

## Review Article

# BIOLOGY OF FOXM1 AND ITS EMERGING ROLE IN CANCER THERAPY

Neha Jaiswal, Sankalpa Chakraborty and Alo Nag\*

Department of Biochemistry, University of Delhi South Campus, New Delhi-110021, India

**Abstract:** The FOXM1 transcription factor has been implicated to play a central role in the regulation of crucial cellular activities. Evidences regarding the significance of FOXM1 in cell cycle control, genomic stability and tumorigenesis are undeniable. This has generated much interest in the field and as a result, past decade has witnessed remarkable progress in FOXM1 research addressing complexity of its function and regulation in tumorigenesis. Its proven role in carcinogenesis and its prospect as a promising therapeutic target against cancer makes it a molecule of considerable clinical interest. A thorough understanding of FOXM1 will be extremely useful in the innovation of strategies for treating and preventing cancer. Here we present a systematic literature review on FOXM1 highlighting its key functions and molecular mechanisms of association in tumorigenesis and its prospects in cancer therapy.

**Keywords:** FOXM1; Cell proliferation; Cell cycle; Tumorigenesis; Cancer therapy and biomarker

### FOXM1: At a Glance

**Name:** Forkhead Box Protein M1 (FOXM1).

**Alternative Names (Before Nomenclature):** Forkhead-related Protein 16 (FKHL-16), Hepatocyte Nuclear Factor3/ Forkhead Homolog 11 (HNF3/FKH11), M-Phase Phosphoprotein-2 (MPP2).

**Family:** Winged Helix Proteins or Forkhead Box Proteins.

**Chromosomal Location:** Chromosomal Band 12p13.33.

**Protein Specifications:**

1. **Variants:** 3 in Human (FOXM1a, FOXM1b and FOXM1c) and 1 additional in rat (Rat WIN).
2. **Size:** 801 Amino Acids (FOXM1a), 748 Amino Acids (FOXM1b), 763 Amino Acids (FOXM1c).
3. **Subunit Structure:** Monomer.

**Cellular Expression:** Expressed In Highly Proliferating cells such as Developing embryo, thymus, testis, small intestine and colon

**Subcellular Localization:** Predominantly in Nucleus, Cytoplasm (As its localization oscillates during cell cycle).

**Functions:** Cell Cycle Regulation, DNA damage and repair, Regulation of Stem Cell Pluripotency, self-renewal and differentiation, Tissue regeneration after Injury, Organogenesis, Angiogenesis and Metastasis.

Corresponding Author: **Alo Nag**

E-mail: [alonag22@gmail.com](mailto:alonag22@gmail.com)

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## Introduction

Forkhead transcription factors are a family of evolutionarily conserved transcriptional regulators, which share a conserved 100 residue, winged helix DNA binding domain and the so called forkhead (FKH) domain. Since the identification of the *forkhead* gene in *Drosophila melanogaster* (Weigel *et al.*, 1989), the founding member of this family whose mutations result in the development of a forkhead like appearance, more than 100 structurally related forkhead transcription factors have been identified. Because FOX family members are involved in a variety of processes during embryogenesis and adult tissue homeostasis, germ-line mutations or variations of FOX family members are often associated with human congenital disorders and diseases.

Forkhead transcription factor (FOXM1) is an important regulatory factor for G1/S and G2/M

phases of cell cycle and maintenance of mitotic spindle integrity. Besides this, FOXM1's involvement in angiogenesis (Wang *et al.*, 2007), metastasis (Dai *et al.*, 2007), apoptosis (Chan *et al.*, 2008; Madureira *et al.*, 2006; Wierstra and Alves, 2007), DNA damage repair (Tan *et al.*, 2007) and tissue regeneration (Kalinichenko *et al.*, 2001) has also been emphasized. Major discoveries related to FOXM1, which directed us towards our present knowledge regarding the FOXM1 biology, are outlined in timeline (Figure 1).

The human Forkhead Box M1 (FOXM1) protein, belongs to a winged-helix transcription factor family, was first identified as a mitotic-phase phosphoprotein (MPP2) from a cervical cancer cell line HeLa (Westendorf *et al.*, 1994). *FOXM1* gene is located on the chromosomal band 12p13.33 (Korver *et al.*, 1997a) and amplification of this locus is frequently observed in breast adenocarcinomas (Curtis *et al.*, 2012), head and

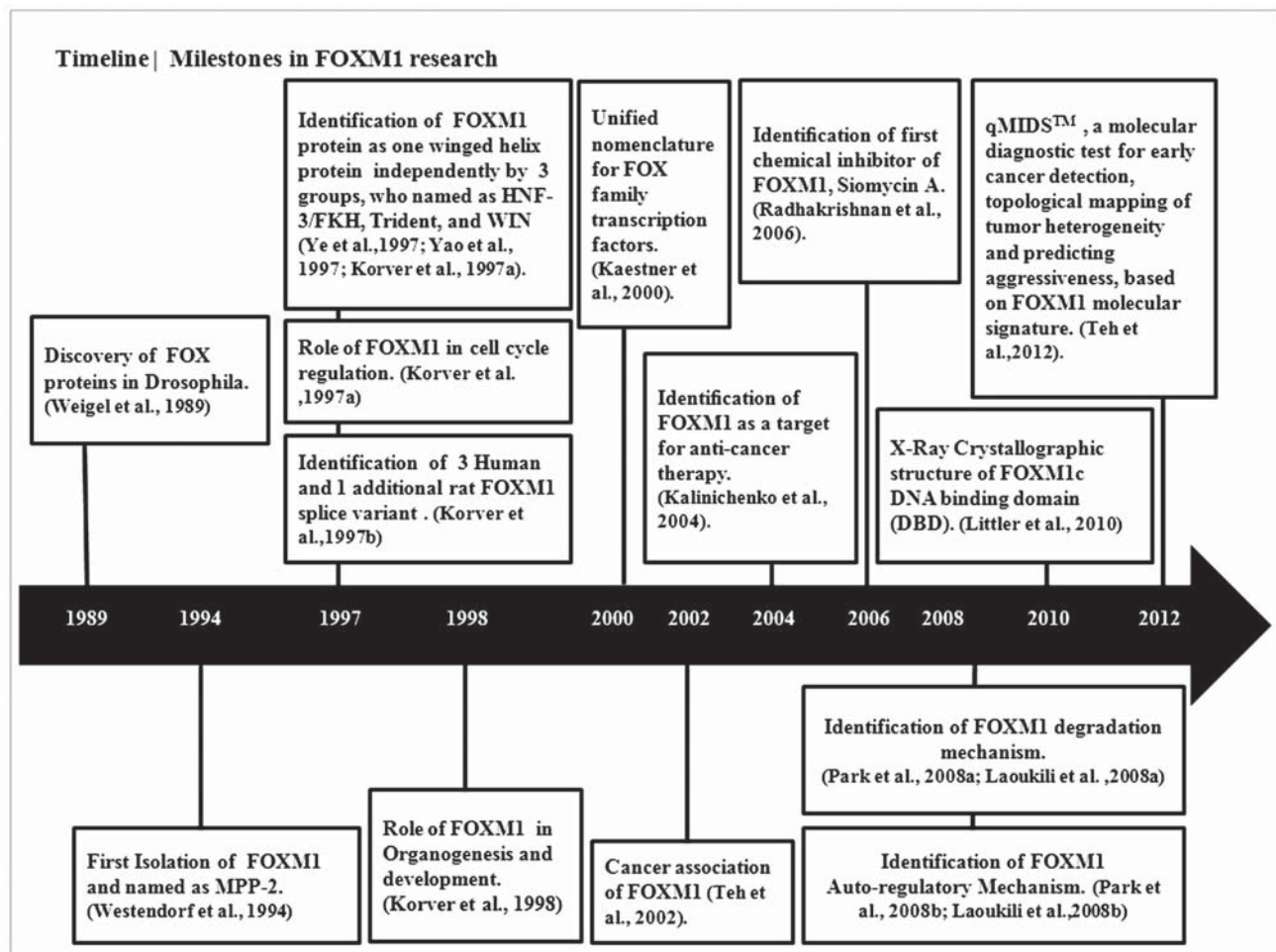


Figure 1: Timeline of Landmark Discoveries on FOXM1.

neck squamous cell carcinomas, basal cell carcinomas (Teh *et al.*, 2002) and cervical cancers (Chan *et al.*, 2008). Due to its critical association in cancer development and progression, **FOXM1 earned the recognition of investigators as one of the most prospective molecules for treatment and diagnosis of cancers.** In this review, we summarized the structural, functional and regulatory features of FOXM1 along with an emphasis on its 'intimacy' with tumor evolution and advancement. We have also attempted to condense our knowledge regarding FOXM1 biology to 'best-fit' this protein in proposed mechanisms of tumorigenicity and discussed some of the possible directions for exploiting FOXM1 as a crucial therapeutic molecule.

