“Ignorance is the curse of God, knowledge the wing wherewith we fly to heaven” ....Shakespeare (Henry VI, Act iv, Sc,7)

Introduction &

Review of Literature
Introduction

1.1 Nuclear matrix organization

Temporal and spatial organization of chromatin in three-dimensional scenario play major role in regulating the gene organization, compartmentalization of regulatory factors and thus allow transcription in a well-defined manner (Stein et al., 2004; Stein et al., 2003a; Stein et al., 2003b). Nuclear matrix is a 3-dimensional dynamic proteinaceous meshwork packed with RNA binding proteins, histones and non-histone proteins. This comprises a higher order organization that maintains the overall size, shape and architecture of the nucleus. Nuclear matrix provides docking sites for attachment of matrix binding proteins and DNA loop during interphase and thus regulates DNA replication, transcription and chromosome arrangement (Bode et al., 2000; Martelli et al., 1997; Stein et al., 1999). DNA that are attached to the nuclear matrix are evolutionary conserved 300-1000 bp long DNA sequences, referred as S/MARs (Scaffold/Matrix Associated Regions) and has well-established role in gene transcription (Bode et al., 2004). Control of gene transcription through dynamic association and dissociation of S/MARs with nuclear matrix is normally important in regulating development (Brown et al., 1999; Vassetzky et al., 2000). A hierarchical order of eukaryotic chromatin organization from gene level to 10 nm/30 nm chromatin fiber and higher order three dimensional nuclear matrix organization is represented as a cartoon (Fig. 1). Gene is organized into promoter, intronic, exonic regions with flanking regulatory elements (Fig. 1 A). DNA is packed into 10 nm fiber in association with histones that is further coiled into 30 nm chromatin fiber (Fig. 1 B and C). These chromatin fibers are then organized into loop domain structure through interaction with matrix binding proteins (Fig. 1 D). All together depending upon the distribution of chromatin, nucleus can be divided into heterochromatin and euchromain (Fig. 1 E). Organization of nuclear sub-compartments like nucleolus (stained with nucleolin), nuclear bodies keep on changing with time and that gives the fourth dimension to the chromatin dynamics (Fig. 1 F).
Fig. 1. **Nuclear matrix organization.** A. Schematic representation of gene where blue boxes indicate coding region, green box indicate promoter region, orange and red boxes indicates enhancers or regulatory domains and black line represents non coding regions of DNA. B and C, Diagrammatic representation of DNA packed with core histones in 10 nm and 30 nm chromatin fiber. D, Higher order chromatin loops in association with nuclear matrix binding proteins. E left panel, Mammalian cell nucleus stained with DAPI showing the distinct euchromatin and heterochromatin regions. E right panel, Nucleolin and p53 staining shown as red and green merged with DAPI represent various nuclear compartments such as nucleolus.
1.2 Dynamics of nuclear matrix during cell cycle and development

Nuclear matrix organization and its dynamic association with matrix binding proteins regulate the accessibility of transcriptional machinery or the silencing factors at the gene locus. This in turn governs the process of gene transcription that goes on in a well-defined stimulus or stage specific manner. Alterations in nuclear matrix organization and sub-cellular distribution of matrix binding proteins have been observed during cell development and differentiation. For example, chromatin movements observed in differentiated cells in eye imaginal discs during larval tissue differentiation in Drosophila (Thakar et al., 2005). Nuclear matrix is also reorganized during erythropoiesis and enucleation. During erythroid burst-forming unit (BFU-E) differentiation to pro-erythroblast Lamin B1 interacts with the nuclear envelope and matrix binding protein Numa, Splicing factor Sm and SC35 resulting in nuclear condensation in a selective manner that allows the critical transcriptional processes to be continued irrespective of extensive chromosomal reorganization (Krauss et al., 2005). Another MARBP Parp is involved in transcriptional regulation, telomere cohesion and mitotic spindle formation during cell division, intracellular trafficking and energy metabolism (Schreiber et al., 2006).

