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Multiple faces of FoxM1 transcription factor

Lessons from transgenic mouse models

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FoxM1 transcription factor (previously called HFH-11B, Trident, FoxM1b, Win and MPP2) is expressed in actively dividing cells and is critical for cell cycle progression. FoxM1 expression is induced in a variety of tissues during embryogenesis, and *Foxm1*$^{-/-}$ mice exhibit embryonic lethal phenotype due to multiple abnormalities in the liver, heart, lung and blood vessels. FoxM1 levels are dramatically decreased in adult tissues, but FoxM1 expression is re-activated during organ injury and numerous cancers. In this review, we discussed the role of FoxM1 in different cell lineages using recent data from transgenic mouse models with conditional "gain-of-function" and "loss-of-function" of FoxM1, as well as tissue samples from human patients. In addition, we provided experimental data showing additional sites of FoxM1 expression in the mouse embryo. Novel cell-autonomous roles of FoxM1 in embryonic development, organ injury and cancer formation in vivo were analyzed. Potential application of these findings for the diagnosis and treatment of human diseases were discussed.

Key words: winged helix DNA binding domain, forkhead transcription factor, FoxM1, embryonic development, cancer, cellular proliferation, cell cycle, transgenic mice

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Introduction

The Forkhead Box (Fox) proteins belong to a large family of transcription factors that are evolutionary conserved in the Winged Helix/*Forkhead* DNA binding domain.[1-3] The name of "Fork head" derives from two head-like structures in fork head mutant Drosophila embryos that exhibited defects in formation of anterior and posterior gut.[4] Fox family includes more than 55 distinct mammalian members grouped into 17 subfamilies according to their sequence homology within the DNA binding domain.[5] Several mutations in Fox genes have been linked to human congenital disorders. Mutations in FoxC1, FoxC2, FoxE3 and FoxL2 cause various eye abnormalities, whereas mutations in FoxE1 and FoxN1 are linked to thyroid hypoplasia, cleft palate and T-cell immunodeficiency.[6] Heterozygous deletions or point mutations in FoxF1 gene locus were recently found in 30% of human patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV), a severe congenital disorder with mortality rate of 100% during the first months of life.[7] FoxP2 mutations are associated with speech and language disorders, and FoxP3 mutations were found in patients with immune deficiency, enteropathy and complex endocrine abnormalities.[6] Duplications of FoxG1 in 14q12 are associated with developmental epilepsy, mental retardation and severe speech impairment.[8]

Fox proteins play important roles in pathogenesis of human cancers. Chromosomal translocations of FoxO proteins have been identified as a causative factor in human alveolar rhabdomyosarcoma and acute lymphoid leukemia.[9,10] The loss of FoxO1A through 13q14 chromosomal deletion was associated with prostate cancer and progression towards androgen independence.[11] Chromosome translocations of FoxP1 have been detected in the subset of diffused large β-cell lymphomas,[12,13] whereas mutations in FoxP3 were associated with the suppression of T-cell immunity that contributed to the growth of ovarian

carcinomas.[14] GATA3-dependent FoxA1 loss was observed in human breast cancers.[15] FoxC2 is overexpressed in invasive breast carcinomas and its increased expression was associated with several adverse prognostic markers, including estrogen receptor negativity and high tumor grade.[16] FoxL2 mutation was found in granulosa-cell tumors of the ovary.[17] Increased expression of FoxM1 and amplifications of the FoxM1 gene locus were found in various human cancers, including prostate adenocarcinomas, non-small cell lung cancers, glioblastomas, breast adenocarcinomas, and head and neck squamous cell carcinomas.[18-22]

Based on these studies, Fox proteins provide novel targets for clinical diagnoses and treatment of various human cancers and congenital disorders. In this review, we will focus on FoxM1 transcription factor (previously known as HFH-11B, Trident, Win or MPP2) and summarize recent transgenic and gene knockout studies highlighting the FoxM1 functions in different cell types and tissues in vivo.

FoxM1 is Critical for DNA Replication and Mitosis

FoxM1 was first identified as a proliferation-specific transcription factor, which is expressed in various tumor cell lines and embryonic tissues.[23-25] Three isoforms of FoxM1 protein have been described: FoxM1b and FoxM1c function as transcriptional activators, whereas FoxM1a is transcriptionally inactive.[23] Multiple in vitro approaches have been used to examine the role of FoxM1 in cellular proliferation. These included FoxM1 mRNA targeting by siRNA, inhibition of FoxM1 by peptides or chemical inhibitors, analysis of a cell line with Doxycycline (Dox)-inducible overexpression of Fox M1b, and analysis of mouse embryonic fibroblasts (MEFs) from *Foxm1*$^{-/-}$ embryos that lacked all FoxM1 isoforms.[26-36] These published studies convincingly demonstrated that FoxM1b and FoxM1c are important regulators of cellular proliferation. FoxM1b induces expression of *cyclin A2*, *JNK1*, ATF2 and Cdc25A phosphatase, all of which are critical for G_1/S transition and DNA replication.[18,30,37] In addition, FoxM1b induces transcription of *Skp2* and *Cks1* which encode subunits of the Skp/Cullin-1/F-box (SCF) ubiquitin ligase complex, which is required for degradation of Cdk2 inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ during G_1 phase of the cell cycle.[30] Thus, FoxM1b negatively regulates the stability of $p21^{Cip1}$ and $p27^{Kip1}$ proteins, leading to increased Cdk2 activity and promoting G_1/S transition.

