CHAPTER 10

MARs AND MARBPs

Key modulators of gene regulation and disease manifestation

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Abstract: The DNA in eukaryotic genome is compartmentalized into various domains by a series of loops tethered onto the base of nuclear matrix. Scaffold/ Matrix attachment regions (S/MAR) punctuate these attachment sites and govern the nuclear architecture by establishing chromatin boundaries. In this context, specific proteins that interact with and bind to MAR sequences called MAR binding proteins (MARBPs), are of paramount importance, as these sequences spool the proteins that regulate transcription, replication, repair and recombination. Recent evidences also suggest a role for these *cis*-acting elements in viral integration, replication and transcription, thereby affecting host immune system. Owing to the complex nature of these nucleotide sequences, less is known about the MARBPs that bind to and bring about diverse effects on chromatin architecture and gene function. Several MARBPs have been identified and characterized so far and the list is growing. The fact that most the MARBPs exist in a co-repressor/ co-activator complex and bring about gene regulation makes them quintessential for cellular processes. This participation in gene regulation means that any perturbation in the regulation and levels of MARBPs could lead to disease conditions, particularly those caused by abnormal cell proliferation, like cancer. In the present chapter, we discuss the role of MARs and MARBPs in eukaryotic gene regulation, recombination, transcription and viral integration by altering the local chromatin structure and their dysregulation in disease manifestation

1. INTRODUCTION

The eukaryotic nuclei once referred to as "merely a bag of chromatin" has now been recognized to be a highly ordered structure or a 'hub' of cellular activities. The nucleus is seen as a three dimensional mosaic of nucleolus, inter-chromatin regions and condensed chromatin, dispersed in a nuclear ground substance

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traditionally called the "Nuclear Matrix" (NM). The NM is a dynamic fibro-granular structure postulated to contain chromatin and ribonucleoprotein domains (Berezney and Coffey, 1977; Smith *et al.*, 1984), serving as the structural milieu for gene function. The multitude of genomic functions occurring in nuclear matrix include gene replication (Smith and Berezney, 1980; Berezney and Buchholtz, 1981), transcription (Jackson and Cook, 1985) and RNA splicing and processing (Mariman *et al.*, 1982; Zeitlin *et al.*, 1987). All the cellular processes are highly coordinated and programmed and this demands that the genome be organized as a set of genes and gene clusters. Such an orderly arrangement of nuclear domains is brought about by anchorage of specific sequences to the matrix at interphase (Berezney and Coffey, 1974) and chromosomal scaffolds during mitosis (Mirkovitch *et al.*, 1984). These signature sequences known as S/MARs (Scaffold/ Matrix Attachment Regions) serve as boundary elements that punctuate chromosomal DNA into topologically restricted functional units, defining borders between chromatin domains (Breyne *et al.*, 1992; Chung *et al.*, 1993). Several studies also indicate the insulator nature of these elements, that together control single or multiple gene expression, by serving as Locus Control Regions (LCRs) (Grosveld *et al.*, 1987). MARs are also known to aid cell specific expression by their co-habitation with enhancers (Forrester *et al.*, 1994) and reduce the position effect variegation from local chromatin structures (Blasquez *et al.*, 1989). This is done by recruiting topoisomerase II (Razin *et al.*, 1991; Berrios *et al.*, 1985) and absorbing torsional stress by their base unwinding ability (Bode *et al.*, 1992). Thus they orchestrate topological organization of functional chromatin domains. Since MARs organize and govern the accessibility to local chromatin structures, they are also targets for viral integration and replication. Recent reports indicate the effect of MARs juxtaposed to retroviral integration sites on human genome. Several studies report that integration is favored near DNaseI hypersensitive sites or active genes (Mooslehner *et al.*, 1990). Small nuclear genome containing tumor viruses like HPV16, HBV, SV40, and HTLV-1 have also been shown to integrate near MARs (Shera *et al.*, 2001). Recent observations regarding the retroviral integrations reveal that HIV-1 and MoMuLV favor active genes for their integration (Schroder *et al.*, 2002; Mitchell *et al.*, 2004) Most of these integration sites (95%) have been shown to be flanked by S/MARs, around 1 Kb region of integration, that could serve as promoters (Kulkarni *et al.*, 2004; Johnson and Levy, 2005). In this context, specific proteins that bind to S/MARs, called MARBPs become significant, as they govern the chromatin accessibility of the region. For example the ratio of SATB1 and Cux/CDP, two well known MARBPs in various tissues for Mouse Mammary Tumor Virus region (MMTV) defines the transcriptional status of the virus. (Zhu *et al.*, 2000; Liu *et al.*, 1999). Similiarly, a study by Stunkel *et al.,* showed that Cux recruits HDAC1 to the LCR MAR of E6 promoter of Human Papilloma Virus (HPV) and represses this oncoprotein (2000).