The CCAAT Displacement Protein CDP, also known as CUTL1, Cut, or Cux-1, homeodomain transcription factor plays an essential role in development and cell cycle progression (Nepveu et al., 2001; Sansregret et al., 2006; Michl et al., 2006). Cux2 is the second cut ortholog that was a result of gene duplication and is specifically expressed in the neuronal cell lines. It exclusively acts as a repressor in NIH3T3 cells unlike Cux1 that can act as repressor or activator in a promoter specific manner (Gingras et al., 2005). Full length CDP (200 kDa) is a negative regulator of mouse mammary tumor virus (MMTV) transcription while its another isoform of 150 kDa that is a cleaved product of the full length protein, exhibits lower DNA binding activity resulting in increased viral transcript (Maitra et al., 2006). CDP/Cux p110 isoform is generated upon cleavage by Cathepsin L and stably interacts with DNA during S phase while at G2 phase this interaction is inhibited through its phosphorylation at Serine 1237 by Cyclin A/Cdk1 (Santaguida et al., 2005). CDP also
has role in hair follicle morphogenesis and differentiation of lung epithelia (Ellis et al., 2001). CDP/Cux p110 overexpressing cells showed increased proliferation rate and faster entry into the S phase while gene inactivation resulted in longer G1 phase and slower proliferation rate. This was correlated to the Cyclin E and A2 expression that was increased upon p110 CDP/Cux expression and decreased in its absence (Sansregret et al., 2006). Protein kinase A (PKA) that inhibits tumor progression directly phosphorylates CUTF1 and decreases its DNA binding activity and thus affects cell cycle progression and cell motility (Michl et al., 2006). Cux also has role in activating notch signaling and thus is involved in embryogenesis (Sharma et al., 2004). Thus, nuclear matrix and MARBPs play important role in regulating cell cycle progression and development.

1.3 Nuclear matrix changes during differentiation

In addition to play role in transcription pertinent to cell cycle and development, changes in nuclear matrix are also associated with the process of cellular differentiation. Erythroblast macrophage protein Emp showed differential pattern of expression wherein it was associated with the matrix in immature macrophage while localized to the cell surface in mature macrophage (Soni et al., 2007). Another MAR binding phosphoprotein, Nucleolin is involved in regulation of gene transcription, chromatin remodeling and RNA metabolism (Dickinson et al., 1995). Its expression is increased in murine hematopoietic stem cells, compared to differentiated tissue and it activates CD34 and Bcl2 expression in CD34 positive hematopoietic cells (Grinstein et al., 2007). MARBP SATB2 is characterized as a multifunctional determinant of craniofacial patterning and osteoblast differentiation (Dobreva et al., 2006). Expression of many nuclear matrix proteins is altered during hexamethylene bisacetamide (HMBA) induced differentiation of human osteosarcoma MG-63 suggesting their role in carcinoma cell growth and differentiation (Fen et al., 2006). Along with MARBPs, some kinases are also associated with the nuclear matrix such as PKC alpha and zeta are associated with nuclear matrix specifically during
differentiation events while PKC delta is always associated with nuclear matrix during DMSO-induced erythroid differentiation (Marchisio et al., 2005).

MARBP, Runt-related transcription factor, Runx2/Cbfa1 is known to promote osteoblast differentiation upon BMP2 osteogenic signal (Bae et al., 2007). Runx2 shows nucleolar localization, colocalize with RNA Pol I transcription factor UBF1 and is associated with the open chromatin. It controls lineage commitment and cell proliferation as well as represses RNA Pol I mediated rRNA synthesis (Young et al., 2007). Other matrix binding transcription factors C/EBP alpha regulate gene transcription of Hp during normal liver development whereas C/EBP beta regulates acute-phase (AP) response during the later phase of differentiation (Dinic et al., 2005). Human osteosarcoma MG-63 and human gastric mucous adenocarcinoma MGc80-3 cells showed significant change in nuclear matrix proteins upon differentiation induced by HMBA (Zhao et al., 2006; Zhao et al., 2005). Another neuronal nuclear matrix protein NRP/B is reported to interact with p110RB through its BTB domain and thus regulate neurite formation in PC12 cells (Kim et al., 2005). Thus role of MARBPs during differentiation is well documented in the literature.