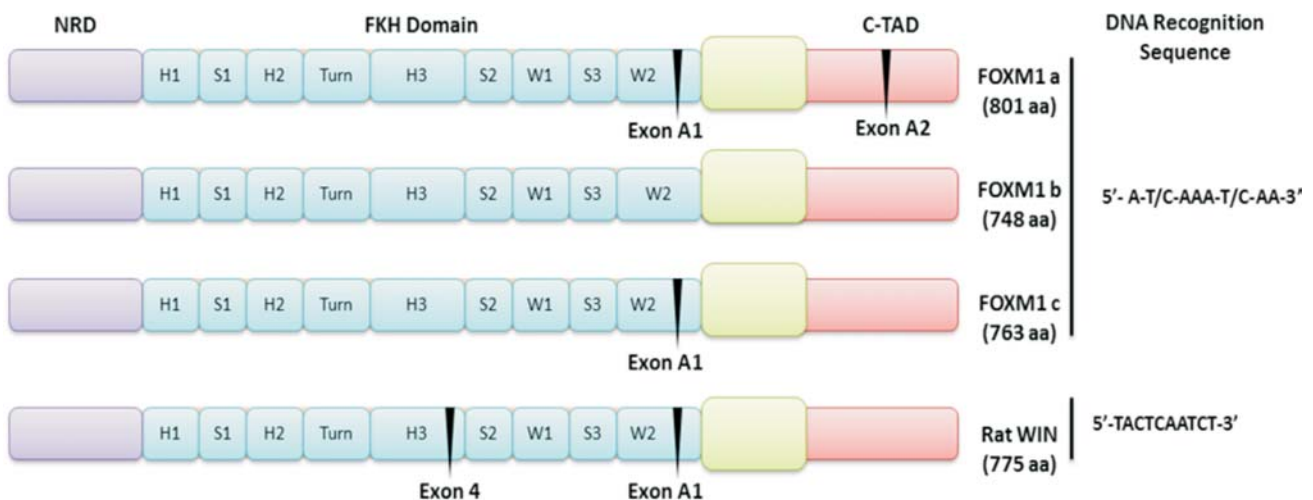
## 2. Biology of FOXM1

### 2.1. FOXM1: Protein and gene level specifications

FOXM1 gene consists of 10 exons, three of which are alternatively expressed, resulting in four alternatively splice variants (mRNA isoforms) that are almost identical in sequence but differ by addition of one or two small exons. In human, there is only 3 splice variants and the other one can be found in rats (Korver *et al.*, 1997b; Yao *et al.*, 1997; Ye *et al.*, 1997). FOXM1 protein contains three major domains: FKH domain, Transactivation domain (TAD) and N-terminal repressor domain (NRD). FKH Domain aids in its

DNA binding activity and evolutionarily well conserved in all types of Forkhead box proteins whereas NRD is important for the autoregulatory activity of FOXM1. In 2010, Littler *et al.*, first resolved the X-ray crystal structure (2.2 Å resolution) of FOXM1c DNA binding domain. The forkhead domain of FOXM1 adopts a structure containing three β-helices (H1, H2, H3), three α-strands (S1, S2, S3), and two loops or wings (W1, W2) and arranged them in H1–S1–H2–turn–H3–S2–W1–S3–W2 order (Littler *et al.*, 2010). FKH domain contains two of the three alternatively spliced exons. The presence or absence of these exons affects the DNA binding specificity and affinity of different splice variants.

In human, FOXM1a, b and c are the variants. FOXM1b (also known as HFH-11B, FKHL16, Trident, Win, MPP2, MPM2) (Kaestner *et al.*, 2000) contains no additional exons while FOXM1c (Trident, Win or MPP2) and FOXM1a (HFH-11A) isoforms contain only exon A1 and both exon A1 and A2, respectively (Korver *et al.*, 1997a; Yao *et al.*, 1997; Ye *et al.*, 1997). Rat WIN lacks exon A2 but contains exon A1 and exon 4 (Yao *et al.*, 1997). Of these variants, FOXM1b and FOXM1c are transcriptionally active (Ye *et al.*, 1997); as depicted in Figure 2. FOXM1a was found to be transcriptionally inactive due to presence of an inhibitory exon (A2) in the C-terminal of its transactivation domain and might also cause



**Figure 2: Structure of protein domains in FOXM1 variants.** FOXM1 consists mainly of 3 domains, N-terminal repressor domain (NRD), Forkhead domain (FKH), C-terminal Transactivation domain (C-TAD). In human, there are 3 variants of FOXM1 and these variants differ only by the presence or absence of 2 exons (A1 and A2), but they all recognize the same DNA sequence. Rat has one additional variant called Rat WIN, which has a different DNA binding specificity.

dominant negative effects as it has retained a functional DNA binding domain (Ye *et al.*, 1997). As mentioned before, the presence or absence of exons in FKH domain affect the DNA binding characteristics of these variants and as H3 is the recognition helix, which confer the DNA binding specificity to any FOXM1 variant, DNA recognition sequence of Rat WIN (5'-TACTCAATCT-3') is completely different from the others (5'-A-T/C-AAA-T/C-AA-3'), due to the presence of exon 4 in H3 (Korver *et al.*, 1997a). Although the presence of exon A1 in wing W2 of the FKH domain does not affect the DNA binding specificity, as W2 domain is not involved in base-specific DNA binding. However, it may alter the DNA binding ability of the variants as it is evident from the EMSA studies with purified GST - FOXM1 variants, where FOXM1b displays higher DNA binding affinity ( $K_D = 0.2 \mu\text{M}$ ) than FOXM1c ( $K_D = 0.4 \mu\text{M}$ ) (Hegde *et al.*, 2011). On the basis of gene organization, the only difference between FOXM1c and FOXM1b lies in the presence of exon A1, which contains an ERK1/2 target sequence, in the c isoform, which can alter the functional specifications of the protein product, possibly transactivating functions (Ma *et al.*, 2005a). FOXM1b was found to be the only isoform showing cell cycle dependent mRNA expression pattern in two different human cell lines. However, it is not clear if this was only due to splicing variations or other additional mechanisms, hence warrants further investigations. Most studies to date focused on FOXM1b and FOXM1c due to their transactivating roles in cell cycle which inadvertently led to lack of studies on the inactive isoform FOXM1a and also the other isoform Rat WIN, hereby their role in cell cycle and other physiological contexts remain unknown.

## 2.2. Regulation

### 2.2.1. Post-translational regulation of FOXM1

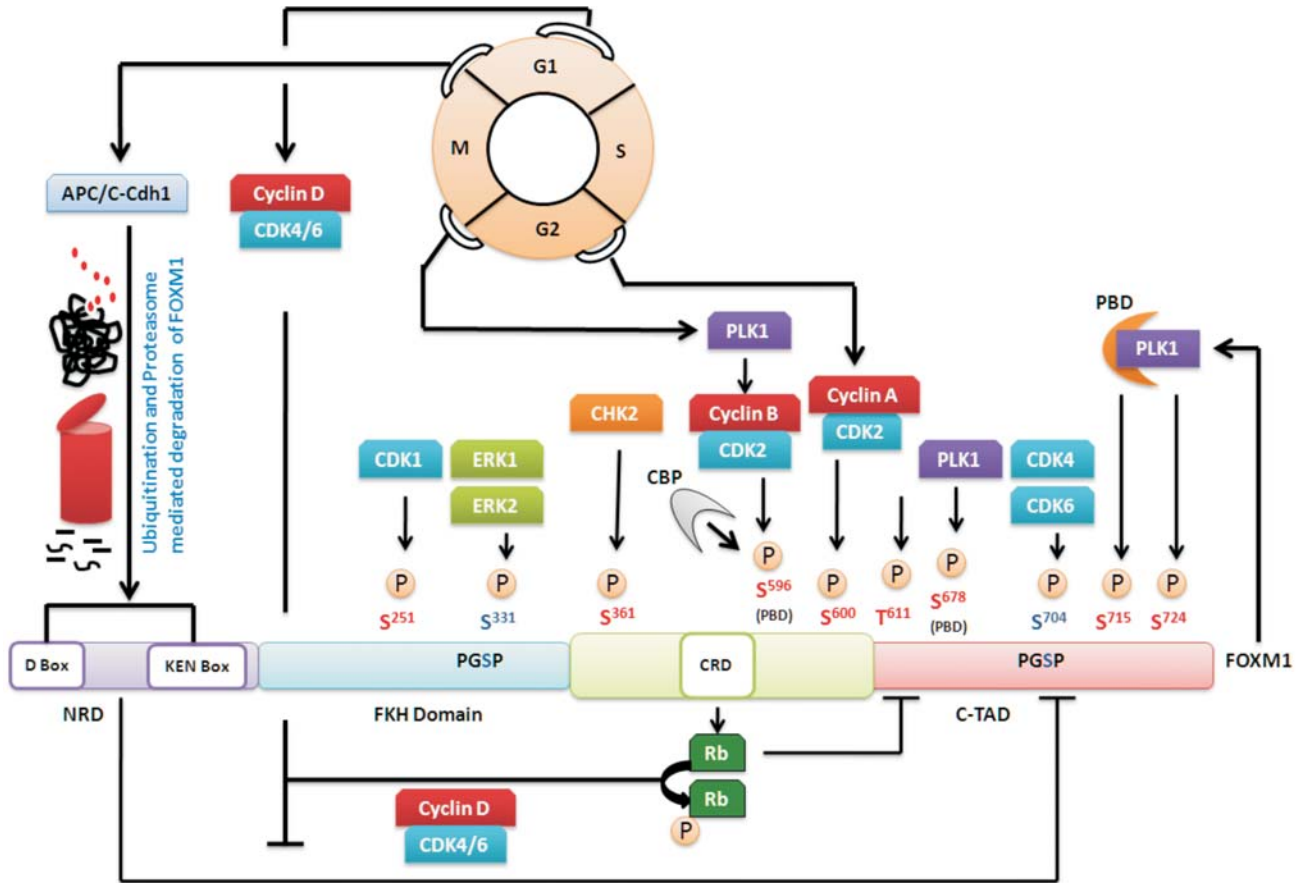
Regulation of multifunctional transcription factor is imperative for spatio temporal fine tuning of broad transcriptional programme during normal development. Thus, pathological conditions like cancer are an obvious outcome of functional deregulation of FOXM1. FOXM1 is regulated by layers of post-translational modifications like

phosphorylation and ubiquitination during an ongoing cell cycle (Figure 3). Better understanding of FOXM1 biology and its regulation would not only help us to generate anti-cancer drugs, but also render it as a crucial biomarker.

**2.2.1.1. Phosphorylation:** Post-translational modifications are implicated in modulation of transcriptional activity of FOXM1 by altering its DNA binding ability, localization, association with interacting partners and stability. Transcriptional activity of FOXM1 is tightly regulated throughout the cell cycle by multisite phosphorylation by different kinases and its counteracting phosphatases. While FOXM1 expression is initiated before S phase entry, its transcriptional activity is suppressed until G2/M phase by hyperphosphorylation. Costa and his coworkers investigated the phosphorylation pattern of endogenous FOXM1 during cell cycle progression. Their findings indicated that FOXM1b is initially phosphorylated at S-phase by cyclinE/A-cdk2 complexes followed by hyperphosphorylation in G2 and M phase of cell cycle by cyclinB-Cdk1 (Major *et al.*, 2004). Thereafter, pioneering research efforts by Raychaudhuri group established a direct correlation between transcriptional activity and phosphorylation status of FOXM1b. Moreover, dephosphorylation of FOXM1 was seen to coincide with exit from mitosis (Chen *et al.*, 2009).