MARBPs have been routinely isolated using high salt extraction of the matrix and their ability to have base unpairing potential (Galande and Kohwi-Shigematsu, 1999). Several studies report the ability of these MARBPs to control

gene expression by binding to MAR sequences within the regulator regions of the gene and activate or repress the gene expression. They are often found in a complex with co-activators or co-repressors, modulating gene function by remodeling or covalently modifying the chromatin structure. Most of these MARBPs have been shown to be drastically affected upon malignant transformations. Since aberrant gene expression gives rise to malignancies and abnormal cell cycle progression, understanding the nature and order of these proteins assumes great importance in the current scenario of chromatin biology and disease manifestation.

2. DEFINING MARs

The available literature on MARs reveals that they can enhance the expression of reporter genes by forming a domain and insulating them from position variegation effect at the integration site (Breyne *et al.*, 1992; Allen *et al.*, 1993). Their main function is to bring together control elements like promoters and enhancers loaded with their transcriptional factors, creating a transcription factor and enzyme rich nuclear microenvironment.

While there is no stead and fast rule for identifying MARs, certain traits make the AT rich DNA elements function as MARs. The AT richness confers DNA unwinding potential, so that in their single stranded form specific DNA unwinding proteins bind to MARs, relieve the torsional stress and the energy is used to relax positive supercoiling generated ahead of transcription elongation point (Bode *et al.*, 1992). Studies by Amati *et al.*, (1990) and von Kries *et al.*, (1991) although suggest that this property of MARs might not be essential for matrix binding. A number of studies show that MARs can allow the induction of DNaseI hypersensitive sites in chromatin. This has been substantiated with several experimental observations that MAR sites overlap with DNase I hypersensitive sites. The proposed mechanism is that MARs become DNAse I hypersensitive at the time of their activation as a result of binding of single strand binding proteins (SSBPs) specifically during transcription and replication due to removal of nucleosomes that preludes ORI activation (Hsieh *et al.*, 1993).

Several triple helical or Z form, cruciform structure of MARs has also been proposed. The cruciform loop part is susceptible to DNaseI and hence characteristic nicking occurs. The role of MARs in chromatin dynamics has also been tested using an artificial MARBP called MATH 20 (Strick and Laemmli, 1995). This protein has numerous linked DNA binding domains called AT hooks, which preferentially bind to AT tracts. Since this protein could then bind MARs and associated chromatin specifically, this was used to study chromatin condensation in Xenopus oocytes. It was found that titration of MARs with MATH 20 specifically inhibited chromatid conversion without inhibiting condensation resulting in abortive mitotic structures. When MATH 20 was added to chromatids, it leads to a structural collapse and formation of chromatid balls. Thus, it is speculated that S/MARs could be target of binding proteins that mediate or facilitate formation and juxtapositioning of

metaphase chromatin loops (Hart and Laemmli, 1998). Similarly, MAR sites have been found to be sites of illegitimate recombination, since these elements are found at the site of DNA insertion, deletion and translocation (Sperry *et al.*, 1989; Shapiro *et al.*, 1987).

List of well known MARBPs and functions

Name	Species	Tissue specificity	Function	Interactome
SATB1/L2a-P1/	*Homo sapiens, Mus musculus*	Predominantly thymus, minute amounts in brain	Repressor	CDP, HDAC1, ACF1, ISWI
Nucleolin	*Homo sapiens*	erythroleukemia cell line K562	Glycosaminoglycan stabilisation	Glycosaminoglycans
SAF-B	*Homo sapiens*	Ubiquitous	Repressor	RNA Pol II
SAF-A	*Homo sapiens*	HeLa, Embryonic kidney cell line 293, K562	Activator Organization of chromosomal DNA & packaging of hnRNA	p300, DNA PK
NFμNR/ Cux/ CDP	*Mus musculus*	Breast, Ubiquitous	Important in organ development	SATB1, HDAC1, SMAR1
p114	*Homo sapiens*	Infiltrating ductal carcinoma tissues, normal breast tissue, benign breast diseases	combined property of PARP and SAF-A (hnRNP-U)	PARP, Ku
Bright	*Mus musculus*	Ubiquitous	B cell regulator of immuno-globulin heavy-chain transcription	Sp100, LYSp100/p140
SP100	*Homo sapiens*	Ubiquitous	Repressor of Bright	Bright
Ku (subunit of PARP)	*Homo sapiens*	SKBR3 cell line	chromosome condensation, subunit of DNA-dependent protein kinase	PARP
DNA-PK	*Homo sapiens*	Breast cancer	downregulated during cellular senescence	PARP, SAF-A