1.4 MAR binding proteins in apoptosis

MARBPs are also involved in apoptosis such as Heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A), is cleaved at the SALD site in a caspase-dependent way during apoptosis. This results in loss of its DNA-binding activity and a concomitant detachment from nuclear structural sites without affecting its function in RNA metabolism (Kipp et al., 2000). SAF-A is an RNA binding protein and contains scaffold-associated region (SAR)-specific bipartite DNA-binding domain and is associated with pre-mRNAs in the nucleus and influence pre-mRNA processing and other aspects of mRNA metabolism (Kiledjian et al., 1992; Fackelmayer et al., 1994a; Fackelmayer et al., 1994b; Gohring et al., 1997; Kipp et al., 2000; Romig et al., 1992). Another MARBP poly (ADP-ribose) polymerase, PARP is involved in mediating DNA damage induced posttranslational modifications of various proteins (Burkle et al., 2006). Loss of Parp-1 in embryonic stem cells results in altered
expression of 9.6% genes wherein 6.3% were downregulated and 3.3% were upregulated by 2-fold or greater, respectively suggesting that it regulates the transcription of a wide variety of genes and thus control various cellular processes (Ogino et al., 2007). It is involved in lowering the intracellular concentration of its substrate, nicotinamide adenine dinucleotide (NAD) and thus reduces the rate of glycolysis, electron transport, ATP formation and subsequently results in cell death during liver injury (Gero et al., 2006). Inhibition of PARP-1 potentiates chemo and radiotherapy for cancer treatment and thus its inhibitors are undergoing the early phase of clinical trials (Plummer et al., 2006). To conclude nuclear matrix proteins (NMPs) are involved during various cellular processes like cell proliferation, development, differentiation, embryogenesis and apoptosis.

1.5 Nuclear lamins

Another major group of proteins, associated with nuclear matrix includes nuclear lamins and lamin-dependent complexes that mediate interaction between nucleoskeleton and cytoskeleton structures (Gruenbaum et al., 2005). Nuclear lamina is shown to control DNA replication, chromatin architecture, gene transcription and thereby regulates various cellular processes (Mattout-Drubezki et al., 2003). Nuclear lamins are altered during C2C12 myoblast differentiation wherein lamin B2 expression was increased and LAP2α is decreased (Markiewicz et al., 2005). Lamin B1 is an important MARBP and is differentially regulated during the development of somatic tissues in chicken (Luderus et al., 1992; Lehner et al., 1987). Further translational control of Lamin B1 mRNA has been reported during oogenesis and early stages of development in Xenopus (Ralle et al., 1999). Thus, association of nuclear lamins with nuclear matrix also contributes in regulating wide variety of cellular functions including development, differentiation, stress response and cellular homoeostasis.
1.6 Nuclear matrix dysregulation in cancer