Following activation of the FOXM1 protein, transcription of the *Cdc25B* phosphatase gene, a direct target of FOXM1b occurs. The increased levels of Cdc25B protein will activate the Cdk1-cyclin B complex through dephosphorylation, allowing Cdk1-cyclinB to maintain phosphorylation of FOXM1b protein at Thr 596 residue and recruitment of the p300/CBP (CREB binding protein) coactivator proteins during the G2 phase of the cell cycle (Major *et al.*, 2004).. Hence, representing a positive-feedback model in which FOXM1 transcriptional activity is regulated. Furthermore, a conserved phosphorylation site (Ser 251) was identified within Forkhead domain, required for Cdk1 dependent phosphorylation of FOXM1 as well as its interaction with the coactivator CBP (Chen *et al.*, 2009). Phosphorylation dependent recruitment





**Figure 3: Regulation of FOXM1 protein during cell cycle.** FOXM1 containing the domains NRD, FKH and C-TAD is shown above. NRD contains D-box and KEN box required for its APC/C-Cdh1 mediated proteasomal degradation during late M and early G1 phase. NRD also inhibits FOXM1 transcriptional activity during G1 phase through its interaction with C-TAD, which is relieved by phosphorylation of FOXM1 by cyclin A/Cdk2 (FOX M1b) and cyclin D/Cdk4 (FOX M1c) complex. During G2 phase, active cyclin B-Cdk1 complex phosphorylates FOXM1 at Thr 596, prerequisite for recruitment of CBP for FOXM1 activation. PLK1 through its PBD interacts with FOXM1b and phosphorylates at Ser 596, Ser 600 and Thr 611. Also PGSP motif of FOXM1c, whose serine residues (331 and 704) undergo phosphorylation via Raf/MEK/MAPK at Ser331 and 704. Negative regulation by Rb is mediated through its interaction with central regulatory domain of FOXM1. This inhibition is relieved upon phosphorylation of Rb by Cyclin D/CDK4.6 complex. Phosphorylation at Ser 361 of FOXM1b in response to DNA damage imparts stability to this protein.

of CBP to FOXM1 has been demonstrated using co-immunoprecipitation, wherein interaction between FOXM1b and CBP was observed. It is possible that acetylation either enhances or represses FOXM1 factor in a target gene specific context (Major *et al.*, 2004). Possibly, formation of the FOXM1-CBP/p300 complex may lead to acetylation of histone proteins and disruption of tight nucleosomal configuration required for transcriptional activation. FOXM1 also undergoes initial priming phosphorylation by cyclinB/Cdk1 complex in G2 phase of cell cycle at Thr 596 and Ser 678 to create docking sites for PBD (Polo-like binding domain) of PLK1 (Polo-like kinase). Subsequently, PLK1 phosphorylates the TAD of

FOXM1 at Ser 715 and Ser 724 residues. This enhances the overall transcriptional activity of FOXM1 allowing high expression of key mitotic regulators like *CyclinB1*, *CENP-F* (Centromeric protein-F), *Cdc25B*, *Plk1*, *AURORA B* (Aurora kinase B). Since *Plk1* is a target gene of FOXM1, this mode of regulation represents a positive feedback loop, leading to further increase in PLK1 level and FOXM1 activity (Fu *et al.*, 2008).

In an independent study, mitogenic signals were shown to stimulate FOXM1 function (Petrovic *et al.*, 2008). Stimulation of FOXM1c by Raf/MEK/MAPK signaling aids in phosphorylation of ERK1/2 sites which facilitates nuclear translocation of FOXM1c thereby

augmenting its transcriptional activity during late S phase or early G2/M phase. Further, while induced activation of Raf/MEK/MAPK pathway was achieved with ATA (aurintricarboxylic acid) due to improved nuclear import, treatment with the MAPK inhibitor UO126 completely abolished the above activity (Ma *et al.*, 2005b). Interestingly, different isoforms of FOXM1 were shown to respond differentially to RAF/MEK/MAPK signaling (Lam *et al.*, 2013). Though FOXM1b exhibits higher transforming ability than FOXM1c isoform, the latter one displayed increased transactivating activity in presence of constitutively active form of MEK1 (Ma *et al.*, 2005b). Phosphorylation of FOXM1 also plays a crucial role in coordinating cellular response to DNA damage such as checkpoint kinase2 (Chk2) by mediating phosphorylation of Ser 361 leading to an increased stability of FOXM1 protein (Tan *et al.*, 2007).

**2.2.1.2. Ubiquitination:** Ubiquitin mediated proteolysis plays a crucial role in controlling the turnover of regulatory proteins involved in cell cycle progress, DNA repair, replication, chromosome rearrangement and cell division. The substrate specificity of degradation is largely conferred by E3 ubiquitin ligases like SCF (Skp1/CUL1/Fbox protein) and APC/C (anaphase promoting complex/cyclosome) complex that controls the timely transition of cell cycle phases. APC/C in coordination with its substrate specific activator Cdh1 had been shown to target FOXM1 for proteolysis at the late M and early G1 phases of cell cycle (Park *et al.*, 2008a). The degradation motifs, D-box and KEN box present in the N-terminal region of FOXM1 were shown to influence the stability of FOXM1 and the mutants lacking D/KEN box were very stable during cell cycle exit (Park *et al.*, 2008a). These observations were also confirmed by Medema group (Laoukili *et al.*, 2008a). Overall, the above findings provide important insights into the cell cycle regulation of the transcription factor FOXM1.

**2.2.1.3. SUMOylation:** Like ubiquitination, SUMOylation is another important post-translational modification which modulates a variety of cellular processes. Our lab was able to SUMOylate FOXM1 in HEK 293T cells and show its effect on protein stability of FOXM1

(unpublished data, Jaiswal, N and Nag, A, presented in several national and international conferences in 2012). Recently, similar observation has been reported in MCF-7 breast cancer cells in response to treatment with epirubicin and mitotic inhibitors like nocodazole. Their study shows that FOXM1 is negatively regulated by SUMOylation as a result of increased translocation to the cytoplasm and enhanced APC/C-Cdh1 mediated ubiquitination. Further investigations also revealed that SUMOylation defective mutant leads to enhanced cell proliferation in comparison to wild type FOXM1 (Myatt *et al.*, 2013). Since SUMO modification is known to influence transcription, cellular localization and protein turnover of transcription factors (Geiss-Friedlander and Melchior, 2007), further understanding of the role of SUMOylated FOXM1 in the context of transcriptional regulation, DNA damage response (Bergink and Jentsch, 2009) and cancer development (Kim and Baek, 2006) would be important.

#### 2.2.2. Auto-inhibition of FOXM1 transcriptional activity

Interestingly, transcriptional activity of FOXM1 has been shown to be negatively regulated by its own N-terminal domain (Laoukili *et al.*, 2008b; Park *et al.*, 2008b). N-terminal region of FOXM1 is proposed to act as an autorepressor domain by forming a direct complex with C-terminal transactivation domain, thereby preventing transcriptional activation of FOXM1 during G1/S phase. However, such intramolecular interaction within FOXM1 was susceptible to disruption by active cyclin A/Cdk (Laoukili *et al.*, 2008b) and cyclin D/Cdk4 (Wierstra and Alves, 2006) complexes in FOXM1b and FOXM1c respectively, which allows full activation of FOXM1 as cells progress to G2 phase. Supporting evidences also came from the experiments performed with the deletion mutant lacking the N-terminal autoinhibitory (N-Del 232) domain of FOXM1. The mutant showed high constitutive activity throughout the cell cycle without any requirement of cyclin-cdk1 for its activation. More importantly, the mutant exhibited increased transforming activity. On the other hand, FOXM1 has been proposed to be involved in positive auto-regulatory loop, where FOXM1 activates its own

mRNA and protein expression (Halasi and Gartel, 2009). Further investigations using transgenic animal models are needed to dissect these interesting regulatory as well as oncogenic mechanisms.

### 2.2.3. Regulation by tumor suppressors

FOXM1 is under the control of three major tumor suppressors: Retinoblastoma (Rb), p53, and p19ARF. Recent study has shown FOXM1 to be a bonafide p53 repression target (Barsotti and Prives, 2009). They showed that ectopic expression of p53 resulted in a reduction of FOXM1 mRNA levels, accompanied by reduction in FOXM1 protein levels. However, DNA damage cooperates with p53 to more potently repress FOXM1 mRNA. Also, DNA damage has been shown to positively regulate FOXM1 protein stability (Tan *et al.*, 2007). This shows that DNA damage regulates multiple signaling pathways to fine-tune FOXM1's cellular level. Mechanistically, p53-mediated inhibition of FOXM1 is partially dependent on p21 and retinoblastoma (Rb), although in some cases p21-independent repression of FOXM1 was also observed (Barsotti and Prives, 2009). Moreover, Rb family members also contributes to FOXM1 mRNA repression (Major *et al.*, 2004). Rb involvement in FOXM1 repression was implicated by the presence of two E2F1 sites in FOXM1 promoter. Besides E2F1, FOXM1 acts as a second proliferation transcription factor to be repressed by Rb. Rb mediated negative regulation of FOXM1 occurs only during G1 but not during S and G2 phase of the cell cycle. This is because hyperphosphorylated Rb fails to interact with FOXM1b protein during this phase. Moreover, the large pocket domain (aa 792-928) of Rb interacts with the central domain (also known as Rb-recruiting negative regulatory domain) of FOXM1c (359-425 aa), thereby repressing FOXM1c TAD in order to exclude its aberrant activity leading to tumorigenesis. This repression was majorly relieved by G1 phase proliferation signal through cyclin D1/Cdk4 and weakly by cyclin E/Cdk2 during G1 when transcriptional activity of FOXM1 is required for stimulation of G1/S transition (Wierstra and Alves, 2006). Regulation of FOXM1 by tumor suppressors is crucial for normal cell proliferation and

alterations in Rb or p53 levels will lead to FOXM1 misregulation and oncogenesis. Tumor suppressor ARF p19 also prevents FOXM1 mediated transactivation as well as FOXM1b induced anchorage dependent growth on soft agar (Gusarova *et al.*, 2007) by targeting it to the nucleolus. More studies in this area will be useful in designing novel therapeutic interventions.