Progression into mitosis requires activation of Cdk1 through the removal of inhibitory phosphates at Thr 14 and Tyr 15 by the Cdc25B and Cdc25C phosphatases.[38-40] FoxM1b inactivation resulted in reduced expression of Cdc25B and delayed accumulation of cyclin B1, inhibiting cyclin B-cdk1 kinase activation and entry into mitosis.[18] Both Cdc25B and cyclin B1 are direct transcriptional targets of FoxM1b.[18] In addition, FoxM1b is a transcriptional activator of various genes critical for chromosome segregation and cytokinesis, such as *Aurora B* kinase and *polo-like kinase 1 (Plk-1)*, *Survivin*, *Cenp A, B* and *F* isoforms.[29,30] Depletion of FoxM1b caused chromosome instability and frequent failure of cytokinesis.[29] Altogether, these published studies demonstrated that FoxM1b regulates expression of proteins that are required for DNA replication and mitosis.

FoxM1 Expression during Embryogenesis and Organ Injury

Previous studies used in situ hybridization and immunohistochemistry to demonstrate that FoxM1 is expressed in various cell types during embryogenesis.[23,41,42] These include hepatoblasts, cardiomyocytes, smooth muscle and endothelial cells, pancreatic endocrine cells, thymocytes, precursors of granule neurons, as well as epithelium and mesenchyme of the embryonic lung, intestine and liver. While recent studies focused on the role of FoxM1 in these cell types, FoxM1 expression pattern in mouse embryos appears to be much broader. We used immunostaining of embryonic paraffin sections with antibodies against the N-terminal region of the mouse FoxM1 protein[43] to identify additional sites of FoxM1 expression in mouse embryos. In developing skin, FoxM1 protein was observed in hair primodiums and keratinocytes of basal layers at embryonic day (E) 15.5 and E18.5 (**Fig. 1A–C**). FoxM1 was also observed in developing kidney (**Fig. 1D–F**) and cartilage (**Fig. 1J–L**), as well as in epithelial and mesenchymal cells of pancreas and stomach (**Fig. 1G–I**). Several additional FoxM1 expressing regions were found in nasal and oral cavities, tongue, tooth primordiums, salivary glands, muscles, as well as in the Rathke's pouch (**Fig. 1M–R**), the latter of which gives a rise to pituitary gland. FoxM1 functions in these tissues remain uncharacterized.

While FoxM1 expression in adult mice is restricted to intestinal crypts, thymus and testes,[23,24,41] FoxM1 expression is re-activated after organ injury and cancer formation. After lung injury with either butylated hydroxytoluene (BHT) or inflammatory mediator lipopolysaccharide (LPS), FoxM1 protein was found in pulmonary epithelial, endothelial and smooth muscle cells.[44,45] FoxM1 expression was induced in hepatocytes and pancreatic endocrine cells following partial hepatectomy or pancreatectomy, respectively.[46,47] Following liver injury with carbon tetrachloride (CCl_4), FoxM1 expression was induced in hepatocytes, bile duct epithelial cells, and inflammatory cells infiltrating periportal liver regions.[43,48] Consistent with the important role of FoxM1 in cell cycle progression, increased levels of FoxM1 mRNA and protein were found in various mouse and human tumors.[10,18,19,49] Although FoxM1 expression patterns appear to be consistent with cellular proliferation, subsequent studies showed that FoxM1 functions are diverse and tissue-specific.

Foxm1$^{-/-}$ Mice is Embryonic Lethal

Mice homozygous for Foxm1 null mutation have been previously generated and characterized by two separate labs[26,50] (**Table 1**). Mice with targeting insertion of PGK-neomycin cassette into the third exon of FoxM1 gene (*Foxm1*$^{-/-neo}$ mice) died immediately after birth due to structural abnormalities in the heart and liver.[50] More severe phenotype was observed in a distinct *Foxm1*$^{-/-}$ mouse line, which contained a complete deletion of exons 4 to 7 that encode the FoxM1 Winged Helix DNA binding and the C-terminal

transcriptional activation domains.[26] The *Foxm1*$^{-/-}$ embryos died in utero between E13.5 and E16.5 due to multiple abnormalities in various organ systems, including liver, lungs, blood vessels and heart.[26,51,52] Abnormal accumulation of polyploid cardiomyocytes and hepatoblasts, resulting from a failure to complete mitosis, was found in both *Foxm1*$^{-/-neo}$ and *Foxm1*$^{-/-}$ mouse lines.[26,50-52] Surprisingly, the polyploid phenotype was restricted to the developing heart, liver and muscle layers of blood vessels, whereas other cell types in *Foxm1*$^{-/-}$ embryos shown no visible changes in either cell sizes or proliferation rates, raising a possibility that FoxM1 functions depend on cellular specificity. These studies demonstrated that FoxM1 is required for proliferation of cardiomyocytes, hepatoblasts and smooth muscle cells during embryogenesis, whereas FoxM1 appears to be dispensable for cellular proliferation in other embryonic cell lineages.