Nuclear matrix and matrix binding proteins dynamics are altered in cancers (Drobic et al., 2006). Nucleus of a tumor cell exhibits gross changes in size, shape, DNA content and chromatin reorganization due to irreversible epigenetic changes in terms of DNA methylation and histone modifications (Santos-Rosa et al., 2005). These epigenetic changes can be correlated to the chromatin modifying enzymes that brings about methylation of DNA and other post translational modifications of core histones (Preston et al., 2007). Epigenetic changes including DNA methylation and chromatin remodeling patterns conferred by environmental stress can affect the DNA repair, cell cycle, genomic stability and gene expression (Verdone et al., 2005). Core histone undergoes various covalent modifications such as acetylation that demarks the permissive and non permissive (repressive) chromatin domains. Tumor suppressor Swi/Snf ATPase chromatin remodeling complex component Snf5 (Ini1/Baf47/Smarcb1) that regulates cell cycle and p53 function is epigenetically silenced in lethal childhood cancers (Sansam et al., 2006). Along with this the dysregulation of MARBPs and their association with the nuclear matrix also contributes to the tumorigenesis. MARBPs like p53, Ku, PARP, SATB1, Cux/CDP are involved in the regulation of various physiological processes that include cell cycle progression, DNA damage-repair, apoptosis etc. (Galande 2006). Among these MARBPs, p53 is frequently mutated in more than 50% human cancer patients (Hollstein et al., 1994). Some of these specific mutations allow p53 to bind to MAR sequences with higher affinity and distort double strand DNA (Gohler et al., 2005; Vogelstein et al., 2000). DNA damage and various other stresses activate p53 and bring about various post translational modifications like phosphorylation, acetylation, sumoylation etc. that regulates its stability and transcriptional activity. This in turn results in execution of its effect upon various target gene expression that mediates cell cycle arrest, apoptosis and cellular senescence (Appella et al., 2001; Prives et al., 2001; Dreijerink et al., 2006). Nuclear protein menin activates or repress transcription depending upon its interaction with JunD and other co-factors. It regulates Cyclin-dependent-kinase inhibitors p18INK4C and p27Kip1 expression. Menin also
maintains proper TGFβ signaling in parathyroid glands. All these functions of menin in turn are critical for multiple endocrine neoplasia type 1 (Esteller 2006). Gene methylation also plays critical role in regulating the gene expression and genome wide screening has revealed that genes involved in cancer, cell cycle control, DNA repair and apoptosis are silenced through promoter region methylation and the number increases during neoplastic progression (Baylin et al., 2006; Esteller 2006). SWI/SNF chromatin remodeling complex plays major role in tumor suppression and its effect on transcription upon different cell cycle specific genes is governed through its interaction with other factors like E2F1, Tip60 and HDAC1/2/3 (Sansam et al., 2006). Recruitment of HP1 protein to the altered pericentromeric heterochromatin sites is dependent upon histone H3 variant deposition and is essential for mitotic progression (Nagl et al., 2007). p300/CBP directly interacts with ATR and loss of p300 in mammalian cells resulted in defective CHK1 phosphorylation in response to stalled DNA replication (Zhang et al., 2007). p21 (WAF1/CIP1) related DNA damage controls the G1 phase of ES through several mechanisms and rapidly inhibit cell cycle progression. This is achieved by blocking E2F dependent genes and H4 transcription through inhibition of transcription factors required for histone gene transcripts, as well as by destabilizing histone mRNAs (Stauffer et al., 2007). Cdc7 kinase phosphorylates MCM4 in MCM complex and stimulates the association of Cdc45 with the chromatin and activates DNA replication (Becker et al., 2007). Also Cdc7-Dbf4 promotes the assembly of Cdc45-MCM complex during S phase and MCM4 phosphorylation is important for proper S phase progression (Masai et al., 2006). Another important kinase Phosphoinositide 3-kinase (PI3K) that is activated immediately after growth factor stimulation is necessary for cell growth and cell cycle entry. Its inhibition leads to c-Myc destabilization and thus affects S phase entry (Sheu et al., 2006). Other cell cycle regulatory proteins include various Cyclins and Cyclin dependent kinase (cdk) complex that are aberrantly expressed in many cancers. Among all the Cyclins, Cyclin D1 expression is one of the hallmarks of breast cancer progression and is considered as a positive diagnostic marker (Sheu et al., 2006; Yu et al., 2001). Catalytic subunit Brahma (Brm) of SWI/SNF chromatin remodeling complex is regulated by Cdt1 (a member of preRC involved in re-replication) that interacts with Geminin and...
facilitates its recruitment onto the chromatin (Lee et al., 2000). Further it is shown that Geminin undergoes cleavage upon caspase-3 activation and induces apoptosis. Geminin cleavage is regulated through its phosphorylation by Casein kinase II (Kumar et al., 2006). The wings apart-like Wapl, another protein implicated in heterochromatin formation and tumorigenesis controls interaction between cohesin and chromatin. WAP1 depletion interrupts the cohesion interaction with chromosomes during the early stages of mitosis and prevents sister chromatids resolution until anaphase (Xouri et al., 2007; Roukos et al., 2007; Kueng et al., 2006). All these data in the literature suggests that MARBPs directly or indirectly affects the nuclear matrix dynamics and their dysregulation is observed during the process of tumorigenesis.