## 2.3. Biological Functions of FOXM1

### 2.3.1. Role in cell cycle regulation, proliferation and senescence

Numerous evidences support that FOXM1 is a proliferation associated transcription factor. (Korver *et al.*, 1997a; Yao *et al.*, 1997; Ye *et al.*, 1997). *In vivo* expression studies in mouse embryo showed high levels of expression in all tissues (Korver *et al.*, 1997a; Ye *et al.*, 1997) whereas adult mice had high levels of FOXM1 in tissues with high proliferation index such as thymus, testis, small intestine and colon. However, significantly lower levels were found in ovary, spleen and lung, which possess less dividing cells (Korver *et al.*, 1997a; Ye *et al.*, 1997). Furthermore, FOXM1 has also been found to be expressed in B and T lymphoid, myeloid and erythroid cell lines as well as various carcinoma cell lines (Korver *et al.*, 1997a) whereas it is not expressed in quiescent or terminally differentiated cells. FOXM1 principally functions in regulating the expression of cell cycle genes (Costa, 2005). It is expressed during G1 phase and maintains an invariant transcript and protein level throughout S-, G2-, and M phase (Korver *et al.*, 1997a). In mammalian cells, FOXM1 controls cell proliferation mainly through inhibiting factors which repress S and M phase entry, such as cyclin-dependent kinase inhibitors (CKI), p21<sup>CIP1/WAF1</sup> and p27<sup>Kip</sup> and by activating cyclins or cyclin dependent kinases (Cdk) activators, such as cyclin A/CDK2 for S-phase entry (Reviewed in (Laoukili *et al.*, 2007)). FOXM1 has also been implicated in the regulation of transcription of Skp2 and Cks1, specificity subunits of the Skp1-Cullin1-F-box (SCF), which is essential for regulating turnover of CDKI during G1/S transition (Wang *et al.*, 2005). In addition, it also plays a crucial role in executing mitosis properly as evident from the development of pleiotropic mitotic defects such as aneuploidy

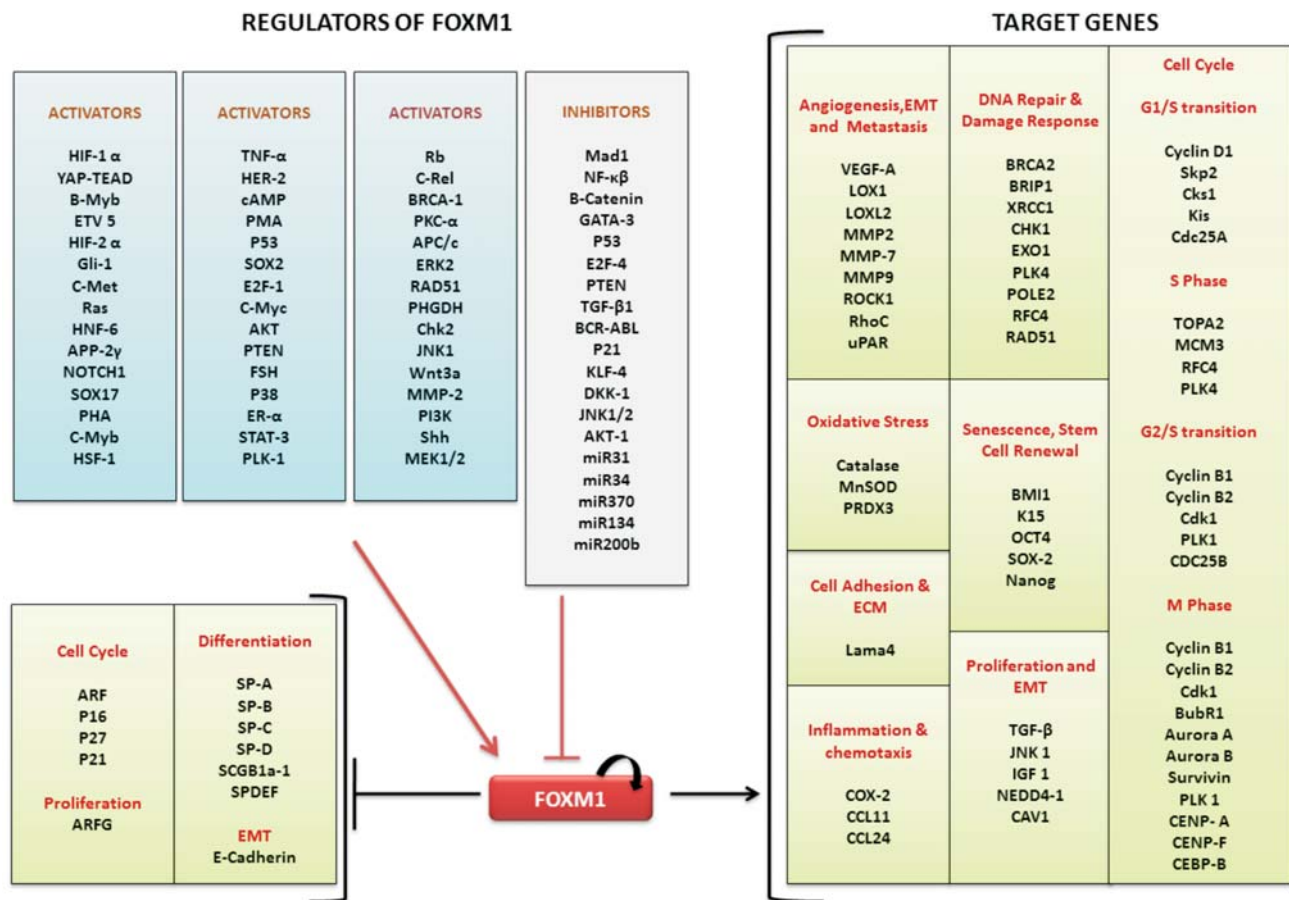


and polyploidy, chromosome segregation anomalies and defects in mitotic spindle formation in FOXM1 deficient cells. Studies involving microarray, chromatin immunoprecipitation (ChIP) and more recently, ChIP-seq analyses had revealed that FOXM1 controls expression of G2 phase genes, which are essential regulators of mitosis like *CCNB1* (CyclinB1), *Cyclin A*, *AURKB*, *Survivin*, *Plk1*, *Cdc25B* (cell cycle progression and mitotic entry); *CENPA*, *CENPB*, *CENPF* (Essential for mitotic spindle checkpoint integrity), *MYC* (c-Myc) (Wang *et al.*, 2005) etc. FOXM1 regulated genes involved in cell cycle regulation and other cellular functions are presented in Figure 4. FOXM1 also inhibits premature cellular senescence. This can be easily explained by phenotypes of FOXM1 knockout MEFs and FOXM1 depleted MEFs

which displayed premature senescence (Laoukili *et al.*, 2005; Wonsey and Follettie, 2005). The phenotypes were reverted by restoring the levels of FOXM1 by overexpression studies. Similar results were also achieved by induction of polycomb protein Bmi-1 via c-Myc (Li *et al.*, 2008).

### 2.3.2. Activation of DNA damage response

Emerging evidences reveal significant contributions of FOXM1 in DNA damage response and maintenance of genomic stability. Consistent with this notion, FOXM1-deficient MEFs displayed anomalies like polyploidy, aneuploidy, defects in cytokinesis, chromosome missegregation as well as high level of DNA breaks (Laoukili *et al.*, 2005). Similar results were obtained upon knockdown of FOXM1 in osteosarcoma cells (Tan *et al.*, 2007). It mainly



**Figure 4:** Regulators of FOXM1 and its effector genes. FOXM1 regulates expression of plethora of target genes through its binding to the consensus DNA sequence. It regulates transcription of cell cycle genes, essential for G1/S, G2/M progression, chromosomal segregation and cytokinesis. FOXM1 also targets genes involved in differentiation, senescence, stem cell renewal, DNA damage response, attenuation of oxidative stress, EMT, angiogenesis, metastasis and pre-metastatic niche formation. Genes that regulate FOXM1 both positively and negatively are also listed above.



plays a crucial role in homologous recombination, as evident from the experimental reports where FOXM1 overexpression prevents the accumulation of double strand DNA breaks in Epirubicin or Cisplatin treated MCF-7 cells. Among the most important FOXM1 target genes, five are HR genes (*brca2*, *xrcc2*, *exo1*, *rad51*, *brip1*). FOXM1 also mediates activation of the DNA repair genes *BRCA2* (breast cancer-associated gene 2) and *XRCC1* (X-ray cross-completing group 1) upon genotoxic stress (Tan *et al.*, 2007). Recently, NBS1, a crucial component of DNA damage repair complex, was also identified as FOXM1 target (Khongkow *et al.*, 2013) gene. Their study revealed that overexpression of FOXM1 enhances NBS1 expression and ATM phosphorylation by modulating the levels of MRN (MRE11/RAD50/NBS1) complex, leading to activation of DNA damage repair signaling. FOXM1 was also found to interact with NF $\kappa$ B in doxorubicin treated breast cancer cells in order to regulate expression of DNA repair genes like EXO1, RFC4, POLE2 and PLK4 and hence protecting the cancer cells from doxorubicin induced double stranded breaks (Park *et al.*, 2012). Moreover, recent evidences show that FOXM1 itself undergoes Chk2 mediated stabilization in response to DNA damage (Tan *et al.*, 2007). This also shows a significant correlation between FOXM1 and DNA damage responses. Recently, it has been shown that FOXM1 is involved in inhibition of DNA damaged induced apoptosis by upregulation of pro-apoptotic factor Bcl2, (Halasi and Gartel, 2012). FOXM1 also plays a crucial role in checkpoint recovery after doxorubicin or IR treatment. Since, overexpression of FOXM1 maintains high levels of PLK1 and Cyclin B1, cells are forced to re-enter the cell cycle even after DNA damage (Alvarez Fernández *et al.*, 2010). Altogether, FOXM1 mediated genotoxic drug resistance is attributed to its involvement in DNA damage repair, recovery after DNA damage and inhibition of DNA damage induced apoptosis.