LYSp100/p140	*Homo sapiens*	B cells	Co-activates Bright	Bright
SMAR1	*Homo sapiens, Mus musculus*	Ubiquitous but predominant in thymus	Represses transcription, downregulated in breast cancer cells	P53, HDAC1, mSin3a
MeCP2/ ARBP	*Homo sapiens*	Ubiquitous, particular high levels in neuron of the post natal brain	Mutations in the MECP2 gene cause Rett syndrome	mSin3a, HDAC1, HDAC2

3. ROLE OF MARBPs IN MODULATING MARs AS FUNCTIONAL ELEMENT

Gene expression status involves changing of attachment points of chromatin loops and hence implies the dynamicity of MARs and its association with nuclear matrix, regulated and governed by cell type (Liebich *et al.*, 2002). The intrinsic activity of MAR is not sufficient to bring about position effects or transcriptional regulation but may depend on the contribution of protein factors that specifically bind to these motifs. These are classified based on their recognition sites as: abundant multifunctional matrix proteins like High Mobility Group proteins (HMGs), transcription factors like H box, Y box and CAAT binding proteins. Some class of proteins like ARBP, MeCP2, SATB1, CDP and SMAR1 also interfere with the MAR- matrix productive effects on transcription, unlike Bright that activate transcription. The varied effects of MARBPs could involve changes in the chromatin structure and activity by the recruitment or interaction with chromatin remodeling complexes. For example MAR binding repressors like ARBP, MeCP2, SATB1, SMAR1, CDP and CRBP mediate transcriptional repression by recruitment of components of histone deacetylating pathways. This is in contrast with activator MARBPs like SAF-A and Bright that recruits SWI or p300 and affect the acetylation and remodeling of chromosomes. Apart from affecting chromatin remodeling, gene regulation is also achieved by the direct interaction of MARBPs like SAF-B with RNA polymerase II and/ or with RNA processing factor. Hence, MARBPs serve as molecular base to assemble transcriptome and chromatin remodeling network. (Fig. 1)

4. MARBPs WORK IN CONSORT

Proteins that mediate MAR function (MARBPs) appear to control numerous genes expressed in differentiated cells. SMAR1, one such MARBP, has been shown to be ubiquitously expressed in all tissues, predominantly in thymus and governs the transition of T cells from DN to DP stage. SATB1 is expressed predominantly in thymus but also in brain and several other organs, while CDP expression occurs

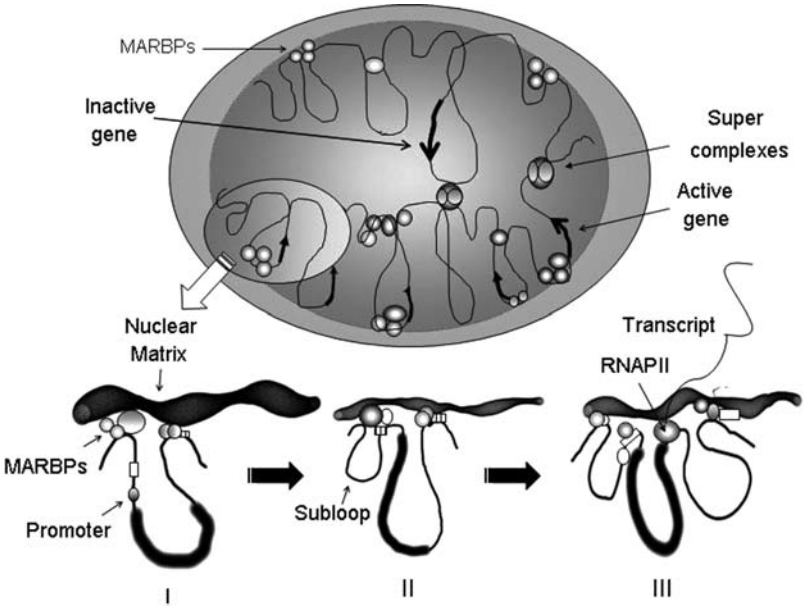

Figure 1. MARs serve to enrich the nuclear milieu by bringing in transcription factors and enzymes to regulate transcription