1.7 MAR binding proteins and tumor metastases

Tumor growth and its metastatic potential are decided by the growth factors available in the surrounding microenvironment. Along with this the transcriptional regulation of genes responsible for cell motility and metastases is perturbed due to dysregulation of the regulatory components. Nuclear matrix protein expression profile is altered in colon cancer associated metastases in liver tissue compared to the adjacent normal liver tissue (Gandhi et al., 2006; Brunagel et al., 2002a). MARBP, High-mobility group (HMG) protein, HMG-I, and its splicing variant, HMG-Y are highly expressed in advanced cancers and their expression is correlated with higher metastases. Heregulin (HRG0-erbB) activates HMG-I(Y) expression that promotes metastatic potential of human breast cancer cell lines (Brunagel et al., 2002b). Another MARBP CDP/CUX/ CUTL1 is recently reported to increase cancer cell motility and invasiveness. CUTL1 is regulated by TGFβ signaling and thus promotes tumor cell migration (Liu et al., 1999). TGFβ plays a dual role in breast cancer by regulating both growth inhibitory and pro-migratory signals in primary and advanced stages of breast cancer respectively, as decided by the extent of Ras activity (Muraoka-Cook et al., 2005). TGFβ signaling involves family of stress-activated kinases that includes phosphorylation of receptor activated Smad2, co-Smad4 to exhibit its effect (Kang et
SMAR1 was identified from mouse double positive thymocytes cDNA library as one of the protein that binds to the MARβ present 400 bp upstream of TCRβ locus. Smar1 gene encodes for 2112 bp long cDNA that corresponds to 60.2 kDa full length (548 amino acids) protein. It exists as two alternatively spliced forms SMAR1\textsuperscript{L} (long) and SMAR1\textsuperscript{S} (short), wherein the short form has a deletion of 39 amino acids at the N-terminus. Homology search and comparative analysis showed that SMAR1 shares homology with other MARBPs such as CDP, Bright and SATB1. MAR binding domain of SMAR1 resides within 352-394 amino acids and is significantly similar to the MAR binding domain of SATB1. SMAR1 has Cut repeat box I (330-371 a.a.) and II (365-381 a.a.) that are homologous with Cux and SATB1 respectively. In addition to this 398-456 a.a. region is homologous with the tetramer domain of Bright. Further northern blot analysis using SMAR1 cDNA as probe has shown that SMAR1 is expressed in thymomas derived from double negative thymocytes as well as in double positive thymocytes suggesting that the expression of SMAR1 is not stage specific. Also it was found to be differentially expressed in other tissues like spleen, bone marrow, kidney, liver, lung, heart etc. although the expression was highest in thymus (Chattopadhyay \textit{et al.}, 2000). Full length endogenous SMAR1 is expressed both in nucleus and cytoplasm while when overexpressed SMAR1\textsuperscript{S} showed specifically nuclear localization. Overexpression of various protein truncations of SMAR1 showed different pattern of expression in nucleus and cytoplasm where in the middle domain of SMAR1 comprised of 160-350 a.a. was localized into the nucleus (Fig. 2). The N-terminal and C-terminal truncations 1-160 a.a. and 350-548 a.a. respectively failed to localize into the nucleus. Further a novel nuclear localization signal was characterized within 160-350 a.a. region (Jalota \textit{et al.}, 2005). This arginine RTAWRRKQR (324-332) and serine SFSRRTPSSSSYSAS (342-356) rich region is important for protein-protein interaction with p53 and has phosphorylation site for protein kinase C (Jalota \textit{et al.}, 2005)

1.8 MAR binding protein SMAR1
et al., 2005). Later on the same region was found to be important for interaction with Cux and HDAC1 (Rampalli et al., 2005). While 160-350 region serves as a protein-protein interaction domain, C-terminal region 350-548 is characterized as MAR binding domain (Rampalli et al., 2005; Kaul et al., 2004). SMAR1 a 548 amino acid long protein has an arginine-serine rich protein-protein interaction domain and C-terminal DNA binding domain (Fig. 2).

**Fig. 2. MAR binding protein SMAR1.** Schematic representation of SMAR1 protein showing N-terminus (skyblue), deleted region in alternatively spliced form (yellow), protein-protein interaction domain (pink), nuclear localization sequence (blue), MAR binding domain homologous to SATB1 (purple), Cut repeat box I and II similar to Cux and SATB1 respectively (orange), tetramer domain homologous to Bright (red) and C-terminal DNA binding domain (green).