Discovery of multiple functions of FOXM1 in DNA damage response also opens up new treatment opportunities in which targeting FOXM1 may be effective in clinically impeding tumour growth. For instance, combining FOXM1 inhibition with PARP inhibitors would sensitize

cells to PARP inhibitors, involved in repairing single stranded DNA breaks during replication (Alvarez-Fernández and Medema, 2013). Hence, better understanding of this area could offer improved therapeutic options for targeting cancer and emergence of new treatment strategies for overcoming chemoresistance.

### 2.3.3. Role in stem cell self-renewal, pluripotency and stem cell fate determination

FOXM1 plays a pivotal role in maintenance of stem cell pluripotency and the self-renewal process. Genes like *Oct4*, *Sox2* and *Nanog*, the critical determinants of stem cell pluripotency and self-renewal capability, gets activated by FOXM1 (Wang *et al.*, 2011b; Xie *et al.*, 2010). It has also been reported that FOXM1 overexpression induces the formation of neurospheres in glioma cells and FOXM1 deficiency or knockdown reduced the formation of neurospheres in neural cortical stem cells and in GIC (GBM initiating cells), indicating a crucial role of FOXM1 in stem cell self-renewal. Recent study in mouse model also suggested involvement of FOXM1 in cell fate determination by regulating expression of GATA-3, a key regulator of breast luminal epithelial differentiation (Carr *et al.*, 2012). Furthermore, FOXM1 has been shown to transactivate an epithelial stem cell marker keratin 15 (*Krt15*) gene in human keratinocytes (Gemenetzidis *et al.*, 2010). Recently, FOXM1 has been found to associate with maternal embryonic leucine-zipper kinase (MELK) in glioma stem cells, resulting in cell cycle progression, cancer cell growth and maintenance of stem cell fate of GBM (Joshi *et al.*, 2013). FOXM1 is also profoundly involved in the acquisition of EMT and CSC phenotypes in pancreatic cancer cells (Bao *et al.*, 2011; Quan *et al.*, 2013).

Hence, it can be proposed that FOXM1 disturbs the balance between stem cell self-renewal and differentiation by inducing self-renewal and disfavoring differentiation. This also augments the process of clonal expansion by inhibition of terminal differentiation of stem cells (Teh, 2012). However, so far FOXM1 has not been shown to revert terminally differentiated cells. Together, these findings also suggest a central role of FOXM1 in tumorigenesis by regulating self-renewal, immortalization and sustained proliferative properties of cells.

The above findings also suggest FOXM1's role in tissue regeneration (liver, lung and pancreas) after injury. Its role in adult tissue repair has been demonstrated for liver regeneration after carbontetrachloride (Wang *et al.*, 2001), partial hepatectomy (Krupczak-Hollis *et al.*, 2003; Wang *et al.*, 2002) injury and Lung regeneration after Butylated hydroxytoluene (Kalinichenko *et al.*, 2003), lipopolysaccharide (Zhao *et al.*, 2006), and *Pseudomonas aeruginosa* induced (Liu *et al.*, 2011) lung injury. Its role in organogenesis has been confirmed by knock-out studies where homozygous FOXM1 mutants displayed a lethal phenotype, indicating that FOXM1 may not play a role in embryogenesis but is crucial for organogenesis (Korver *et al.*, 1998). Based on these studies, FOXM1 can be proposed to be a promising candidate for developing strategies for regenerative medicine. In this context, it will be extremely important to explore the conditions that will specifically stimulate the regenerative properties of FOXM1 without arousing its unwanted oncogenic potential.

### 3. FOXM1: Involvement in tumor development and progression

#### 3.1. FOXM1: A central performer in cancer

FOXM1 signaling maintains a balance between cell proliferation, differentiation, and apoptosis and an abnormal activation of FOXM1 gene is a hallmark of many human cancers. Amplification of the 12p13 chromosomal band containing the FOXM1 gene have been reported in numerous tumors such as cervical squamous cell carcinomas, breast adenocarcinomas, pancreatic cancer, lung cancer, gastric cancers, nasopharyngeal carcinomas, and head and neck squamous cell carcinomas (Laoukili *et al.*, 2007). Moreover, gene expression profiling of cancers has also identified FOXM1 as one of the most commonly upregulated genes in human solid tumors. This observation reaffirms the link between FOXM1 deregulation and cancer progression. The oncogenic potential of FOXM1 is mainly based on its ability to transcriptionally activate genes that are involved in different facets of cancer development.

Multiple oncogenic signaling pathways have been reported to cross talk with FOXM1 pathway.

Hedgehog signaling pathway has been found to upregulate FOXM1 gene in pancreatic cancer (Wang *et al.*, 2007), basal cell carcinoma (Teh *et al.*, 2002) and lung cancer (Gialmanidis *et al.*, 2009). Several components of this signaling pathway are correlated with FOXM1. For instance, Gli1 overexpression was observed in NSCLC which in turn is known to induce FOXM1 transcriptional activity in basal cell carcinoma. Furthermore, Gli2 has been shown to play a predominant role in hepatocellular carcinoma and basal cell carcinoma (Teh *et al.*, 2002). Notch signaling also plays a very crucial role in prostate cancer cell survival, where it mediates its effect via downregulation of FOXM1 and Akt leading to inhibition of cell growth and induction of apoptosis. Presence of FOXM1 binding elements in Caveolin-1 (Cav1) promoter, which is known to play a critical role in pancreatic cancer progression and EMT further reveals role of FOXM1 in pancreatic cancer pathogenesis and aggressiveness (Huang *et al.*, 2012). Future studies in relation to FOXM1-Cav1 signaling pathway would be useful in designing better treatment modalities in controlling this deadly cancer. Like FOXM1, COX-2 is also upregulated in many cancers and is implicated in different malignancies. COX2 promoter contains FOXM1 responsive element, where it binds and stimulate COX2 promoter activity (Xu and Shu, 2013) causing lung cancer (Wang *et al.*, 2008a). FOXM1 has been implicated in the development as well as progression of many cancers and is implicated in tumor angiogenesis, invasion and metastasis (Koo *et al.*, 2012). Recent studies have shown that downregulation of FOXM1 inhibits cell growth, migration and invasion in breast cancer cells (Wang *et al.*, 2007; Yang *et al.*, 2013b), pancreatic cancers (Bao *et al.*, 2011; Huang *et al.*, 2014), hepatocellular carcinoma (Wu *et al.*, 2010), gastric cancer (Li *et al.*, 2009) etc. by inhibiting the expression of many factors that are involved in the degradation of extra cellular matrix and angiogenesis such as uPA, uPAR, MMP-2, MMP-9, and VEGF (vascular endothelial growth factor). FOXM1 is also considered to be part of the breast tumor proliferation cluster, which includes genes that are known to enhance proliferation rates of tumors. Studies revealed that FOXM1 is a physiological regulator of ER $\alpha$  expression in

breast carcinoma cells (Madureira *et al.*, 2006). The regulation of FOXM1 by ER $\alpha$  also supports tumorigenesis and hormone-insensitivity in breast cancers. Furthermore, recent evidence suggested that the anti-proliferative role of ER $\alpha$ 1 in the development of breast cancer is mediated through the negative regulation of FOXM1 expression via ER $\alpha$ . Another study by Bektas group also suggested a positive correlation

between FOXM1 expression and HER2 status, thereby pointing to the potential role of FOXM1 as a new drug target in HER2 resistant breast tumors (Wang *et al.*, 2007). In a recent study by Yang *et al.*, overexpression of FOXM1 was shown to promote EMT in breast cancer by stimulating promoter of Slug, a EMT related gene (Yang *et al.*, 2013a). FOXM1 association with different cancers is summarized in Table 1.

**Table 1**  
**Deciphering association of FOXM1 with cancer**

TYPE OF CANCER	FINDINGS	EXPERIMENTAL SYSTEMS			FOXM1-Targeting	Ref.
		Cell Lines	<i>In vivo</i>	Clinical Samples		
Breast Cancer	Down regulation Of FOXM1 inhibited the growth of breast cancer cells. It also reduced invasion and migration capabilities of cancer cells.	MDA-MB 231, SKBR3, SUM 102, SUM 149	X	X	FOXM1 has been successfully targeted in breast cancer through RNAi, Siomycin A, Thiostrepton, DIM both in vivo and <i>in vitro</i> . Docetaxel, Thiostrepton-Bortezomib combination has also been proved to reduce xenograft tumor growth.	(Ahmad et al., 2011; Ahmad et al., 2010; Bektas et al., 2008; Caldwell et al., 2010; Francis et al., 2009; Halasi et al., 2010; Kwok et al., 2008; Wang and Gartel, 2011, 2012; Yang et al., 2013b)
	FOXM1 can be used as an crucial diagnostic marker for Breast Cancer	BT474,SKBR3,M CF-7, MDA-MB 231, MDA-MB 453	MCTV-c-Neu mice	112 BCa patient samples		
		MCF7,T47D,ZR75 -1, MDA-MB 231,SKBR3, MDA-MB435s,BT20	X	204 BCa patient samples		
Cervical Cancer	FOXM1 is significantly associated with cervical cancer progression and pathogenesis and involved in normal epithelium-cervical intraepithelial neoplasia (CIN) transition. Its Expression is also associated with Ki67 expression and can be used as a similar prognostic marker as Ki67.	Nos NC104, NC105, Hela, SiHa, CasKi, C33A and C4-1.	X	108 cervical cancer patient samples	Only RNAi has been proved to be successful only <i>in vitro</i> . Further extensive research needed.	(Chan et al., 2008)
Colorectal Cancer	FOXM1 is crucial for growth and proliferation of colorectal cancer	X	FOXM1 knock-out Mice	X	No clinically relevant FOXM1 targeting. Further research is needed.	(Li et al., 2013); (Yoshida et al., 2007); (Chu et al., 2012)
		X	Orthotopic mouse models	203 patient samples		
	FOXM1 over-expression can be used as a molecular marker for predicting metastatic potential and poor prognosis of colorectal cancer	LoVo and SW480	X	112 CRC tissue		