Conditional Inactivation of FoxM1 during Embryogenesis

Given the broad expression pattern of FoxM1 during embryogenesis,[23,41,42] several recent studies used conditional knockout mouse models to address cell-specific roles of FoxM1 in different cell types (**Table 1**). FoxM1-floxed (fl) mice, containing LoxP sites flanking exons 4 to 7 of the FoxM1 gene, were generated in Robert Costa's laboratory.[53] To inactivate the FoxM1 gene in a cell-restricted manner, *Foxm1*$^{fl/fl}$ mice were bred with various mouse lines containing cell-specific Cre recombinase transgenes. Hepatoblast-specific deletion of FoxM1 in AFPp-Cre *Foxm1*$^{fl/fl}$ embryos caused embryonic lethality by E18.5 due to disruption of hepatic cords and vasculature, and lack of intrahepatic bile ducts, indicating a cell-autonomous role of FoxM1 in hepatoblasts.[26] A conditional deletion of FoxM1 from precursors of cerebellar granule neurons (Math1-Cre and Nestin-Cre transgenes) caused a delay in brain development by interfering with Shh-induced signaling.[54] Deletion of FoxM1 from T lymphocyte lineage using either lck-Cre or CD4-Cre transgenes caused a decrease in proliferation of early thymocytes and activated mature T cells without any effects on apoptosis or T-cell differentiation.[55] Mice with either endothelial-specific (Tie2-Cre *Foxm1*$^{fl/fl}$) or macrophage-specific FoxM1 deletion (mLyz-Cre *Foxm1*$^{fl/fl}$) developed normally,[43,45] indicating that FoxM1 is not required in these cell lineages during embryonic development. Interestingly, although embryos with pancreatic-specific deletion of FoxM1 (Pdx1-Cre *Foxm1*$^{fl/fl}$) displayed normal development of pancreas,[56] the male Pdx1-Cre *Foxm1*$^{fl/fl}$ mice developed severe abnormalities in postnatal β-cell mass expansion, causing impaired islet function and diabetes.[56] Female Pdx1-Cre *Foxm1*$^{fl/fl}$ mice developed gestational diabetes during pregnancy.[57] These results are consistent with different requirements for FoxM1 at different stages of pancreatic development.

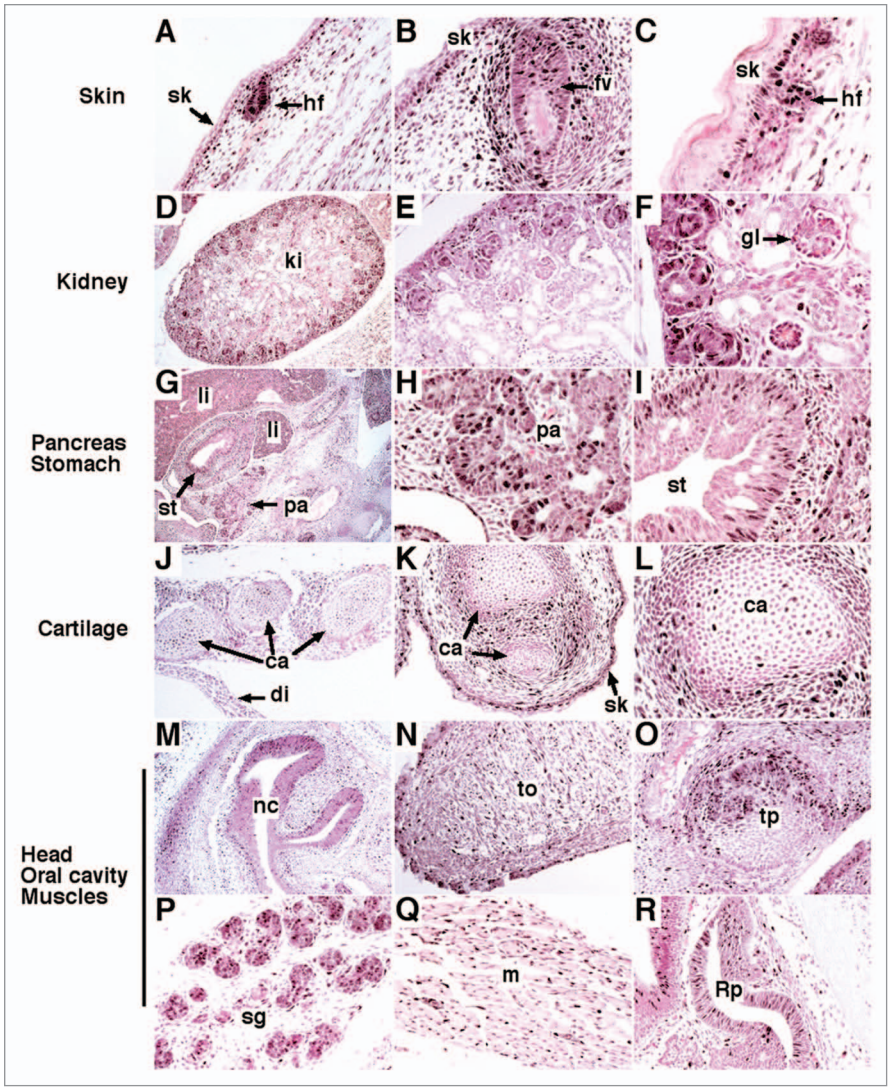

Figure 1. FoxM1 expression in the mouse embryo. Paraffin sections were prepared from mouse wild type embryos harvested at E13.5 (G–I and R), E15.5 (A, B, D and J–P) or E18.5 (C, E, F and Q). Sections were used for immunohistochemistry with FoxM1 antibodies (brown) and counterstained with nuclear fast red (red nuclei). Nuclear FoxM1 staining was detected in hair follicles (A and C), follicles of vibrissae (B) and keratinocytes of basal layers (A–C). FoxM1 was also observed in developing kidney (D–F), cartilage (J–L), epithelial and mesenchymal cells of pancreas and stomach (G and I). FoxM1 expressing cells were detected in nasal cavity (M), tongue (N), tooth primordium (O), salivary glands (P), muscles (Q) and the Rathke's pouch (R). sk, skin; hf, hair follicle; fv, follicle of vibrissae; ki, kidney; gl, glomeruli; li, liver; pa, pancreas; st, stomach; ca, cartilage; di, diaphragm; nc, nasal cavity; to, tongue; tp, tooth primordium; sg, salivary gland; m, muscle; Rp, Rathke's pouch. Magnification: (D, G, J and M) x50; (C, F and L) x400; (A, E and N–P) x100; remaining parts, x200.

