Review

FOXM1 and its oncogenic signaling in pancreatic cancer pathogenesis

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ABSTRACT

Pancreatic cancer is a devastating disease with an overall 5-year survival rate less than 5%. Multiple signaling pathways are implicated in the pathogenesis of pancreatic cancer, such as Wnt/β-catenin, Notch, Hedgehog, hypoxia-inducible factor, signal transducer and activator of transcription, specificity proteins/Krüppel-like factors, and Forkhead box (FOX). Recently, increasing evidence has demonstrated that the transcription factor FOXM1 plays important roles in the initiation, progression, and metastasis of a variety of human tumors, including pancreatic cancer. In this review, we focus on the current understanding of the molecular pathogenesis of pancreatic cancer with a special focus on the function and regulation of FOXM1 and rationale for FOXM1 as a novel molecular target for pancreatic cancer prevention and treatment.

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1. Introduction

Pancreatic cancer is currently one of the leading causes of cancer deaths in industrialized countries, and its incidence appears to be increasing [1]. Every year about 40,000 Americans are diagnosed with pancreatic cancer, and almost an equivalent number die of it annually, making pancreatic cancer the fourth leading cause of cancer-related deaths in the United States [2]. The near uniform lethality of pancreatic cancer can be attributed to the fact that most patients have locally advanced or distant metastatic disease at the time of presentation, precluding radical resection because of the insidious, aggressive natural history of the disease [3]. Even though outcomes in patients with pancreatic cancer have been improved by the use of potentially curative resection and adjuvant treatments such as chemotherapy and biological therapies, early recurrence and metastasis still occur in a substantial proportion of them after these treatments [4,5]. Thus, understanding the molecular mechanisms underlying the aggressive biology of pancreatic cancer is very important.

Recently, authors described Forkhead box M1 (FOXM1) as a major oncogenic transcription factor in tumor initiation, promotion, and progression [6,7]. Extensive studies demonstrated that elevated FOXM1 expression is found in patients with cancers of the brain, lung, breast, esophagus, stomach, colon, cervix, ovary, or pancreas [8–16]. FOXM1 regulates the expression of a number of targeted genes important to cell differentiation, proliferation, and apoptosis; cell-cycle progression; and tumor angiogenesis, migration, invasion, and metastasis (Table 1) [17], suggesting it has a general role in tumor development and progression.

Arguably, dysregulated expression and function of FOXM1 are not necessarily unique to certain types of cancer, *i.e.*, aberrant FOXM1 activity is likely common for all types of tumor [6,7,17,18]. Our present review focuses on recent advancements in identification of aberrant FOXM1 signaling in pancreatic cancer cells and its possible underlying mechanisms, including potential roles of FOXM1 in pancreatic tumor growth, angiogenesis, invasion, metastasis, and epithelial-to-mesenchymal transition (EMT), as well as its roles in pancreatic cancer stem cells (CSCs). Also, we discuss the prognostic value of FOXM1 protein expression and potential of targeting FOXM1 for pancreatic cancer treatment.

2. Molecular pathogenesis of pancreatic cancer

Pancreatic cancer is one of the most lethal cancers, with an incidence rate equaling its mortality rate. Pancreatic ductal adenocarcinoma (PDAC) and its variants account for approximately 90% of all primary pancreatic cancers. Therefore, the term PDAC is often used synonymously with pancreatic cancer. PDAC develops *via* three different ductal precursor lesions: pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasia, and mucinous cystic neoplasia [19–21]. Identification of these precursor lesions in pancreatic ducts has led to the formulation of a progression model of PDAC: the pancreatic ductal epithelium progresses from normal epithelium to increasing grades of pancreatic intraepithelial neoplasia to invasive cancer [22].