Head and Neck Cancer	FOXM1 induces a global DNA methylation pattern mimicking cancer methylome landscape	SCC4, SCC9, SCC15, SCC25, SqCC/Y1, UK1, VB6, CaDec12, SPT, H357, SVpgC2a, SVFN1-8.	X	HNSCC tumor tissues	No clinically relevant FOXM1 targeting. Further research is needed.	(Teh et al., 2012a); (Waseem et al., 2010)
	CEP55 and HELLS are the downstream targets of FOXM1 and can be used as a cancer progression marker.	SCC9, SCC15, SCC25, SqCC/Y1, UK1, UB6, CaLH2, CaDec12, SPT, H357.	X	20 patient samples		
Gastric Cancer	FOXM1 is critical for gastric cancer development and progression.	NCI-N87, AGS, HTB 103, HTB 135 SNU1, SNU16, Sk-GT5	X	GC patient samples	RNAi has been proved to be successful both <i>invitro</i> and <i>invivo</i> . Further extensive research needed.	(Li et al., 2009; Zeng et al., 2009)
	FOXM1 inhibition leads to p27 <sup>kip1</sup> mediated cellular senescence in gastric cancer	AGS, BGC-823, HGC-27, KATO-III	X	42 patient sample		
Glioma	FOXM1 promotes glioma formation and metastasis	Hs683, U118MG, LN 229, U87 MG, HF U 251 MG	BALB/c nude mice	X	RNAi has been proved to be successful both <i>invitro</i> and <i>invivo</i> . Further extensive research needed.	(Dai et al., 2007; Dai et al., 2010; Liu et al., 2006; Zhang et al., 2011; Zhang et al., 2008)
		SW1783, Hs683, HF-U251 MG, U87 MG.	X	X		
		Hs683, HFU 251	BALB/c nude mice	X		
	FOXM1 interacts with $\beta$ -Catenin and controls canonical wnt signaling, required for glioma formation	Hs683, SW1783	X	X		
Pancreatic cancer	FOXM1 critically regulates development and metastasis of pancreatic cancer cells	AsPC-1, BxPC-3, COLO-357, HPAC, L3.6PL, MIAPaCa, PANC1	X	X	Genistein in <i>invitro</i> studies and RNAi in both <i>invivo</i> and <i>invitro</i> has been	(Bao et al., 2011; Bhat et al., 2011; Huang et al.,



		AsPC-1, CaPan-1, MiaPaca-2, PANC1, MDAPanc-28, MDAPanc-48.	X	70 primary patient sample	proved to be successful	(2012; Wang et al., 2010; Wang et al., 2007; Xie et al., 2014)
Prostate Cancer	NOTCH-1 signaling mediated activation of FOXM1-Akt required for prostate cancer growth and apoptosis.	PC-3, DU145, C4-2B, LNCaP.	SCID mouse	X	RNAi, SiomycinA and Thioestreptone treatment has been proved to be successful <i>in vitro</i> . Genistein and natura- $\alpha$ displayed prosperous results both <i>in vivo</i> and <i>in vitro</i>	(Kalin et al., 2006; Li et al., 2011; Pandit and Gartel, 2010; Wang et al., 2011a)
	Increased level of FOXM1 induces development and progression of prostate cancer	X	TRAMP and LADY transgenic mice	X		
Lung Cancer	FOXM1 expression is an independent poor prognostic factor in lung carcinoma.	X	X	69 patient tissue sample	Only RNAi has been proved to be successful only <i>in vitro</i> . Further extensive research needed.	(Halasi and Gartel, 2013; Kim et al., 2006; Yang et al., 2009)
	FOXM1 stimulates the tumor proliferation during lung cancer development	X	Mx-Cre FOXM1 <sup>-/-</sup> mice	X		

FOXM1 also stimulates proliferation of lung tumor cells during progression of NSCLC (Wang *et al.*, 2008a). This was clearly evident from decreased expression of cell cycle promoting cyclinA2 and cyclinB1 genes, diminished DNA replication and reduced anchorage-independent growth of FOXM1 depleted A549 lung cancer cells (Kim *et al.*, 2006). The functional significance of FOXM1 in human cervical cancer is also poorly understood even though FOXM1 has been shown to interact with HPV16-E7 oncoprotein and enhance transformation of cervical cancer cells (Lüscher-Firzlaff *et al.*, 1999). Recently, it has been found that HPV16 E7 oncogene contribution in cellular proliferation occurs through its interaction with DREAM (DP, Rb-like, E2F and MuvB) complex during cell cycle (DeCaprio, 2013). More recently, Pang *et al.* (2013) demonstrated the contribution of functional interaction of E7 and B-Myb-MuvB complex, in activation of S and M phase genes (Pang *et al.*, 2013). This provided novel insights into mechanism of oncogenesis and development of cervical carcinoma. Further studies are required to investigate more convincing links between

FOXM1 and HPV oncoproteins in cervical cancer. Using *in vitro* and animal models, the underlying mechanism of altered FOXM1b expression on gastric cancer growth and metastasis was also investigated (Li *et al.*, 2009) where abnormal activation of FOXM1b caused overexpression of multiple angiogenic molecules like VEGF, which in turn render tumor cells highly angiogenic whereas knockdown of FOXM1b did the reverse (Li *et al.*, 2009). Therefore, FOXM1b also plays a crucial role in gastric cancer pathogenesis (Li *et al.*, 2009). FOXM1 signaling network is also reported to be critical for glioma by promoting cell proliferation, invasion, angiogenesis and cancer stem cell renewal. FOXM1, by regulating expression of SKP2, promotes degradation of p27Kip1, which results in an aberrant cell cycle and glioma tumorigenicity (Liu *et al.*, 2006). FOXM1b is the predominant form of FOXM1, present in the glioma tissue and mainly contributes to glioma angiogenesis and invasion through upregulation of VEGF expression (Zhang *et al.*, 2008). Glioma formation is also accelerated by FOXM1 mediated  $\beta$ -catenin activation as well (Abla *et al.*, 2012; Zhang *et al.*, 2011). Another

study suggested that FOXM1 is a HSF1 (heat shock factor) target and promotes cell cycle progression through Cdc2, Cdc20 and Cdc25B (Dai *et al.*, 2013). This finding was consistent with the finding which showed high levels of HSF1 and FOXM1 in glioma tissue samples. Further studies in determining whether FOXM1 can cooperate with other HSPs to promote cancer progression will be interesting.

Recently, miRNAs have been reported to modulate the expression of FOXM1 and it has been identified as a direct target of miR-134, whose levels are inversely correlated with the invasive potential of some NSCLC cells (Li *et al.*, 2012). FOXM1 is also repressed by miR-370 in acute myeloid leukemia (Zhang *et al.*, 2012), gastric cancer (Feng *et al.*, 2013) and laryngeal squamous cell carcinoma (LSCC) (Yungang *et al.*, 2013). Additionally, FOXM1 and miRNA signaling pathway was also exploited for pancreatic cancer treatment (Shi *et al.*, 2014). With multifaceted oncogenic roles in myriads of human cancer, FOXM1 can therefore be exploited as a cancer biomarker for clinical benefits.

### 3.2. Epigenetic regulation and cancer: Role of FOXM1

Epigenetic changes or alterations in the gene expression profile of oncogenesis driver genes may have profound effect on the development of cancer. First evidence of FOXM1's link to epigenetic regulation was the identification of HELLS, a chromatin remodeling/DNA helicase, as a downstream target of FOXM1 in head and neck squamous cell carcinomas. Aberrant upregulation of FOXM1, was found to reprogram the normal cells by changing its 'methylation' landscape towards those found in cancer cells, through the recruitment of HELLS and two DNA methyltransferases DNMT1 and DNMT3B (Teh *et al.*, 2012a). Furthermore, using genome wide methylated arrays, number of FOXM1 regulated genes, which get differentially methylated were identified including *SPCS1*, *FLNA*, *CHPF*, *GLT8D1*, *MGAT1*, *NDUFA10*, *PAFAH1B3* and *C6orf136* (Hwang *et al.*, 2013). Although these basic findings support the involvement of FOXM1 in inducing epigenetic alteration through expression of downstream epigenetic modulators, further exploration of this domain is immediately

required for making the understanding of diverse molecular mechanisms by which FOXM1 induces oncogenesis. Compelling evidences also suggest the use of FOXM1 expression in combination with CEP55 and HELLS (Janus *et al.*, 2011; Teh *et al.*, 2012b) as a biomarker set for early cancer detection of malignant conversion and progression. Thus, understanding of aberrant epigenetic alteration involving DNA methylation is prerequisite to finding predictive and early cancer biomarkers. This would have tremendous clinical applications in population screening to identify individuals with cancer predisposition or at the risk of developing cancer.