### 1.8.1 SMAR1 regulates V(DJ) recombination

V(D)J recombination is an important phenomena in T-cell development that takes place at TCRβ locus during transition of double negative stage to double positive and single positive (Godfrey et al., 1994). SMAR1 was initially identified from mouse
thymocytes during double positive stage (Chattopadhyay et al., 2000). It was found to directly bind to Eβ enhancer present 400 bp upstream of the TCRβ locus (Chattopadhyay et al., 2000). Further it was observed to interact with another MARBP Cux at MARβ present at TCRβ locus (Kaul et al., 2005). SMAR1 transgenic mice exhibit spleenomegaly and lymphadenopathy. Massive infiltration of lymphocytes was observed in spleen and lymph nodes of SMAR1 transgenic mice. T-cell development was also perturbed and majority of T-cell were halted at double negative stage (CD44+CD25+). It also affects the T cell maturation and V(D)J recombination specifically at Vβ 5.1/5.2, Vβ 8.1/8.2, Vβ8.3 and Vβ13 (Kaul et al., 2004). These studies establish that SMAR1 plays a role in V(D)J recombination through modulation of transcription by virtue of its MAR binding activity and its interaction with other MARBPs (Fig. 3).

Fig.3. SMAR1 downregulates Eβ mediated transcription. SMAR1 binds to HS1 MAR along with another MARBP Cux and represses Eβ mediated transcription at TCRβ locus

1.8.2 SMAR1 stabilizes p53

The p53 is an important tumor suppressor protein that is involved in controlling major cellular processes like DNA damage repair, cell cycle and apoptosis. It has an N-terminal transactivation domain (TAD), a potential conformational element consisting of a proline rich-domain (PRD), a large DNA-binding domain (DBD), a
tetramerization domain (TmD) and a basic C-terminal domain (CTD) (Toledo et al., 2006). Mutations in p53 are reported in about 60% of all human tumors, making it the most frequent target for genetic alterations in cancer (Hollstein et al., 1991; Levine et al., 1997; Vousden et al., 2002; Lacroix et al., 2006). These mutations facilitates interfere with the tumor suppressor activities of the wild-type (wt) p53, although in some forms of tumors there is gain of function of these mutant p53 that contributes to its overt oncogenic activities (Deppert 1996). Wild type p53 expression is elevated in response to various genotoxic stresses such as DNA damage induced by irradiation, UV, chemical carcinogens and cytotoxic drugs (Appella et al., 2001; Saito et al., 2002). It is also activated under various physiological stress conditions such as hypoxia, nucleotide depletion, oncogenic activation and microtubule disruption resulting in either cell cycle arrest and/or apoptosis (Giaccia et al., 1998; Lane 1992; Saito et al., 2002; Haupt et al., 2003; Yu et al., 2005; Harms et al., 2004; Green et al., 2006). Therefore p53 function is important in controlling cell cycle and apoptosis and thus in turn controlling the process of tumorigenesis.

SMAR1, a novel MARBP interacts with p53 and stabilizes the phospho-serine 15 p53 in the nucleus. In normal conditions p53 is rapidly degraded through the ubiquitin-proteasome pathway by MDM2 while upon stress p53 levels are stabilized through phosphorylation at the N-terminus of p53 that prevents the interaction of MDM2 with p53 (Haupt et al., 1997; Kubbat et al., 1997; Daujat et al., 2001; Momand et al., 2000; Rodriguez et al., 2000). On the other hand, p53 binds specifically to the MDM2 gene and stimulates its transcription (Wu et al., 1993). This duality defines an autoregulatory negative feedback loop, which probably serves to keep p53 in tight check. SMAR1 interacts with the p53 and protects it from MDM2 mediated degradation by facilitating the phosphorylation of p53 at serine 15 that inhibits its interaction with MDM2. SMAR1 is phosphorylated at serine residue 347 by protein kinase C that in turns facilitates the serine 15 phosphorylation of p53 (Jalota et al., 2005). Thus SMAR1 mediated stabilization of p53 allows its retention in the nucleus and modulates the p53 target genes expression (Fig. 4).
Fig. 4. **Diagrammatic model showing SMAR1 mediated stabilization of p53.**
Diagrammatic representation showing SMAR1 (red) interaction with p53 (blue) that enhances the phosphorylation of p53 at serine 15 residue (yellow star) and hence prevent it from MDM2 (green) mediated degradation.