Table 1. Transgenic mouse models showing the role of FoxM1 in embryonic development

Mouse model	Affected cells	Phenotypes and references
Foxm1$^{-/-neo}$	Gene disruption in all cell types	Postnatal lethality, accumulation of polyploid cardiomyocytes and hepatoblasts.[50]
Foxm1$^{-/-}$	Deletion in all cell types	Embryonic lethality between E13.6 and E17.5, diminished numbers of hepatocytes, absence of intrahepatic bile ducts.[26] Diminished proliferation of cardiomyocytes and ventricular hypoplasia in the heart.[52] Defects in differentiation of pulmonary mesenchyme into mature capillary endothelial cells, structural defects in blood vessels.[51]
smMHC-Cre Foxm1$^{fl/fl}$	Deletion in smooth muscle cells	Postnatal lethality due to severe pulmonary hemorrhage and structural defects in arterial walls and esophagus, diminished proliferation of smooth muscle cells.[42]
AFPp-Cre Foxm1$^{fl/fl}$	Deletion in hepatoblasts	Embryonic lethality by E18.5, absence of intrahepatic bile ducts, diminished numbers of hepatoblasts resulting from diminished DNA replication and a failure to enter mitosis causing a polyploid phenotype.[26]
Math1-Cre Foxm1$^{fl/fl}$ and Nestin-Cre Foxm1$^{fl/fl}$	Deletion in neural precursor cells	Mice are viable. Entry into mitosis was delayed. Cerebellar granule neuron precursors displayed defects in spindle assembly and centrosome amplification. Differentiation of neuron precursors was normal[54]
SPC-rtTA/TetO-Cre Foxm1$^{fl/fl}$	Deletion in respiratory epithelial cells	Postnatal lethality due to respiratory failure after birth, delayed lung maturation, decreased surfactant production. Proliferation of non-mature respiratory epithelial cells was normal.[41]
Pdx1-Cre Foxm1$^{fl/fl}$	Deletion in pancreatic epithelial cells	Mice are viable, delayed postnatal β-cell mass expansion, impaired islet function and diabetes in male mice by 9 week of age.[56] Gestational diabetes was observed in female mice during pregnancy[57]
Ick-Cre Foxm1$^{fl/fl}$ and CD4-Cre Foxm1$^{fl/fl}$	Deletion in T lymphocytes	Mice are viable, decreased proliferation of early thymocytes and activated mature T cells, no effects on apoptosis or T cell differentiation[55]
SPC-rtTA/TetO-FoxM1 activated mutant	Overexpression in respiratory epithelial cells	Mice are viable, decreased lung sacculation in embryos, increased proliferation of respiratory epithelial cells, focal airway hyperplasia resulted from overgrowth of Clara cells.[74]

Recently, we generated transgenic mice in which FoxM1-floxed allele was conditionally deleted using Cre recombinase transgene driven by a smooth muscle myosin heavy chain promoter (smMHC-Cre *Foxm1*$^{fl/fl}$ mice).[42] Although FoxM1 deletion did not influence differentiation of smooth muscle cells, the majority of smMHC-Cre *Foxm1*$^{fl/fl}$ mice died immediately after birth due to severe pulmonary hemorrhage, structural defects in arterial wall and esophageal abnormalities.[42] Reduced levels of cell cycle regulatory genes, such as cyclin B1, Cdc25b, Plk1, JNK1 and cMyc, were found in muscle layers of smMHC-Cre *Foxm1*$^{fl/fl}$ arteries, as well as in cultured arterial smooth muscle cells after siRNA-mediated FoxM1 depletion.[42] Interestingly, decreased myocyte proliferation was specifically found in muscle layers of embryonic blood vessels and esophagus, whereas proliferation rates in either intestinal or peribronchial smooth muscle cells were normal. These results suggest different requirements for FoxM1 in different populations of developing smooth muscle cells. Previously published studies demonstrated that vascular and intestinal smooth muscle cells use distinct transcription factor complexes to activate smooth muscle-specific gene promoters. For example, the Serum response factor (SRF), GATA-5 and Foxf1 transcription factors displayed fundamental differences in either expression pattern or DNA-binding properties between different smooth muscle subtypes.[58-61] Although, molecular mechanisms underlying the FoxM1 selectivity in smooth muscle cells remain unknown, it is possible that FoxM1 cooperates with other transcriptional co-activators to regulate different set of genes in subpopulations of smooth muscle cells.

In our recent studies,[41] FoxM1 was deleted conditionally in developing respiratory epithelium using the surfactant-associated protein C (SPC) promoter (*SPC-rtTA/TetO-Cre Foxm1*$^{fl/fl}$ mice). Deletion of FoxM1 did not alter proliferation rates in respiratory epithelial cells, indicating that FoxM1 is not required for epithelial proliferation during lung development. However, the FoxM1 deletion impaired lung maturation and caused respiratory failure after birth.[41] Maturation defects in *SPC-rtTA/TetO-Cre Foxm1*$^{fl/fl}$ lungs were associated with decreased expression of surfactant-associated proteins SPC, SPB and SPA, and a delay in differentiation of type I cells from their epithelial precursors.[41] Thus, FoxM1 is dispensable for epithelial proliferation but is critical for surfactant homeostasis and lung maturation during lung development. Altogether, various conditional knockout models demonstrated that FoxM1 plays unique roles in different tissues during embryonic development.