### 3.3. Unwinding the knot: Understanding the mechanism of FOXM1 action in oncogenesis

Malignant tumors arise from a small population of self-renewing cancer stem cells (CSCs). These cells, being highly resistant to chemo/radiation therapy help in the development of resistant tumors and disease recurrence after therapy. According to the current understanding of CSC development, they arise from the adult progenitor cells, which, due to their high proliferation rate, can easily accumulate mutations, resulting in generation of CSCs. CSCs mostly follow the pattern and features of adult stem cells. These give rise to highly proliferative progenitor cancer stem cells, which then generate terminally differentiated cellular population in a tumor (Lobo *et al.*, 2007). FOXM1, due to its influence in diverse cellular processes like cell cycle, EMT, stem cell differentiation and self-renewal, drives the process of tumor initiation and progression. Encouragement of self-renewal of CSCs and inhibition of CSC differentiation are the basic criteria for successive tumor development. Recent findings implicate important role of FOXM1 in the maintenance of cancer stem cells via inhibition of differentiation (Teh, 2012). Moreover, interaction of FOXM1 with  $\beta$ -catenin has been shown to assist stem cell self-renewal (Zhang *et al.*, 2011). As the extent of therapeutic resistance should increase with the amount of remaining CSC population, it can also explain the therapeutic resistance of FOXM1 upregulated cancers. After the generation of tumor, EMT is a crucial mechanism of metastatic progression. It is also important for the process called

'Phenotypic transition', which maintains the proportional heterogeneity in a tumor cell population. FOXM1 has been proved to upregulate mesenchymal cell phenotype markers involved in EMT, such as, ZEB1 (Zinc-finger E-box binding homeo-box1), ZEB2, Snail2, vimentin, fibronectin, N-cadherin and downregulate epithelial marker E-Cadherin. FOXM1 has also been found to transcriptionally activate VEGF expression by directly binding to Forkhead binding elements of its promoter (Zhang *et al.*, 2008). Suppression of FOXM1 in glioblastomas, gastric, pancreatic and hepatocellular carcinomas resulted in low VEGF expression substantiating the idea that FOXM1 is required for VEGF induced angiogenesis (Zhang *et al.*, 2008). Similar role of FOXM1 in the regulation of MMP2 and MMP9 expression has also been documented in breast carcinoma, colorectal carcinoma and glioblastoma. FOXM1 generally regulates MMP2 through its binding to forkhead consensus site, while FOXM1 regulates MMP9 expression indirectly via its downstream target JNK1 (Wang *et al.*, 2008b). Moreover, FOXM1 has been shown to transcriptionally activate stathmin, thereby increasing cell motility by destabilizing microtubules (Park *et al.*, 2011). FOXM1 binding to promoters of Lysyl oxidase (LOX) and LOXL2 has also been found to stimulate induction of premetastatic niche (Park *et al.*, 2011). Overexpression of FOXM1 also upregulates cancer stem cell surface markers such as CD44 and EpCAM in human pancreatic cancer cells (Bao *et al.*, 2011). Thus, as FOXM1 can positively regulate the process of EMT and angiogenesis, it can also assist invasion, metastasis and maintenance of the stem cell population through 'Phenotypic transition' of non-stem cell population in the tumor. These results provide sufficient evidences in support of the role of FOXM1 signaling in tumor cell aggressiveness through the acquisition of EMT phenotype in cancer cells. Therefore, targeting FOXM1 would be useful for reversing EMT phenotype.

Another important process that FOXM1 negatively regulates is cellular senescence. It is an intrinsic cellular response that restricts unlimited cell proliferation and has a key physiological role in tumor suppression through

preventing cancer initiation and progression (Acosta and Gil, 2012; Ben-Porath and Weinberg, 2005). Recent evidences, such as triggered p53 and p16<sup>INK4A</sup> independent senescence through p27<sup>Kip1</sup> expression in FOXM1 depleted gastric cancer cells or onset of premature senescence in FOXM1 depleted MEFs established the idea that FOXM1 is critically involved in the inhibition of cellular senescence (Zeng *et al.*, 2009). This has been correlated with upregulation of polycomb group protein Bmi-1, a major negative regulator of the Ink4a/ARF/Ink4b locus that encodes p19 ARF as well as the CDK inhibitors p16 and p15 and help out the cells in overcoming senescence (Li *et al.*, 2008). Reports have also suggested participation of FOXM1 in cytoprotection of cancer cells by inducing anti-oxidant genes and reducing oxidative stress (Li *et al.*, 2008). It has also been reported that it may further counteract ROS levels by inducing expression of ROS scavenger genes, such as catalase, *MnSOD* and *PRDX3* (Park *et al.*, 2009). Detailed analysis confirmed the presence of conserved FOXM1 binding sites on the promoters of these genes. Evidences also suggest a HIF-1 $\alpha$  mediated upregulation of FOXM1, upon encountering hypoxic stress (Xia *et al.*, 2009).

FOXM1 also plays an integral part in the development of acquired drug resistance to hamper effectiveness of most chemotherapeutic drugs. For example, FOXM1 has been shown to confer resistance to Herceptin, Paclitaxel, Cisplatin in breast cancer cells (Carr *et al.*, 2010). FOXM1 has also been implicated in genotoxic drug resistance. Studies from Medema's group suggested that over-expression of FOXM1 in Epirubicin sensitive cells confer resistance to Epirubicin. Whereas depletion of FOXM1 in epirubicin resistant cells was found to re-sensitize these cells to the drug (Khongkow *et al.*, 2013). This supports FOXM1's role in cancer drug resistance (Millour *et al.*, 2011) (de Olano *et al.*, 2012). Hence, critical exploration of roles of FOXM1 during tumorigenesis can provide us with valuable insights regarding mechanism of FOXM1 action in cancer development and advancement. In light of above findings, we propose a comprehensive model depicting probable mechanism of FOXM1 action in tumorigenesis (Figure 5).



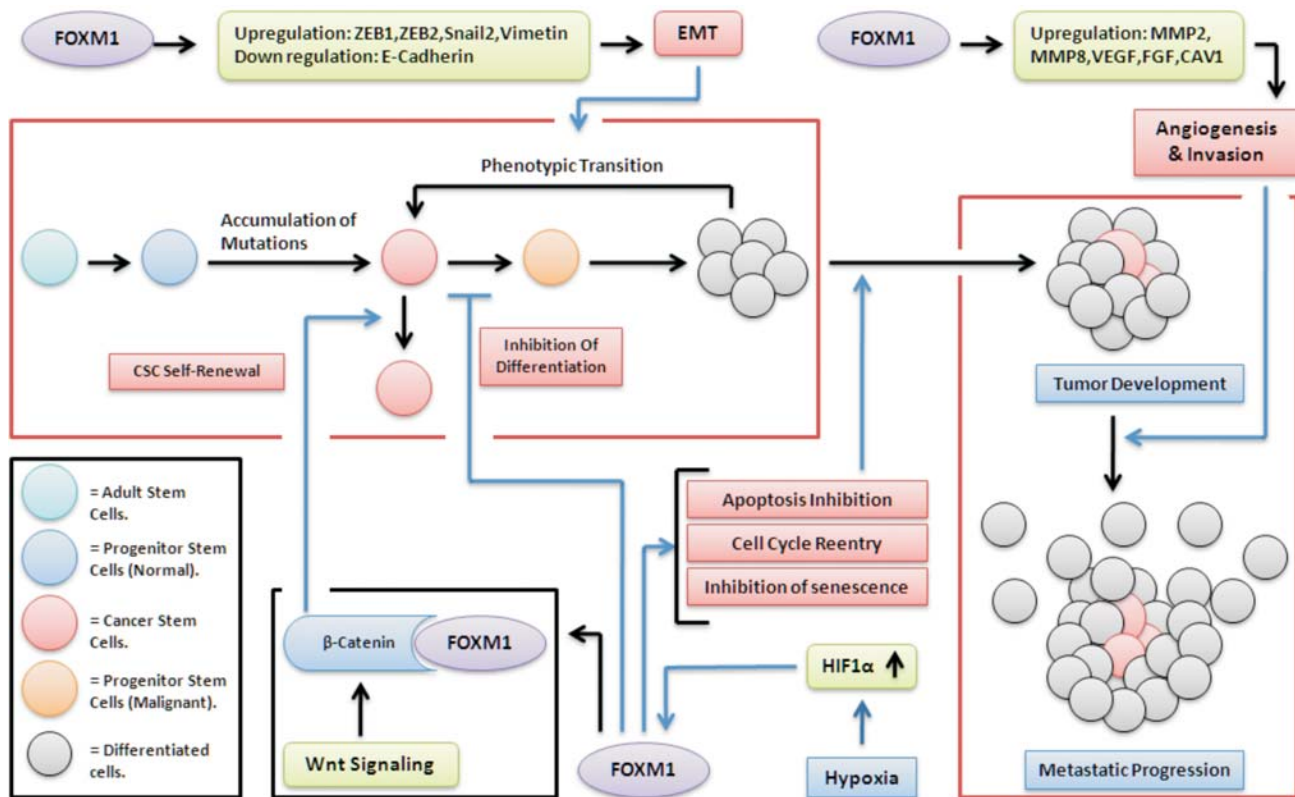


Figure 5: Probable Mechanism of FOXM1 action in tumorigenesis. FOXM1 induces many of the genes involved in stem cell self-renewal, angiogenesis and metastasis, and inhibits genes involved in cellular senescence and differentiation. These FOXM1 driven processes play a crucial role in cancer stem cell maintenance and tumor development.

### 3.3. 'Biological Sense of Cancer': Analyzing the oncogenic role of FOXM1 from a different viewpoint

In the year 2006, O. S. Bustuoabad and R. A. Ruggiero proposed a new hypothesis, where they made an effort to unravel the mystery behind 'Origin of Cancer' (Ruggiero and Bustuoabad, 2006). With their hypothesis they actually supported the preliminary idea of Zajicek (Zajicek, 1996), Bissell (Kenny and Bissell, 2003), Duesberg (Duesberg and Rasnick, 2000), and Soto (Maffini *et al.*, 2004), who first tried to look at cancers as the ultimate survival mechanism of the cellular system. According to their hypothesis, tumor formation is the ultimate effort of the physiological systems to restore the functional and structural viability of any degenerated organ (Ruggiero and Bustuoabad, 2006). In a simpler way, if the tissues of any organ get damaged either due to ageing or harmful environmental factors and lost the capability of responding to cellular proliferative signals, organs try to make the ultimate attempt for

survival by generating 'tumor', which would be the only capable fraction of cells, within the damaged organ, that can respond to the signals and meet the requirement of proliferation. As according to the theory, the basic reason behind 'tumor generation' is tissue injury, a brief glance at FOXM1 function in oncogenesis from this point-of-view may support our further understanding of tumor development and progression.