### 1.8.3 SMAR1 and cell cycle regulation

Cell cycle dysregulation and uncontrolled cell proliferation is one of the major feature of tumor cells (Hartwell *et al.*, 1994; Yamasaki *et al.*, 2004; Diffley *et al.*, 2000). Genetic alterations like gain-of-function mutations are known to contribute to cancer development (Aaronson 1991). These mutations leads to onset of “oncogenes” that are the mutant versions of the normal cellular “proto-oncogenes”. Most of these oncogenes function as inducers of cellular proliferation. The best characterized of these is Cyclin D1, also known as Prad1, cloned as a gene involved in the translocation in parathyroid adenomas (Motokura *et al.*, 1991). Cyclin D1 is the Bcl1 oncogene, associated with certain B-cell lymphomas. Cyclin D1 gene amplification is also related in subset of breast, esophageal, lung, bladder and squamous cell carcinomas (Wong *et al.*, 2003; Zhang *et al.*, 2003; Donnellan *et al.*, 1998). Deregulated expression of Cyclin D1 has also been reported to directly contribute to tumorigenesis in animal models (Bodrug *et al.*, 1994; Wang *et al.*, 1994; Lovec *et al.*, 1998).
1994). Cyclins D2 and D3 have also been reported to be overexpressed in some tumors (Hunter et al., 1994; Leach et al., 1993; Bergsagel et al., 2005).

SMAR1 is located at 16q24.3 locus that harbors tumor suppressors involved in breast cancer. RT-PCR analysis for the SMAR1 in various transformed cancerous cell lines revealed that the expression of SMAR1S is drastically downregulated and this suggested that SMAR1S might also function as a tumor suppressor protein. To study the role of SMAR1S specifically in various cellular processes stable clone expressing SMAR1S were generated in mouse melanoma B16F1 cells. When overexpressed SMAR1S negatively regulated the cell proliferation and allowed cell cycle arrest in G2/M phase (Kaul et al., 2003). These results suggested that SMAR1 exhibit growth inhibitory function. Further investigation into the mechanism of cell cycle regulation by SMAR1 showed that it induces p53 mediated activation of p21 and thus allows cell cycle arrest. It failed to activate p21 in p53 defective cell line K562 that again suggested the requirement of p53 for p21 activation (Kaul et al., 2003). Further investigation showed that SMAR1 overexpression also resulted in increased cdc2 phosphorylation at Tyr-15 that inhibited the cell cycle progression (Jalota et al., 2005). SMAR1 also repress the transcription Cyclin D1, one of the major cyclin involved in G1 to S phase transition during cell cycle (Rampalli et al., 2005). Thus SMAR1 controls the cell cycle progression through modulating the expression of various cell cycle regulatory proteins (Fig. 5).

1.8.4 SMAR1 in regulating transcription through MARs

SMAR1 directly bind to the MAR regions and the DNA binding domain lies in the C-terminal (Chattopadhyay et al., 2000; Jalota et al., 2005). It regulates the Eβ mediated transcription in synergism with another MARBP Cux (Kaul et al., 2004). SMAR1 binds to MAR present in Cyclin D1 promoter (Rampalli et al., 2005). SMAR1 interacts with HDAC1-msin3a corepressor complex and recruits them at the MAR site and respresses the transcription of Cyclin D1 (Rampalli et al., 2005). In brief above results suggests that SMAR1 can regulate transcription through its binding to the
MAR regions and recruitment of HDAC1 dependent repressor complex at its binding site.

Fig.5. **SMAR1 regulates cell cycle progression.** SMAR1 interacts with p53 and stabilizes p53 in the nucleus and thus activates p53 target gene p21 expression. SMAR1 also downregulates Cyclin D1 transcription through its direct binding to the Cyclin D1 promoter MAR and recruitment of HDAC1 dependent repressor complex.
“Experience is the only teacher”  .....Swami Vivekananda

SMAR1-p53: A positive feedback loop and its dysregulation in breast cancer