. Binding of the nuclear matrix protein primary antibodies to fixed cells would be followed by a secondary antibody containing a fluorescent dye or enzyme linked to it. Visualization results from emitted light upon excitation or chemical cleavage producing color, respectively [61].

Antibodies have been produced to tumor-specific nuclear matrix proteins. Antibodies recognize specific nuclear matrix proteins in rat hepatoma but not normal rat liver [54,62]. In humans, monoclonal antibodies have been shown to react with nuclear proteins from malignant cells of primary breast tumors [56]. Immunoblotting of normal breast from reduction mammoplasty showed no signal.

Thus, a basis for screening malignant tissue to render a diagnosis for treatment can be quick and efficient with the appropriate antibodies to nuclear matrix proteins.

4.3. Nuclear matrix proteins as diagnostic agents for other diseases

Diagnosis of cellular transformation to a malignant state will be an enormously beneficial use of nuclear matrix proteins as the incidence of cancer is already significant and is constantly rising. However, the diagnostic capabilities of nuclear matrix proteins extend beyond cancer into other diseases. Autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, scleroderma, and mixed connective tissue disease can be characterized by the presence of antinuclear matrix antibodies in the patient's serum [63,64]. For each disease antinuclear matrix antibodies appear to recognize different antigenic components of the matrix. Systemic lupus erythematosus patients have antibodies which recognize the RNase-resistant antigen Sm (basically represented by six small nuclear RNA molecules, snRNA) while mixed connective tissue disease patients possess antibodies which recognize ribonucleoprotein (RNP) [65,66]. Patients with rheumatic diseases possess antibodies which recognize neither of these nuclear antigens [67]. The immunofluorescent pattern elucidates a different nuclear staining than either of the nuclear antibodies of the other diseases.

In addition, the detection of early stages of slow growing viral infection is possible. Virus must replicate using cellular components and cellular replication is associated with the nuclear matrix. Evidence has been demonstrated that alterations in the nuclear matrix structure result after adenoviral infection [68]. Electron micrographs illustrate virus-related inclusions appearing in the nucleus. These virus centers consist of amorphously dense regions, granular regions, and filaments connecting these regions. In addition, the nuclear lamina becomes crenulated and chromatin condenses onto the nucleoli. Although two-dimensional polyacrylamide gels were not performed to illustrate specific protein changes in the isolated nuclear matrix, they probably do exist. Since the nuclear matrix is involved with many of the ongoing functions in the nucleus diagnosis of addition-

al diseases will become more evident as diagnostic agents are developed for known diseases with nuclear matrix alterations.

5. CONCLUSIONS

Nuclear matrix proteins play important functional roles in the cell. Changes in cellular condition also cause changes in the nuclear matrix. These nuclear matrix protein changes can be detected by two-dimensional gel electrophoresis. Utilization of this information can lead to the development of diagnostic agents to detect various diseased conditions of the cell and organism.

6. ACKNOWLEDGEMENT

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