in all but terminally differentiated cells. Binding of SATB1 and CDP to regulatory elements has been associated with transcriptional repression of numerous genes expressed in differentiated cells. For instance, SATB1 regulates gene expression both spatially and temporally during T-cell development. Recently, SATB1 null-mice were generated, and genome-wide expression profiling analysis indicated that about 2% of all genes become significantly derepressed in thymocytes (Alvarez *et al.*, 2000). In contrast, the activator Bright is present in differentiated cells, where it may compete with repressors for MAR-binding sites. Thus, there would be a dynamic process of activation/ repression forming temporary sites of DNA attachment. Bright overexpression was found to enhance transgene expression, indicating that the normal activator protein level is limiting. In contrast, cotransfection of CDP abrogated Bright transactivation and reduced the basal expression level, indicating that CDP is able to override the DNA binding and/or transactivation capacity of Bright (Herrscher *et al.*, 1995). Some MARBPs, depending on the context, either function as transcriptional repressors or activators. MARBPs such as Cux/CDP and SATB1 can function as transcriptional repressors in non-B cells by interacting with their target MAR sequences flanking the IgH intronic enhancer (Alvarez *et al.*, 2000). On the other hand, the MARBP Bright acts as a transcriptional activator in B cells (Schubeler *et al.*, 1996) and this activation is context-dependent as it requires an intact IgH enhancer core (Zahn-Zabal *et al.*, 2001).

Different degrees of repression could be mediated by competition between repressors and activators for MAR binding**.** For example, MARβ, which resides 400 bp upstream of TCRβ enhancer (Eβ), is the docking site for three MARBPs: SMAR1, Cux and SATB1 (Chattopadhyay *et al.*, 2000). MARβ (HS1) is the major DNase I hypersensitive site induced during the TCR co-receptor $CD4^{-}CD8^{-}$ double negative (DN) to $CD8^{+}CD4^{+}$ double positive (DP) stage of thymocyte development. This induction is concomitant with halt of TCRβ V(D)J recombination in DP thymocytes. Studies by Chattopadhyay *et al.*, (1998) identified that SMAR1 binds to this region along with Cux and SATB1 (Fig. 2).

The 114 Kd protein PARP, exclusively found as a mixture with DNA-PK has been implicated in repair after DNA damage. Evidences show that PARP and Ku 70/86 interact together and synergistically enhance their binding to DNA (IgH MARs)