FoxM1 in Organ Injury and Regeneration

Since FoxM1 expression is re-activated in various organ injury models, several recent studies addressed the role of FoxM1 in organ injury and repair (**Table 2**). Following partial hepatectomy, mice with hepatocyte-specific FoxM1 deletion (Albumin-Cre *Foxm1*$^{fl/fl}$) showed reduced DNA replication, which was due to reduced expression of Cdc25A phosphatase and increased nuclear accumulation of p21^{Cip1} protein in regenerating hepatocytes, causing decreases in Cdk2 activation and hepatocyte progression into S phase.[53] Likewise, a significant reduction in numbers of

Table 2. Transgenic mouse models showing the role of FoxM1 in organ injury

Mouse model	Affected cells	Injury/organ	Phenotype
Albumin-Cre Foxm1$^{fl/fl}$	Deletion in hepatocytes	Partial hepatectomy/ Livers	Mice showed diminished proliferation of hepatocytes after partial hepatectomy[53]
Tie2-Cre Foxm1$^{fl/fl}$	Deletion in endothelial cells	LPS treatment/Lungs	Reduced vascular repair, decreased proliferation of endothelial cells and impaired reannealing of endothelial adherens junctions were found.[45,78]
Pdx1-Cre Foxm1$^{fl/fl}$	Deletion in pancreatic epithelial cells	Partial pancreatectomy/ Pancreas	Mice showed decreased proliferation of alpha- and beta-cells following partial pancreatectomy. No differences in proliferation of acinal or ductal cells were found.[47]
		Pregnancy/Pancreas	Gestational diabetes was observed in female mice during pregnancy[57]
mLyz-Cre Foxm1$^{fl/fl}$	Deletion in macrophages and granulocytes	Carbon tetrachloride/ Liver	No defects were found in macrophages and granulocytes of bone marrow and peripheral blood. Following acute liver injury by carbon tetrachloride, a delay in liver repair was found. Mice showed decreased recruitment of monocytes into the injured liver, as well as impaired differentiation of monocytes toward mature macrophage lineage.[43]
Rosa26-FoxM1b	Overexpression in all cell types	BHT treatment/Lungs	Increased proliferation of respiratory epithelial, endothelial and smooth muscle cells was found after BHT-mediated lung injury.[44]
TTR-FoxM1b	Overexpression in hepatocytes	Partial hepatectomy or carbon tetrachloride/ Liver	Increased proliferation of hepatocytes was found after partial hepatectomy[46] or carbon tetrachloride-mediated liver injury.[48]
		Partial hepatectomy and aging/Liver	Expression of FoxM1b resulted in protection of old mice against age-associated decline in liver regeneration.[62]

hepatocytes undergoing mitosis was found in regenerating Albumin-Cre *Foxm1*$^{fl/fl}$ livers. Mitotic abnormalities in Albumin-Cre *Foxm1*$^{fl/fl}$ livers were due to diminished mRNA levels and nuclear expression of Cdc25B phosphatase and delayed accumulation of cyclin B1, causing a decrease in Cdk1 activation.[53] Consistent with the important role of FoxM1 in hepatocyte proliferation, overexpression of FoxM1 in hepatocytes of TTR-FoxM1b transgenic mice reduced p21^{Cip1} expression and increased proliferation of hepatocytes after partial hepatectomy[46] or CCl_4-mediated liver injury.[48] These results convincingly showed that FoxM1 plays a key role in proliferation of hepatocytes during liver regeneration. Interestingly, hepatocytes of old TTR-FoxM1b mice were protected against age-associated decline in liver regeneration, which normally occurred in regenerating livers of old wild-type mice.[62] Increased regenerating potential in old TTR-FoxM1b livers suggests that FoxM1 can be used as an attractive therapeutic target to restore age-associated decline in liver regeneration by inducing hepatocyte proliferation.

The importance of FoxM1 in pancreatic regeneration was recently found.[47] After partial pancreatectomy, Pdx1-Cre *Foxm1*$^{fl/fl}$ mice exhibited specific impairments in β-cell mass regeneration and islet growth. Although cellular proliferation was reduced in pancreatic α- and β-endocrine cells, no impairments were found in proliferation of acinar or ductal cells.[47] Although this study suggests different requirements for FoxM1 in different populations of regenerating pancreatic cells, molecular mechanisms for this selectivity remain unknown.

Mice with endothelial-specific FoxM1 deletion (Tie2-Cre *Foxm1*$^{fl/fl}$) displayed significantly increased lung vascular permeability and increased mortality in response to LPS-mediated injury.[45] These complex abnormalities in endothelial repair were due to inability of FoxM1-deficient endothelium to restore endothelial barrier function injured by LPS. FoxM1 directly induced expression of β-catenin protein and was critical for reannealing of endothelial adherens junctions after lung injury. Furthermore, gain-of-function studies with Rosa26-FoxM1b transgenic mice confirmed a critical role of FoxM1 in lung repair. After BHT-mediated lung injury, increased proliferation of alveolar endothelial cells and other respiratory cell types was found in Rosa26-FoxM1b mice.[44]

Recently, we generated transgenic mice with FoxM1 deletion from myeloid cell lineage (mLyz-Cre *Foxm1*$^{fl/fl}$), which includes macrophages, monocytes, neutrophils and their precursors. A significant delay in liver repair was found in mLyz-Cre *Foxm1*$^{fl/fl}$ mice after CCl_4-mediated liver injury.[43] Surprisingly, FoxM1-deficiency did not influence proliferation of myeloid cells during liver repair, but dramatically reduced recruitment of macrophages and their precursors, monocytes, into injured livers. Expression of L-selectin and the CCR2 chemokine receptor, both critical for monocyte differentiation and recruitment to injured tissues, was decreased in mLyz-Cre *Foxm1*$^{fl/fl}$ mice.[43] In cotransfection experiments, FoxM1 directly induced transcriptional activity of the mouse CCR2 promoter, indicating that CCR2 is a direct transcriptional target of FoxM1. Furthermore, FoxM1 expression in myeloid cells was critical for liver injury because adoptive transfer of wild type monocytes to injured mLyz-Cre *Foxm1*$^{fl/fl}$ mice restored liver repair and rescued liver function after CCl_4 injury.[43] These studies demonstrated that FoxM1 is critical for