Over the past 20 years, oncologists' knowledge of pancreatic cancer pathogenesis has advanced significantly because of an astonishing and exciting explosion in understanding of its molecular biology. Like many other human tumors, pancreatic cancer is caused by a myriad of genetic and epigenetic alterations, and pancreatic cancer progression is promulgated by an even larger number of genetic alterations. The altered genes include not only the dozens of characterized oncogenes, which serve as accelerators to activate the cell cycle and promote cellular growth, but also the tumor suppressor genes, which serve as brakes to slow the growth of tumor cells under normal conditions. Recently, Jones et al. [23] reported on the sequencing of 23,219 transcripts representing 20,661 protein-coding genes in 24 PDAC cases. In this landmark study, they found that all of the tumors harbored mutations of the KRAS oncogene. The rates of mutation of the tumor suppressor genes KRAS, p16/cyclin-dependent kinase (CDK) inhibitor 2A, TP53, and SMAD4 were 100%, 80%, 80%, and 60%, respectively. Presumably, alterations in these oncogenes and tumor suppressor genes have diverse effects that lead to deregulation of signaling pathways that control tumor initiation, promotion, and progression.

However, how these genetic alterations link with the insidious and aggressive nature of pancreatic cancer is unknown. Generally, these alterations are perceived to regulate numerous signal transduction pathways that affect their downstream signal genes. Ultimately, transcription factors participate at the ends of all aberrant activation signal transduction pathways by causing upregulation or downregulation of expression of specific genes [24]. Therefore, recent research efforts

Table 1
Genes targeted by FOXM1 in human tumor progression.

First author/year	Targeted genes	Tumor type	Reference
Wang/2005	Skp2, Cks1, Cdc25B, Aurora B kinase, survivin,centromere protein A and B	Osteosarcoma	[83]
Radhakrishnan/2006	Cdc25B, survivin, centromere protein B	HCC	[84]
Kalin/2006	p27 (Kip1), cyclin A2, cyclin B1	Prostate cancer	[71]
Kim/2006	Cyclin A2, cyclin B1	Lung cancer	[9]
Madureira/2006	Estrogen receptor alpha	Breast cancer	[10]
Dai/2007	MMP-2	Glioma	[116]
Zhang/2008	VEGF	Glioma	[41]
Wang/2007	Cyclin B, cyclin D1, Cdk2, MMP-2, p21, p27, MMP-9, VEGF	Pancreatic cancer	[78]
Li/2009	VEGF	Gastric cancer	[88]
Ahmad/2010	uPA, MMP-2, MMP-9, VEGF	Breast cancer	[97]
Wu/2010	Cyclin B1, cyclin D1, uPA, MMP-2	HCC	[96]
Dai/2010	NEDD4-1	Glioma	[42]
Huang/2012	Caveolin-1	Pancreatic cancer	[101]

have focused on this activation mechanism and targeting of the activity of transcription factors, which ultimately control the expression of genes involved in almost all aspects of pancreatic cancer cell biology [25,26].

Over the past 10 years, we focused on three important transcription factors: specificity protein 1 [27–29], Krüppel-like factor 4 (KLF4) [30,31], and signal transducer and activator of transcription 3 [32–35]. Our data demonstrated that these factors play important roles in pancreatic cancer initiation and progression by regulating the expression of a number of genes that are critical to tumor cell growth, angiogenesis, invasion, and metastasis. Recently, some studies demonstrated that another important transcription factor, FOXM1, also plays an important role in pancreatic cancer progression [16,36]. Thus, we chose to focus on FOXM1 signaling and its oncogenic roles in pancreatic cancer pathogenesis.

3. FOXM1 isoforms and regulation of their expression

3.1. FOXM1 isoforms

FOXM1, also known as HNF-3, HFH-11, MPP2, Win, and Trident, is a transcription factor in the FOX protein superfamily that is characterized by a conserved winged helix DNA-binding domain [37]. The human FOXM1 gene consists of 10 exons, two of which, exon Va (A1) and exon VIIa (A2), are alternatively spliced. These splicing events give rise to three distinct isoforms: FOXM1a, FOXM1b, and FOXM1c (Fig. 1) [38–40]. FOXM1a, which harbors both exon Va and exon VIIa, is transcriptionally inactive owing to disruption of the transactivation domain by exon VIIa. On the other hand, both FOXM1b, which contains neither of these two exons, and FOXM1c, which contains exon Va only, are transcriptionally active and can activate their target gene expression via different mechanisms [39].