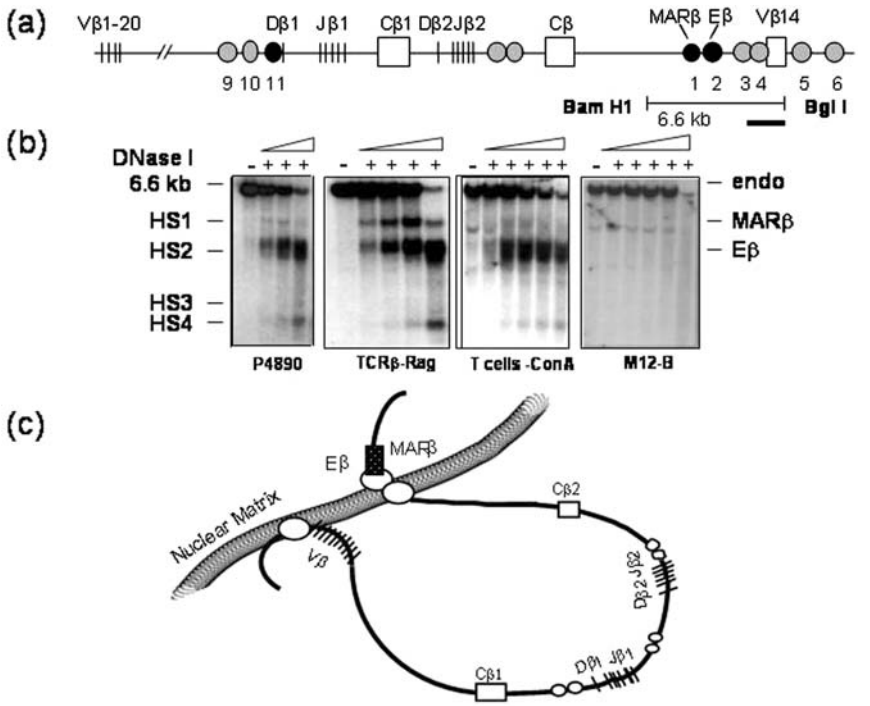

Figure 2. TCRβ locus showing eleven DNase1 hypersensitive sites. (a) HS1 and HS2 represent Eβ enhancer and MARβ sites respectively that are located upstream of Vβ14 segment. (b) DNaseI hypersensitivity assay showing four sites within a 6.6 kb BamH1-BglII genomic fragment. Panel 1: P4890 representing Rag mutant double negative (DN) lymphoma T cell line. Panel 2: DP cells of Rag mutant TCRβ transgenic mice. Panel 3: Mature single positive T cells from lymph node stimulated with ConA. Panel 4: Mature B cell line. No DNaseI hypersensitive sites were observed in B cells indicating that all four hypersensitive sites are specific for T cell and (c) A current model of possible role of MAR sites in maintaining cross-talk between Eβ enhancer, MARs and MARBPs that together control V(D)J recombination and transcription at TCRβ locus

(Galande and Kohwi-Shigematsu, 1999). Similarly, PARP and SAF-A contribute to the MAR binding ability of p114. Together, these observations suggest that the different MAR-binding proteins associate with diverse sets of proteins involved in transcriptional regulation and also cross-talk amongst each other, constituting a functional nuclear matrix.

5. MARBPs AND DISEASE MANIFESTATION

Since most cases of malignant transformations occur by means of deregulation of genes or viral integrations, the role of MARs and MARBPs become crucial. The composition of nuclear matrix has been reported to be altered during the course of transformation and the MARBPs that associate with transcriptional units might be involved in the progression/ cessation of disease. The uncontrolled cell proliferation and invasion of the cancer cells would naturally require an altered organization of the chromatin, to assure the differential expression of a subset of proteins compared to their normal counterparts. These genes have to be expressed or repressed ectopically. For example, the expression pattern of nuclear matrix proteins is considerably different between normal breast epithelial cells and malignant cells (Khanuja *et al.*, 1993).

The abnormal levels of MARBPs, apart from their altered cellular distribution, seems to govern the progression of proliferative diseases. Studies to identify the nuclear matrix binding proteins associated with aggressive cancer phenotype have lead to the identification of PARP, Ku, High mobility group proteins (I/Y), NMP, SAF-A/B that have binding affinity to double stranded BURs. The expression of these proteins is dramatically increased upon malignant transformation and marks the advanced cancer phenotype leading to metastasis. Unlike other MARBPs that are highly expressed at the onset of malignant transformation, SMAR1 level is downregulated in breast cancer derived lines, which may be explained in part to its regulation of Cyclin D1 gene, a hallmark of breast and prostate cancer (Rampalli *et al.*, 2005). In most cases of cancer, there is gene duplication or loss of transcriptional control of this gene that leads to uncontrolled cell proliferation, leading to cancer. We have shown that SMAR1 forms a co-repressor complex with HDAC1 and mSin3a, recruits this complex to the Cyclin D1 promoter on a segment that is rich in ATCs (putative MAR sequence as evaluated by MAR finder program MAR-WIZ). The recruited complex then deacetylates histones (H3K9 and H4K10) at the loci, leading to chromatin condensation and eventually shuts down transcription. (Fig. 3)

Several reports have highlighted the ability of such MARBPs to recruit chromatin modifying enzyme complexes to control gene transcription, showing that differential expression of these proteins is critical too. For example in breast cancer derived cell lines, SMAR1 levels are downregulated that correlates to an induced Cyclin D1 levels. Another example is HMG-I, an architectural chromatin protein that binds to minor groove of double stranded DNA. Recent reports suggest the role of HMG-I, C and Y proteins in cellular proliferation and neoplastic transformation

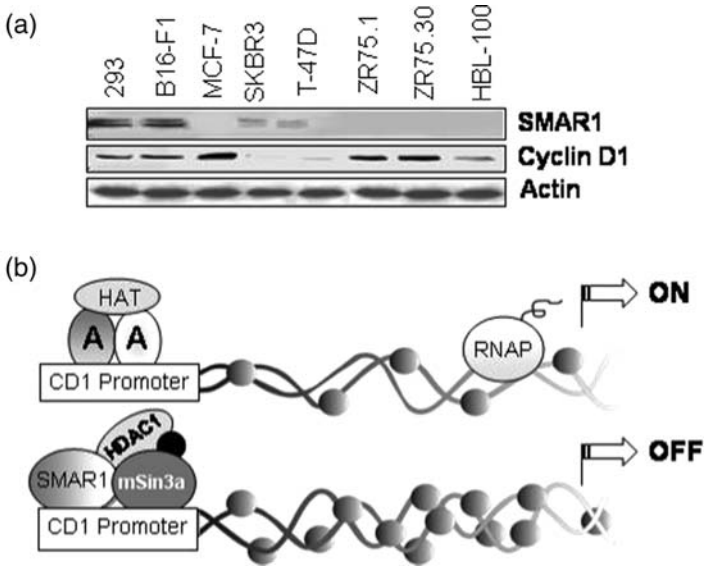

Figure 3. (a) Western blot analysis of breast cancer derived cell lines showing an induced Cyclin D1 levels that correlates to a decreased SMAR1 expression. (b) A schematic representation of SMAR1 recruitment of HDAC1 on CyclinD1 promoter, that switches off the transcription