Table 3. Transgenic mouse models showing the role of FoxM1 in tumorigenesis

Mouse model	Affected cells	Tumor induction	Type of carcinogenesis	Phenotype
Rosa26-FoxM1b	Overexpression in all cell types	MCA/BHT	Lung adenomas	Increased numbers and sizes of lung adenomas and increased proliferation of tumor cells were found. FoxM1b induced chronic lung inflammation with macrophage infiltration and lung remodeling.[71]
		AOM/DSS	Colon adenocarcinomas	Increase in the number and size of colorectal tumors and increased proliferation of tumor cells were found.[66]
		Breeding with TRAMP or LADY transgenic mice	Prostate adenocarcinomas	FoxM1b cooperated with SV 40 T Antigen to accelerate development of prostate adenocarcinomas in TRAMP and LADY transgenic mice.[19]
TTR-FoxM1b	Overexpression in hepatocytes	DEN/phenobarbital	Hepatocellular carcinoma (HCC)	Increased proliferation of hepatocytes in preneoplastic regions, but no effects on progression of HCC was found.[73]
SPC-rtTA/TetO-FoxM1 activated mutant	Overexpression in respiratory epithelial cells	Breeding with K-Ras transgenic mice	Lung adenocarcinomas	Expression of FoxM1 mutant alone was insufficient to induce lung adenocarcinomas. However, the FoxM1 transgene cooperated with activated K-Ras to accelerate lung tumor growth in vivo.[74]
Mx-Cre Foxm1$^{fl/fl}$	IFN-inducible deletion in all cell types	Urethane	Lung adenomas	Decreased proliferation of lung tumor cells and reduced numbers and sizes of lung adenomas were found.[22]
		DEN/phenobarbital	HCC	Decreased growth and progression of HCC was found.[72]
Albumin-Cre Foxm1$^{fl/fl}$	Deletion in hepatocytes	DEN/phenobarbital	HCC	Mice showed diminished proliferation of tumor cells and decreased formation of HCC.[28]
Villin-Cre Foxm1$^{fl/fl}$	Deletion in colonic epithelial cells	AOM/DSS	Colon adenocarcinomas	Reduction in the development and growth of colorectal tumors were found.[66]
SPC-rtTA/TetO-Cre Foxm1$^{fl/fl}$	Deletion in respiratory epithelial cells	Urethane or MCA/BHT treatment	Lung adenomas	FoxM1 deletion prior to tumor induction prevented lung tumorigenesis. Deletion of FoxM1 in pre-existed lung tumors delayed tumor growth and decreased tumor cell proliferation.[75]
Tie2-Cre Foxm1$^{fl/fl}$	Deletion in endothelial cells	Urethane or MCA/BHT treatment	Lung adenomas	Tumor numbers and sizes were increased. Perivascular infiltration by inflammatory cells was observed. Activity of canonical Wnt signaling was increased in lung tumor cells.[79]

liver repair and required for recruitment of monocytes to the injured liver.

Role of FoxM1 in Carcinogenesis

Since FoxM1 is re-activated during benign and malignant transformations, this protein was extensively studied in various tumor cell lines as well as in tumor tissues obtained from cancer patients. Microarray analysis of human solid tumors demonstrated that FoxM1 is one of the most common overexpressed genes.[63] FoxM1 was overexpressed in human non-small cell lung cancers (NSCLC), head and neck squamous carcinomas, hepatocellular carcinomas (HCC), intrahepatic cholangiocarcinomas, colon carcinomas, basal cell carcinomas, infiltrating ductal breast carcinomas, anaplastic astrocytomas, glioblastomas, pancreatic carcinomas, gastric cancer, acute myeloid leukemia and other human tumors and human neoplastic cell lines.[10,22,28,49,64-68] Our previous studies have demonstrated that elevated levels of FoxM1 correlated with high proliferation rates in human prostate adenocarcinomas[19] and NSCLC.[22] Positive correlation was also found between FoxM1 overexpression and increased angiogenesis in human glioblastomas.[69] In breast cancer patient samples, FoxM1 levels strongly associated with expression of estrogen receptor alpha.[70] FoxM1 was overexpressed in HCC from patients that responded poorly to treatment.[64]

To study the role of FoxM1 during carcinogenesis, various genetic mouse models were generated (**Table 3**). We previously developed a transgenic *Rosa26-Foxm1* mouse line in which FoxM1b levels were increased in all cell types.[44] Ubiquitous overexpression of FoxM1b elevated proliferation of lung tumor cells and increased the number and size of lung tumors induced by tobacco smoke derived carcinogen 3-methylcholanthrene (MCA) and promoted by butylated hydroxytoluene (BHT).[71] Likewise, an increase in the number and size of colorectal tumors was found in *Rosa26-Foxm1* mice treated with azoxymethane (AOM) and dextran sodium sulfate (DSS).[66] FoxM1b transgene cooperated with SV 40 T Antigen to accelerate initiation and progression of prostate adenocarcinomas in *Rosa26-Foxm1*/TRAMP and *Rosa26-Foxm1*/LADY double transgenic mice.[19] Deletion of FoxM1 from all cell types in *Mx-Cre/Foxm1$^{fl/fl}$* mice decreased urethane-mediated lung tumorigenesis[22] and delayed the growth and progression of hepatocellular carcinoma (HCC) induced by DEN/phenobarbital treatment.[72] Although these studies suggested a critical role for FoxM1 in lung, prostate, colon and liver tumorigenesis, specific requirements for the FoxM1 in different cell populations were not addressed.