Several lines of evidence reported in the literature support a crucial role for FOXM1b in the regulation of expression of its targeted genes and suggest that FOXM1b plays important roles in human cancer initiation and progression [8,41,42]. However, because FOXM1a can bind to DNA, it is considered to be a dominant-negative regulator of expression of other FOXM1 isoforms [6]. In addition, FOXM1c is transcriptionally active and can activate its target gene expression. Therefore, more studies focused on the expression and function of FOXM1a and FOXM1c in human cancer pathogenesis are needed. Moreover, we recently discovered two new variants of FOXM1b: FOXM1b1 and FOXM1b2 (Fig. 1). We have designed specific polymerase chain reaction (PCR)

primers for these two variants and are investigating their expression and critical roles in pancreatic cancer cases.

3.2. FOXM1 regulation

There are many models of regulation of FOXM1 expression in normal and tumor cells. Here we describe a few models of FOMX1 regulation in pancreatic cancer. Those models may apply in other types of cancer.

3.2.1. Oncogenes and oncogenic signals activate FOXM1 in human cancer cells

FOXM1 expression is often induced by oncogenes (Fig. 2A) [43]. For example, activated RAS increases expression of FOXM1, and this increased FOXM1 expression is critical for RAS-induced transformation [44]. Mechanistically, RAS increases the expression of FOXM1 by inducing cellular levels of reactive oxygen species (ROS). This ROS-regulatory function of FOXM1 protects proliferating normal and tumor cells against oxidative stress and promotes their survival. Consistent with that, tumor cells expressing ROS-inducing oncogenes (e.g., RAS, Akt) are addicted to FOXM1 for their survival. In addition, investigators demonstrated that ROS alone activated expression of FOXM1 [44].

The transcriptional activation function of FOXM1 depends on phosphorylation by cyclin and CDKs and by polo-like kinase 1 (Plk1). Cyclin/CDK-dependent phosphorylation of the C-terminal region of FOXM1 appears to be particularly important with respect to transcriptional activation, as it recruits a transcriptional co-activator, the histone deacetylase p300/CREB-binding protein [45]. Furthermore, mutations at the cyclin/CDK and Plk1 phosphorylation sites render FOXM1 transcriptionally inactive [46,47]. In addition, the presence of appropriate mitogenic signals is required for FOXM1 transcriptional activation. The mitogen-activated protein kinase (MAPK) pathway apparently plays an important role in this respect. Researchers showed that MAPK-mediated phosphorylation of FOXM1 regulated its subcellular localization and transcriptional activation [48]. Inhibition of the MAPK signaling pathway resulted in a great reduction in FOXM1-dependent transcription. Moreover, they showed that Raf/MAPK kinase/MAPK signaling stimulated nuclear translocation of FOXM1 via phosphorylation in late S phase [48]. Additionally, FOXM1 is a downstream component of the Wnt signaling pathway and is critical to β-catenin transcriptional function in tumor cells. Wnt3a increases the level of expression and nuclear translocation of FOXM1, increasing FOXM1 nuclear localization and transcriptional activity [49]. These findings suggest that FOXM1
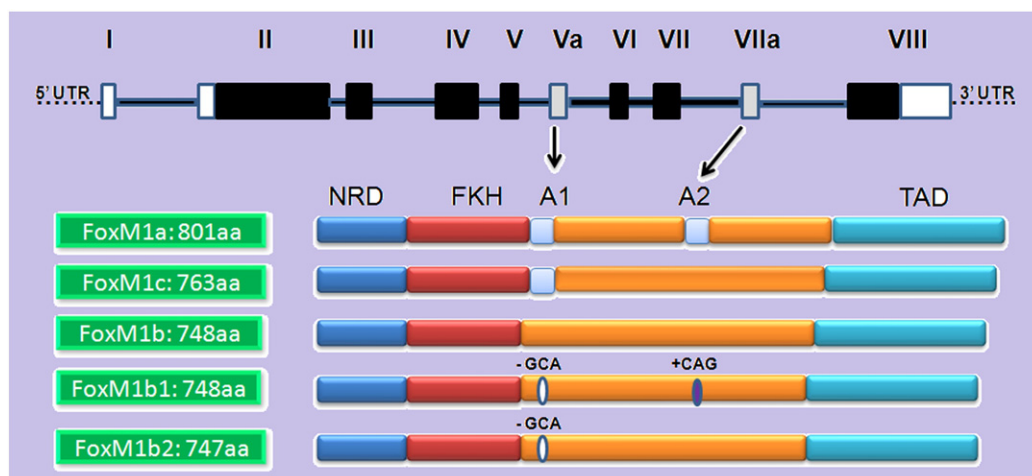