As discussed, FOXM1 is a crucial factor for regeneration of liver, lung and pancreas after injury. Normally, FOXM1 can repair the damaged tissue and help in its regeneration. But if the injury is severe, it is even impossible for FOXM1 and other tissue repair factors to regenerate the tissue, which creates a condition of severe 'Crisis'. Under such critical condition, continuous upregulation of FOXM1 may trigger oncogenic proliferation cascades, which may lead to 'Tumor development' and genomic instability as depicted in Figure 6. However, the hypothesis needs to be validated by substantial experimental data.



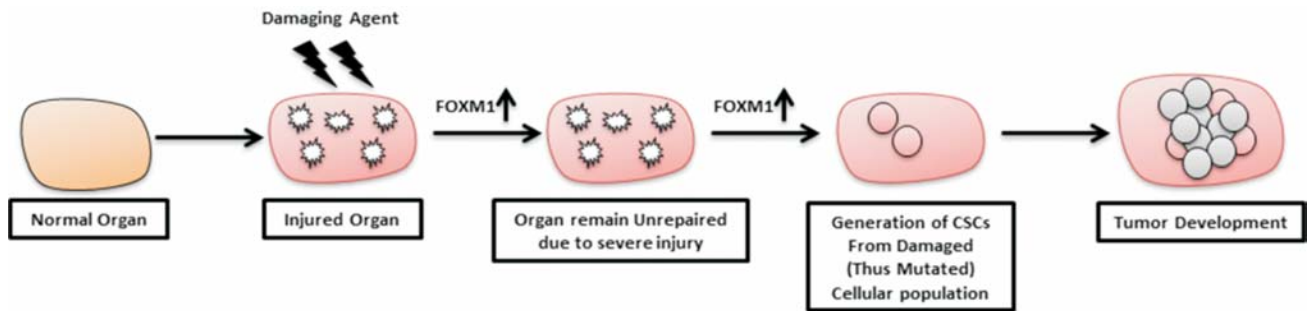


Figure 6: Probable alternative mechanism of FOXM1 action. According to a theoretical model of cancer generation, cancer is the ultimate survival mechanism for any severely injured cellular system. Due to FOXM1's intimate association with tissue repair and injury, their model can be supported as an alternative mechanism of tumor generation.

#### 4. FOXM1 as a potential target for anti-cancer therapy

The International Society for Molecular and Cell Biology and Biotechnology Protocols and Research (ISMCBBPR) has recognized FOXM1 as the Molecule of the Year, 2010 because of its growing potential as a target for developing promising cancer therapies. This has led to the discovery of numerous novel agents for targeting FOXM1 for anti-cancer therapy.

##### 4.1. Small peptide proteasome inhibitors

Using screening of chemical libraries, several small molecule inhibitors against FOXM1 has been identified such as thiopeptide antibiotics like Siomycin A (Radhakrishnan *et al.*, 2006) and Thiostrepton (Bhat *et al.*, 2008; Kwok *et al.*, 2008). Both Siomycin A and Thiostrepton, represses endogenous FOXM1 mRNA and protein levels expression, as well as the transcriptional activity of exogenous FOXM1b and act as proteasome inhibitors. Both of these peptides directly bind FOXM1, blocking its binding to the promoters of its target genes. The initial problem regarding Thiostrepton treatment emerged due to its hydrophobicity, which was addressed by encapsulating the peptide into micelles assembled from amphiphilic lipid-polyethylene glycol, where hydrophobic Thiostrepton molecules were solubilized into the lipid component of the micelle shell. This approach was proved to be successful and inhibited growth of human cancer xenografts and suppressed FOXM1 expression in tumors. It was also reported to confer apoptosis and reduced cell migration, invasiveness and transformation in breast cancer cells. Moreover, as none of these

thiazole antibiotics affect the transcriptional activity of any other factor other than FOXM1 and due to micromolar level sensitivity of cancer cells to these drugs, specificity and toxicity issues in anti-cancer therapy can be addressed in future.

Other proteasome inhibitors including Bortezomib, MG132 are also reported to suppress FOXM1 similar to Thiostrepton and Siomycin A (Halasi and Gartel, 2013). A model stating that all proteasome inhibitors will inhibit FOXM1 auto-regulation and FOXM1 expression through the stabilization of hypothetical NFRM (Negative regulator of FOXM1) had been proposed by Gartel and his colleagues (Bhat *et al.*, 2009).

##### 4.2. Inhibition through RNAi

Targeting FOXM1 using RNA interference approach has been proved successful so far. Although the therapeutic utility of this technique is debatable, at least *in vitro* and *in vivo* preclinical studies were positively conclusive. Gartel and co-workers showed suppression of FOXM1 and its targets in breast cancer xenografts by anti-FOXM1 siRNA encapsulated in polyethylimine-based cationic polymer (Wang and Gartel, 2011). Using this approach, expression levels of FOXM1 and its transcriptional targets Cdc25B and Aurora B kinase were also decreased, while p27, an indirect target of FOXM1 (via suppression of Skp2), was increased in tumors treated with FOXM1-siRNA (Wang and Gartel, 2011). Later, different research groups have showed that knock-down of FOXM1 by RNAi can suppress the proliferation of Breast (Ahmad *et al.*, 2010), Pancreatic (Wang *et al.*, 2007), Prostate (Kalin *et al.*, 2006), Lung (Kim *et al.*, 2006), Cervical (Chan *et al.*, 2008) and Colon (Yoshida *et*

*al.*, 2007) cancer cells. Further, FOXM1-siRNA was also shown to reduce migration, invasion and angiogenic potential of these cancer cells. Recently, use of micro-RNAs have also been proposed for controlling the levels of FOXM1. Hence, targeting FOXM1 by RNAi represents an appealing approach for treatment of cancer (Halasi and Gartel, 2013).

#### 4.3. Alternative approaches

Among various other FOXM1 inhibitory agents reported so far, except a cell penetrating p19 ARF peptide inhibitor of FOXM1 (Carr *et al.*, 2010), others do not have potential to stand up to the previous two approaches. This modified membrane transducing peptide from ARF protein was found to interact with FOXM1b and inhibits transcriptional activity. Some of the encouraging findings with ARF peptide inhibitor has been its negative effects on cell proliferation and angiogenesis after 4 weeks of treatment in hepatocellular carcinoma (HCC) mouse model (Gusarova *et al.*, 2007). Moreover, this peptide was found to induce apoptosis in the p53 null sarcoma and lymphoma, leading to a strong inhibition of their metastatic colonization. Thus, ARF peptide mediated inhibition of FOXM1b transcriptional activity represents a promising therapy for hepatocellular carcinoma.

FOXM1 inhibitors in the form of NPM (Nucleoplasmin) peptides (Bhat *et al.*, 2011), which can disrupt the interaction between FOXM1 and NPM, could also represent a novel drug against cancer as recent studies suggested that NPM interacts with FOXM1 and NPM knockdown in cancer cells leads to significant down regulation of FOXM1.

Various anti-cancer drugs such as Genistein (Wang *et al.*, 2010; Zhang *et al.*, 2011), TMPP (Nakamura *et al.*, 2010), 3,3-Diindolylmethane (DIM) (Ahmad *et al.*, 2011), Natura-alpha (Li *et al.*, 2011) and 9-diindolylmethane (9D) (Caldwell *et al.*, 2010) were also shown to effectively down regulate the FOXM1 mRNA expression in pancreatic, AML, breast and prostate cancer cells and consequently inhibit the growth of these cells. However, recent findings are indicating a need to switch towards use of combinatorial approach for improved treatment efficacy. It has been

shown that FOXM1 inhibition via ARF peptide or siRNA render increased sensitivity of the cancer cells towards commonly used anticancer drugs like herceptin or paclitaxel and can be proved to be useful for targeting chemo/radiation resistant cancer cell population (Carr *et al.*, 2010; Pandit and Gartel, 2011). Findings related to FOXM1 directed anti-cancer therapy are summarized in Table 1.

#### 5. Summary and future perspective

In this article, we have made an effort to discuss the recent advancements in our understanding of FOXM1 as an oncogenic transcription factor to recapitulate its significance in tumorigenesis. Impressive numbers of evidences reviewed above implicate that FOXM1 and its associated signaling pathways play a critical role in pathogenesis and progression of several malignancies. Consistent findings also indicate that deregulation of FOXM1 is a major driving force for multiple steps of tumor progression. Therefore, delineation of various signaling pathways involved in deregulation or aberrant expression of FOXM1 may provide opportunities for development of anti-cancer modalities. Again, a significant number of findings show a strong correlation between downregulation of FOXM1 with suppression of tumorigenesis, underscoring its therapeutic potential. So far many anti-cancer drugs have been used to target FOXM1, but none of them entered clinical trials. Thus, there is an urgent need to find a way for successful therapeutic strategy by exploiting the best possible approaches. While selective elimination of FOXM1 appears to be an attractive anti-cancer therapy, complications may arise due to long term medications. Hence, this approach needs to be tested by studying long-term effects of FOXM1 inhibition. Several studies also emphasize on the fact that there exist functional and regulatory differences between different isoforms of FOXM1. Thorough understanding of these aspects are necessary for developing effective therapies. Another way FOXM1 can prove to be clinically beneficial is its consideration as a cancer biomarker. Since FOXM1 expression signatures in several cancers strongly correlates with the predisposition and progress of the disease, analysis of its expression may help in early cancer

screening, identification of high and low risk patients and better prognosis of patients. However, clinical assessment of these ideas remains a major challenge and deserves more attention in future investigations.

In conclusion, an increased understanding of FOXM1's function and its regulatory mechanisms would not only provide deeper knowledge of tumorigenesis but would also influence our perspective of drug resistance in the clinic and help us discover novel approaches to selectively target cancer cells.

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### Abbreviations

FOXM1, Forkhead transcription factor 1; NRD, N-terminal repressor domain; CBP, CREB binding protein; Plk1, Polo-like kinase; PBD, Polo-like binding domain; EMT, Epithelial-Mesenchymal transition; CSC, cancer stem cells; CENPA/B/F, Centromeric protein A/B/F; MMP2/9, Matrix metalloproteinase 2/9.

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