To dissect cell-autonomous roles of FoxM1 in cancer lesions, selective targeting of this transcription factor was performed in vivo. Hepatocyte-specific overexpression of FoxM1b in TTR-FoxM1b transgenic mice was insufficient to induce HCC.[73] After DEN/phenobarbital treatment TTR-FoxM1b livers showed increased proliferation of hepatocytes in preneoplastic regions, but no effect on progression of HCC was found.[73] In our recent studies, specific expression of activated FoxM1b mutant protein in respiratory epithelial cells (SPC-rtTA/TetO-FoxM1b ΔN mice) caused epithelial hyperplasia but was insufficient to induce lung adenocarcinomas.[74] These studies demonstrated that FoxM1b might require a "second hit" to transform differentiated hepatocytes or lung epithelial cells into malignant phenotype. The fact that simultaneous overexpression of FoxM1b-ΔN and activated K-Ras accelerated lung tumor growth in vivo[74] provides a direct support for this concept.

FoxM1 was found to be required for growth and expansion of HCC and epithelial tumors. Mice with hepatocyte-specific FoxM1 deletion (Albumin-Cre *Foxm1*$^{fl/fl}$) displayed diminished proliferation of tumor cells and decreased formation of HCC after DEN/Phenobarbital induction.[28] Likewise, reduced growth of colorectal tumors was found in Villin-Cre *Foxm1*$^{fl/fl}$ mice treated with AOM/DSS.[66] Our recent studies demonstrated that deletion of *FoxM1* from lung epithelial cells (*SPC-rtTA/tetO-Cre/Foxm1*$^{fl/fl}$ transgenic mice) prior to the tumor initiation with urethane or MCA/BHT caused a striking reduction in the number and size of lung adenomas.[75] Decreased lung tumorigenesis in *SPC-rtTA/tetO-Cre/Foxm1*$^{fl/fl}$ mice was associated with diminished proliferation of tumor cells and reduced expression of *Topoisomerase-2α (TOPO-2α)*, a critical regulator of tumor cell proliferation. We also demonstrated that FoxM1 directly bound to and induced transcription of the mouse *TOPO-2α* promoter region, indicating that *TOPO-2α* is a direct target of FoxM1 in lung tumor cells.[75] Interestingly, a deletion of FoxM1 in pre-existing lung tumors dramatically reduced tumor growth in the lung,[75] indicating that FoxM1 is a promising target for anti-tumor therapy in cancer patients.

Lung cancer lesions contain not only tumor cells, but also diverse stromal and inflammatory cells, that create a tumor promoting microenvironment. Vascular endothelial cells provide essential support to the tumor microenvironment, directly contributing to proliferation and progression of tumor cells. Recently, we used mice with endothelial cell-specific FoxM1 deletion (*Tie2-Cre/Foxm1*$^{fl/fl}$) to study the role of FoxM1 in tumor-associated blood vessels. Surprisingly, numbers and sizes of lung tumors were increased in *Tie2-Cre/Foxm1*$^{fl/fl}$ mice treated with urethane or MCA/BHT.[79] Thus, FoxM1 may function as a tumor suppressor in tumor-associated blood vessels. In addition, perivascular infiltration by inflammatory cells was elevated and numbers of inflammatory cells in BAL fluid were increased in *Tie2-Cre/Foxm1*$^{fl/fl}$ lungs. FoxM1 was shown to be a direct transcriptional activator of endothelial-specific genes, such as VEGF receptor-2 (*Flk-1*) and *FoxF1* transcription factor, both of which are critical for lung inflammation.[79] Moreover, FoxM1 was directly bound to the promoter region of *Sfrp1* gene, a known inhibitor of canonical Wnt/β-catenin signaling. As a consequence of decreased *Sfrp1* expression in FoxM1-deficient endothelium, *Tie2-Cre/Foxm1*$^{fl/fl}$ tumors displayed increased canonical Wnt signaling as was demonstrated by activation of TOPGAL transgene,[79] a known reporter for Wnt/β-catenin signaling. Taken together, these in vivo studies suggest that endothelial-specific expression of FoxM1 limits lung inflammation and canonical Wnt signaling in lung epithelial cells, thereby restricting lung tumorigenesis.

FoxM1 Functions are not Limited to Regulation of the Cell Cycle

Requirements for FoxM1 in proliferation of tumor cell lines and MEFs in vitro are well described and accepted (reviewed in ref. 18 and 76). However, recent studies demonstrated that FoxM1 deletion in vivo did not always inhibit cellular proliferation. For example, *Foxm1*$^{-/-}$ embryos survived until late gestation and exhibited several well-developed embryonic tissues without proliferation defects,[26] suggesting the role of FoxM1 is cell type specific. Detailed analysis of *Foxm1*$^{-/-}$ phenotypes led to discovery of additional FoxM1 functions (**Fig. 2**). FoxM1 was found to be critical for cellular differentiation. For example, livers of *Foxm1*$^{-/-}$ embryos failed to form intrahepatic bile ducts,[26] implicating FoxM1 in differentiation of hepatoblast precursor cells toward the biliary epithelial cell lineage during liver development. Likewise, *Foxm1*$^{-/-}$ lungs exhibited defects in differentiation of pulmonary mesenchyme into mature capillary endothelial cells during the canalicular stage of lung development.[51] Although differentiation of either cardiomyocytes or smooth muscle cells was not altered in FoxM1-deficient embryos,[42,52] FoxM1 was required for differentiation of non-mature respiratory epithelial cells toward alveolar type II and type I lineages.[41] These results are consistent with different requirements for FoxM1 in various embryonic cell types (**Fig. 2**).

Lung epithelial-specific deletion of FoxM1 during embryogenesis did not influence epithelial proliferation.[41] However, FoxM1 deletion from respiratory epithelium of adult mice inhibited proliferation of lung tumor cells and dramatically decreased lung tumorigenesis induced by either urethane or MCA/BHT.[75] Furthermore, FoxM1 was not required for proliferation of pancreatic endocrine cells in utero, but it was found to be critical for proliferation of β-cells after birth.[77] These published studies support the hypothesis that the role of FoxM1 may change under various biological conditions.