(Tamimi *et al.*, 1993). HMG-I has been identified as a target gene for c-myc and is involved in c-myc mediated neoplastic transformation. Distant metastasis is reported in animals injected with HMG-I or -C (Wood *et al.*, 2000a, b). The mechanism of transformation has been attributed to the identification of HMG-C AT hooks in chimeric proteins associated with lipomas and mesenchymal tumors (Schoenmakers *et al.*, 1995; Rogalla *et al.*, 1997). These chimeras are thought to function by binding to DNA via the AT hooks and alter the gene regulation by transcriptional regulatory domains they acquire by rearrangement (Ashar *et al.*, 1995, Wunderlich and Bottger, 1997; Bustin, 1999). Apart from this, HMG-C, truncated transgenic mice display gigantism and lipomatosis while the null mice develop pygmy phenotype. (Battista *et al.*, 1999; Zhou *et al.*, 1995). This reveals that the levels of the MARBPs must be kept under a tight leash to avoid abnormal cellular proliferation and transformation.

Protein modifications of MARBPs also assume great importance as their binding properties can be altered. MARBPs like SATB1 and SAF-A get modified with special reference to a particular cellular process. For example, the cleavage of SATB1 by Caspase 6 disrupts the PDZ domain mediated dimerization, resulting in the formation of SATB1 monomers that do not have DNA binding ability. This causes detachment of the protein from chromatin that eventually leads to rapid and efficient disassembly of higher order chromatin structure and facilitates apoptosis in T cells, an important event in T cell receptor rearrangement (Galande *et al.*, 2001).

Likewise, the SAR binding domain of SAF-A loses its DNA binding potential upon proteolytic cleavage during apoptosis (Gohring and Fackelmayer, 1997). There are reports that nuclear matrix proteins associate with granular nuclear bodies and undergo modifications in cells that undergo apoptosis (Zweyer *et al.*, 1997). In most of the cases, the loss of DNA binding ability of MARBPs becomes central to the altered function. For example, Ku deficiency leads to extreme radiation sensitivity and high levels of chromosomal aberrations (Gu *et al.*, 1997). This is due to the fact that the Ku heterodimer (Ku 70/80) binds to DNA double strand breaks and facilitate repair by non-homologus end joining pathway (Walker *et al.*, 2001). In case of invasive breast cancer, a specific nuclear matrix binding protein called NMP has been identified, that recognizes a unconventional MAR in the promoter, stimulates the levels of NFκB, that in turn increases the DNA binding activity of NFκB observed in c-erb2 and BRCA1 positive human breast tumors, suggesting a role in breast cancer progression (Raziuddin *et al.*, 1997). HET/SAF-B was originally cloned as a nuclear matrix protein that binds to the MAR of hsp27 promoter and represses it in breast cancer cells (Oesterreich, 1997). In addition, it has been shown to bind to ER and function as its co-repressor (Townson *et al.*, 2000). There are also reports suggesting that the overexpression of HET causes growth inhibition in M phase of cell cycle and causes multicellularity. Consistent with this, it is also known to cause aneuploidy in breast tumor specimens and causes lower proliferation (Townson *et al.*, 2000). This suggests that these factors might have important and distinct roles in tumorigenesis independent of their transcriptional functions. The MARBPs have also been identified to play major role in normal tissue differentiation and organogenesis. For example, Cux has been shown to play role in specifying the identity of external sensory organs during peripheral nervous system development. It has also been implicated in controlling proliferation and differentiation (Nepveu, 2001). Cux knock out mice show retarded growth, curly whiskers, late development of lung epithelia, defective hair follicle development, infertile male progeny and less number of T and B cells and more myeloid cells (Sinclair *et al.*, 2001, Tufarelli *et al.*, 1998). On the other hand, Cux-1 transgenic mice show organomegaly and hyperplasia of different organs (Ledford *et al.*, 2002).

