SUMMARY

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The nuclear matrix is a non-histone proteinaceous skeletal structure in the interior of the nucleus, having attachment sites for DNA replication. The term 'nuclear matrix' was assigned to this nucleoprotein fibrillary network by Berezney and Coffey (1974). DNA tightly associated with the nuclear matrix contained A-T rich sequences and therefore termed as matrix associated regions (MARs) or scaffold associated regions (SARs). In eukaryotes the nuclear matrix has been implicated in DNA replication, trascription, regulation of gene expression, post-transcriptional processing, RNA splicing, carcinogen binding, oncogene proteins and binding to certain hormone receptors. The nuclear matrix is now considered as a definitive structure with few defined functions and several yet to be identified functions.

The nuclear matrix can be structurally compartmentalized into nuclear envelope, internal chromatin matrix and residual nucleoli. In terms of its composition, the nuclear matrix contains protein (≈ 10 %), RNA (≈ 2 %), DNA (≈ 1 %) and phospholipids (≈ 2 %). Though the quantitative composition of the nuclear matrix components is approximately identical among all most all cells, qualitatively and functionally the nuclear matrix protein composition has been shown to vary. Further, the knowledge on proteins associated with the nuclear matrix apart from Ianiins is very limited and in view of their functional importance, characterization of the nuclear matrix associated proteins from brain has been attempted in this study.

Brain is a complex tissue having regional, temporal and cellular heterogenity. Of all the variations, cell heterogenity is further intricate due to the co-localization of non-dividing neurons and dividing glial cells.

The characterization of proteins associated with the nuclear matrix is not fully analyzed excepting for lamins A, B and C in liver. Whether or not the nuclear matrix protein composition has any tissue specific pattern is also not known. Since, the nuclear matrix has been implicated in DNA replication, does this structure has something to do with cell division ?, If so, whether there are changes in the matrix protein composition in non-dividing and dividing cells such as neurons and glia. When this protein composition data is generated from dividing and non-dividing cells, will it give any clues (or can it be used) in identifying or to understand the molecular mechanisms in abnormally dividing cells such as Cancer cells.

Attempts are made in this study to separate the nuclear matrix proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two dimensional electrophoresis (2DE) involving Iso-electrofocussing (IEF) and SDS-PAGE, purification by diethylaminoethyl cellulose (DEAE) & high performance liquid chromatography (HPLC) and characterization by single strand deoxyribonucleic acid (ssDNA) binding activity. Polyclonal antibodies were developed against the nuclear matrix proteins and the antibodies were used for immunological characterization of the nuclear matrix proteins interms of a) regional heterogenity in brain, b) tissue specific expression, c) development specific expression and d) screening against nuclear extracts of chosen cancer cell lines having same cellular origin.

The nuclear and nuclear matrix protein composition was analyzed in 1. neurons (postnatally after 15 days of birth the differentiation stops) 2. glial cells (differentiating cell type) of different age groups of rats (10 day, 45 days, 180 days 3. cancer cell lines such as C6 glioma (induced by nitroso-methyl urea), Neuro-2A (spontaneous neuroblastoma), IMR-32

(mixture of neuroblastoma and hyline fibroblast), SK-N-SH (bone marrow epithelial like neuroblastoma having neurogenic origin) and U-373MG (epithelial like glioblastoma, astrocytoma grade III, from a malignant glioma brain).

The isolation of neuronal and glial nuclei was done according to Thompson (1973) from cerebral cortex of rat with minor modifications. The purity of neuronal and glial nuclei was improved by using sucrose cushions in every centrifugation. Clear cut differences were observed in SDS-PAGE and two dimensional elctrophoresis (2DE) patterns of nuclear protein composition in neuronal and glial nuclei. There have been considerable differences in the composition of nuclear proteins in 10 day, 45 days and 180 days samples of neuronal and glial cells, suggesting that nuclear protein composition is dependent on developmental and cell type heterogenity in brain. However, in the nuclei from 45 days old rat brain, maximum number of proteins are present when compared to 10 days and 180 days rat. But for this, difference in protein composition between 45 days to 90 days was minor. So, 45 days old rat brain has been selected for further experiments.

The nuclear matrix was prepared by two methods, the high salt extraction method (HSM) and low salt extraction method (LSM) using lithium diiodosalicylate according to Mirkovitch *et al.*, (1984). The protein composition of nuclear matrix in neurons and glia showed slight variations, which mainly depends on the methods of nuclear matrix preparations (HSM and LSM). Scanning elctron microscopic (SEM) studies showed that nuclear matrix preparation whether by HSM or LSM do not show any morphological variation.

The low salt method preparation yields a matrix having relatively less protein composition than by high salt method. However, lamins are retained irrespective of high salt or low salt preparation. The purification of matrix associated proteins by DEAE chromatography or by HPLC are only partially successful. The elute fractions obtained in chromatography apparently show homogenity on SDS-PAGE gels, but when resolved on two dimensional electrophoresis show very high polypeptide heterogenity. Some of the nuclear matrix proteins other than lamins showed binding to single strand DNA, which were eluted at 0.2 M and 0.4 M ionic strength suggesting regulatory functional importance.

In view of difficulties in purification, polyclonal antibodies were developed for total matrix associated proteins in neurons and glia. The four antibodies, NHSM-Ab (antibodies were raised against the neuronal nuclear matrix proteins extracted by high salt method), NLSM-Ab (antibodies were raised against the neuronal nuclear matrix extracted by low salt method), GHSM-Ab (antibodies were raised against the glial nuclear matrix proteins extracted by high salt method), GLSM-Ab (antibodies were raised against glial nuclear matrix proteins extracted by low salt method) in general, showed immune reactivity having several similarities. But the antibodies prepared by low salt method gave a better immune reactivity in both neurons and glia.

Two proteins ≈ 71 kDa and 60 kDa showed immune response in glial cells by LSM antibodies, which are yet to be characterized. The regional heterogenity studies showed that brain stem had a high immune reactivity while mid brain showed least.

The data for tissue specific pattern suggests that some of the nuclear matrix proteins are brain specific. However, two minor proteins are

noticed to be shared by kidney and heart. In particular the LSM antibodies are highly specific for brain and other tissues showed least reactivity.

When the antibodies were used for screening nuclear proteins from tumors by employing nuclear extracts of tumor cell lines (such as C6 glioma, Neuro-2A, U-373MG, SK-N-SH and IMR-32) immune responses were obtained in tumors having identical cellular origin. The neuronal nuclear matrix antibodies were not of much use in terms of their immune reactivity with the tumor cell types.

The GHSM-Abs showed 66 kDa, 45 kDa and 36 kDa reactive species in tumor cell lines having identical cellular origin like neuroblastoma and glioblastoma (C6 glioma). The GLSM-Abs showed a 29 kDa band in all these tumors having identical cellular origin. A tumor cell type with different cellular ancestory like that of IMR-32 did not react with these antibodies. The immune responses with specific proteins in tumors of identical cellular origin suggest possible utility of these antibodies in early tumor development.

In summary, this study showed that nuclear matrix protein composition is not dependent on cell division status, such as non-dividing and dividing cells. But some of the nuclear matrix proteins could be possibly tissue specific, while selective nuclear matrix proteins could still be possibly used in cancer detection, as cancer cell lines of similar cellular origin show up specific nuclear matrix patterns.