Additional FoxM1 functions were reported in recent studies. FoxM1 induces cell migration and invasion of cultured osteosarcoma U2OS cells and MEFs through transcriptional activation of the MAPK kinase JNK1 and metalloproteinases MMP-2 and MMP-9.[37] Increased angiogenesis in FoxM1-expressing pancreatic adenocarcinoma cell lines was directly linked to the activation of VEGF gene by FoxM1.[33] FoxM1 directly binds to and transcriptionally activates the promoter region of Cyclooxygenase 2 (Cox2) and *TOPO-2α* genes, implicating FoxM1 in prostaglandin synthesis and DNA

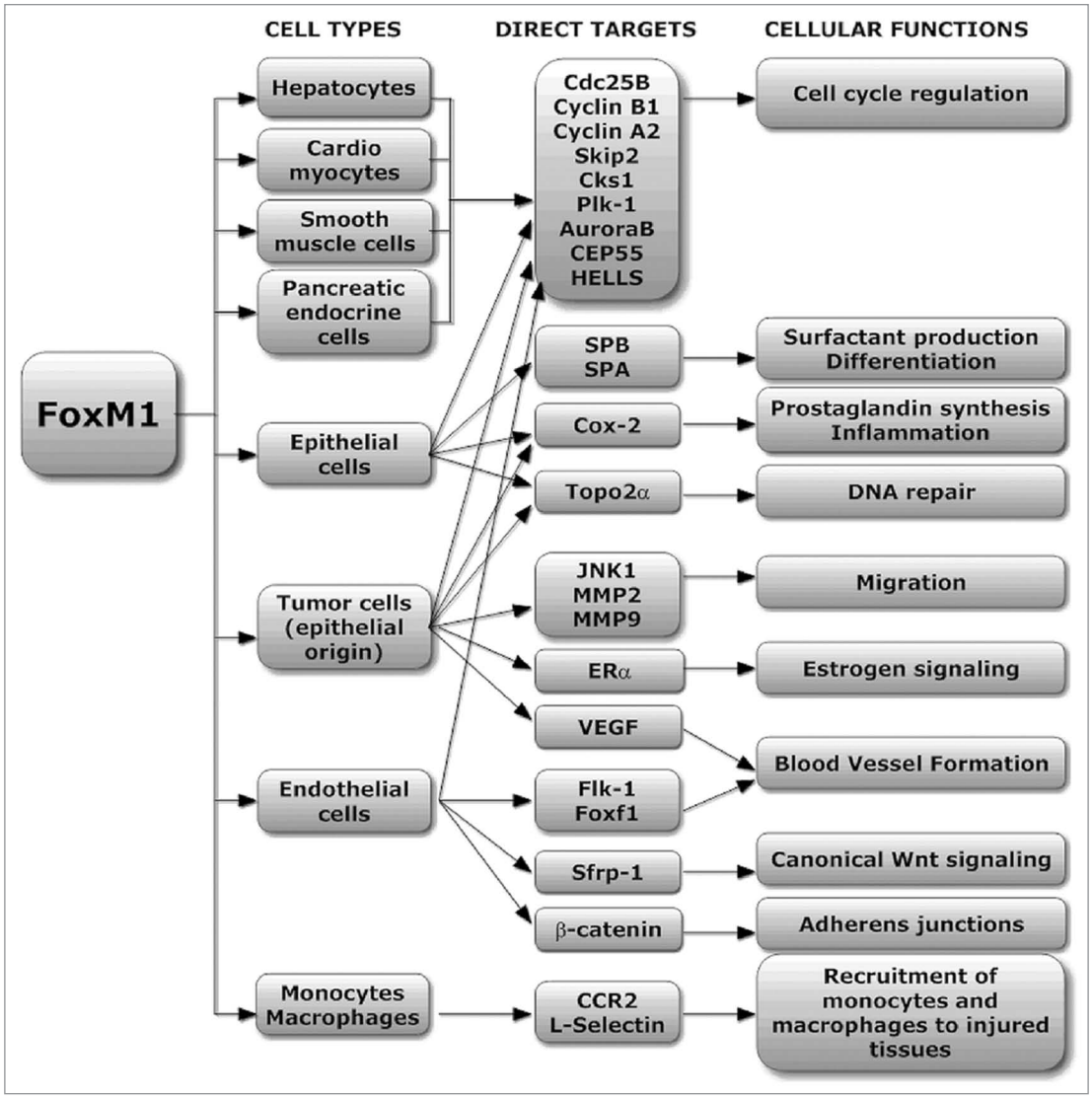

Figure 2. Diagram showing direct FoxM1 target genes in different cell lineages.

repair.[71,75] In addition, FoxM1 was found to be critical in reannealing of endothelial adherens junctions by directly inducing the β-catenin protein.[78] Finally, FoxM1 influenced surfactant homeostasis by activating expression of surfactant-associated proteins SPC, SPB and SPA in respiratory epithelial cells.[41] Thus, FoxM1 is important for the execution of distinct cellular functions in various differentiated cells (**Fig. 2**).

Summary

(1) Although FoxM1 positively regulates cellular proliferation in cultured cell lines in vitro, FoxM1 functions in vivo are diverse and dependent on cellular specificity; (2) FoxM1 is critical for various cellular functions, such as proliferation, differentiation, migration, DNA repair, surfactant production and formation of cellular junctions; (3) FoxM1 functions change dynamically during embryonic development, carcinogenesis and various biological conditions. Identification of novel FoxM1 functions will enable us to determine whether FoxM1 is a promising therapeutic target for various human diseases and cancers.

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