Certain novel isoforms of MARBPs have been suggested to play key role in disease manifestation. Two isoforms of Cux/CDP were previously known till Goulet *et al.,* showed that a novel isoform of Cux (p75) showed a higher binding affinity to DNA and represses the transcription of CDK inhibitor p21 and activated DNA polymerase a gene promoter. They also identified that a novel intronic transcription initiation was responsible for the expression of this isoform. Although this isoform is predominant in $CD4^+/CD8^+$ and $CD4^+$ T cells, expression was also activated in breast tumor cell lines and in primary human breast tumors. The stable lines expressing p75 isoform could not form tubule structures in collagen but developed as solid undifferentiated aggregates of cells. Some studies also indicate that Cux is a downstream target of Notch, activated in T cell leukemia. Cux could thus serve as a downstream target marker for Notch activation (Goulet *et al.*, 2002). Activation of CDP/Cux at the G_1/S transition involved the proteolytic processing of the protein

to generate a shorter isoform in uterine leiomyomas (Moon *et al.*, 2002). Similarly, CUTL1 activity is associated with increased migration and invasiveness in numerous tumor cell lines, both *in vitro* and *in vivo* transcriptional target of transforming growth factor beta (TGFβ) and a mediator of its promigratory effects CUTL1 expression is significantly increased in high-grade carcinomas and is inversely correlated with survival in breast cancer. This suggests that CUTL1 plays a central role in coordinating a gene expression program associated with cell motility and tumor progression (Michl and Downward, 2006).

SMAR1 is shown to exist in two isoforms, the shorter form having a deletion of 117 bp at the N terminus (Chattopadhyay *et al.*, 2000). The 39 aa deleted shorter form has been shown to be more effective in regressing B16F-10 induced melanoma (Kaul *et al.*, 2003). SMAR1 transgenic mice showed abnormal V(D)J recombination and organomegaly of lymphoid organs with cellular infiltrations, suggesting a necessity for fine tuning of protein expression to continue cellular process (Kaul-Ghanekar *et al.*, 2005) (Fig. 4). This reveals that various isoforms of MARBPs may have different roles in the context of cellular functions.

Apart from serving as modulators of transcription, MARBPs alter cellular functions by their protein interactions. Nucleolin first described by Orrick *et al.*, (1973) is a major nucleolar protein involved in ribosome biogenesis. Recent reports also suggest that nucleolin binds to cell surface adhesion molecules like L-selectin expressed on leucocytes and hemopoeitic stem cell progenitors (Harms *et al.*, 2001). Modulation of hepatitis delta viral replication is also well documented (Lee *et al.*, 1998). This protein is also linked to actin cytoskeleton and inhibits HIV infection by cytokine midkine. Reports by Christian *et al.*, (2003) show that nucleolin on cell surface is a marker for endothelial cells and its interaction with Acharan sulfate (AS) may be a key in solving the mechanism of AS mediated inhibition of tumor growth. The identification of cancer cell specific markers led to the identification of a subset of nuclear matrix proteins (NMPs) that exist in prostrate, bladder, colon and renal cancer (Konety *et al.*, 1998). These NMPs can be detected in the serum samples of the patients because of these factors as tumor cells undergo degeneration and lysis. NMP 22 has been now routinely used to assess bladder cancer and similarly L4 is a candidate for identification of colon cancer (Brunagel *et al.*, 2002).

Several ubiquitous transcription factors like Lys 100/ p140, SP100 are also known to bind to MARs. While p140 is known to co-activate transcription by Bright, SP1 represses transcription. AP-1 family transcription factor is also known to associate with nuclear matrix (van Wijnen *et al.*, 1993) and allow the expression of Igκ gene in response to LPS stimulation (Schanke *et al.*, 1994). p53 another well known transcription factor, the function of which depends on its DNA binding ability to different promoters. Wild type p53 is known to be a tumor suppressor while mutant form exerts oncogenic functions of its own (Dittmer *et al.*, 1993; Levine *et al.*, 1995). The "gain of function" of mutant p53 has been demonstrated partly due to its high affinity to bind to MARs (Will *et al.*, 1998). These MARs have a typical AATATATTT unwinding motif, promoting structural alterations in

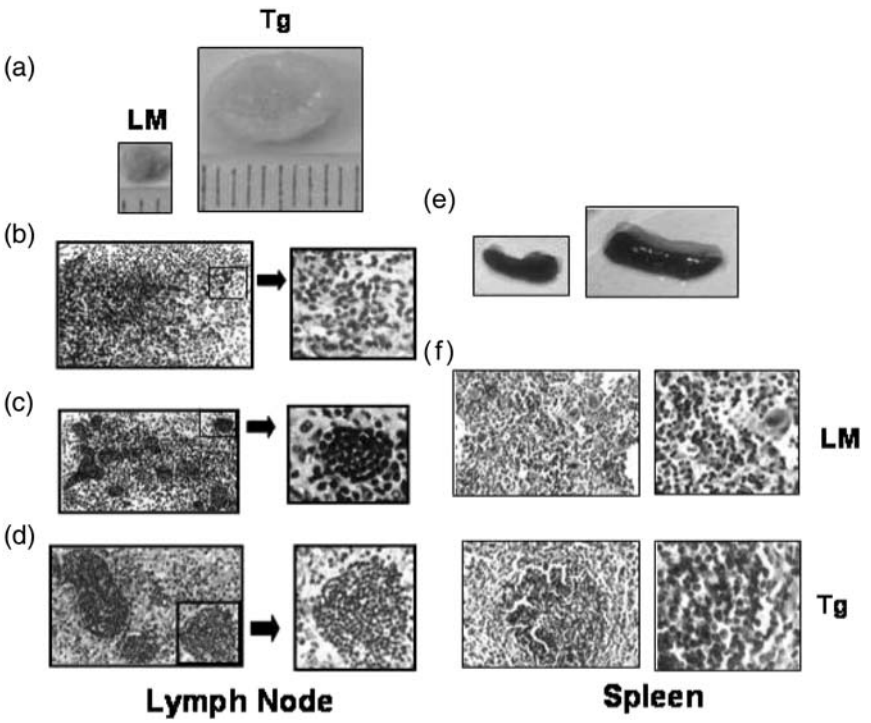

Figure 4. Comparison of lymph node and spleen architecture in SMAR1 transgenic and control littermate mice. (a) Lymph node size of non-transgenic (N) and transgenic (T) mice are shown at the same scale. (b) Histological analysis of lymph node from control at 10X and 40X magnification are displayed. (c and d) Histological sections of Lymph node at 10X and 40X. (e) Enlargement of spleen size shown in transgenic mice compared to control mice. (f) Histological sections of spleen from Littermate normal (LM) and SMAR1 transgenic mice showing strong infiltration of T cells into the lymph node. (See Colour Plate 15.)

chromatin, thereby affecting cellular replication. Since mutations in p53 constitute the most frequent alteration in a single gene in human cancer (Hainaut *et al.*, 1997), the MAR binding potential of the mutant form could form the molecular basis of oncogenic potential documented for mutant p53.

6. MARBPs AS PROGNOSTIC MARKERS AND FUTURE PERSPECTIVES

The aberrant expression of MARBPs upon malignant transformation makes them a reliable marker for diagnosis of advanced diseased conditions. For instance, the NMP 22 levels in urine have now been routinely used to identify bladder and ductal cancer. Similarly, p114 MAR-binding activity has been detected in aggressive tumors, while significantly weaker p114 activity has been observed in

less aggressive tumors. Hence, PARP, an interactor of p114 could be used as a marker for identifying invasive breast cancer. The novel isoform of Cux (p75) is a reliable marker for identifying breast cancer. MAR binding protein, p230, is detectable in rat hepatoma cells but not in normal liver and suggests that this protein is a diagnostic and prognostic marker for liver cancer. It is clear that nuclear matrix proteins hold a considerable promise as diagnostic tools for pathologists.

MARBPs with their newly discovered role as linkers between chromatin remodeling, signal transduction and cell cycle regulation form an important part of chromatin biology. Most importantly, direct correlation of disease manifestation and the MARBPs will make them an important tool in understanding disease progression. Present evidence, suggests that nuclear matrix proteins may be useful biomarkers of neoplastic diseases in the serum, body fluids, and tissues. Nuclear matrix proteins are also potential candidates for the use as tumor prognostic factors and targets of anticancer drugs through apoptosis.

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GLOSSARY

SAR/MARs
Scaffold Attachment Region
SAF-A: Scaffold Attachment factor A
Nuclear matrix binding proteins
Matrix Associated Region Binding lproteins(MARBPs)
Tumor suppressor p53
Nucleases
DNase I hypersensitivity assay
Locus control Region (LCR)
SMAR1
Human immunodefficiemcu Virus 1
HTLV
MMTV
TCRβ locus
Cyclin D1
mSin3A complex

V(D)J recombination
SATBI: Special AT-rich sequuence binding protein I
Cux/CDP
CUTL1
Eβ enhancer

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