Fig. 1. Schematic representation of splice variants of human FOXM1 protein. FOXM1 protein has three isoforms owing to differential splicing of exons Va and VIIa: FOXM1b and FOXM1c function as transcriptional activators, whereas FOXM1a is transcriptionally inactive. FOXM1b1 and FOXM1b2 are closely related to FOXM1b and exhibit similar functions.
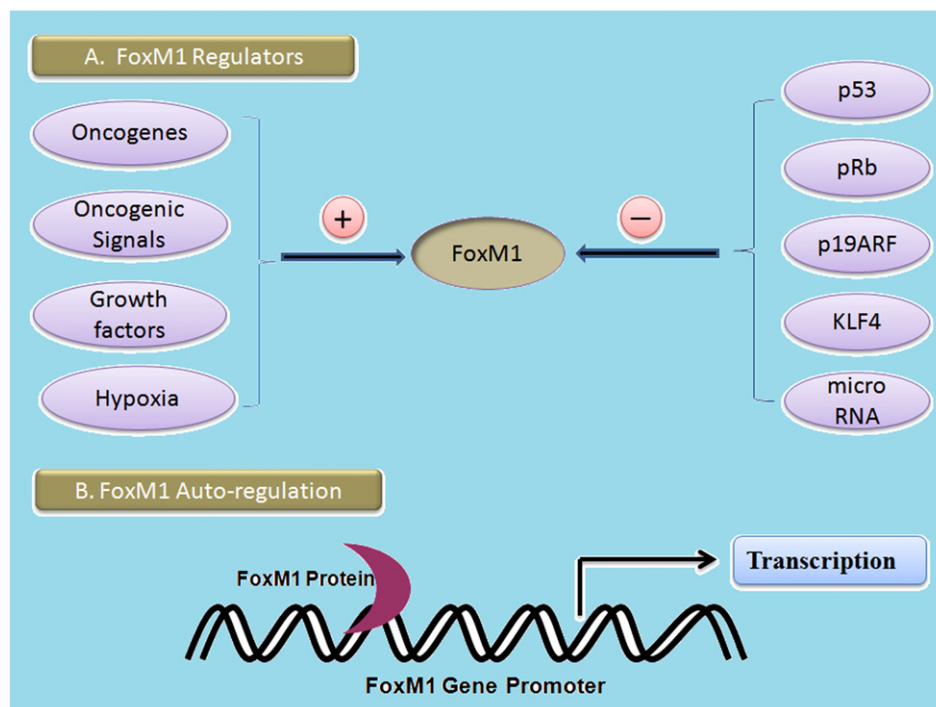
Fig. 2. Schematic representation of FOXM1 expression and its regulation. (A) FOXM1 expression is upregulated by oncogenes, oncogenic signals, growth factors, and hypoxia and downregulated by p53, pRb, p19Arf, KLF4, and miRNA. (B) FOXM1 expression is autoregulated by FOXM1 protein.

phosphorylation and transcriptional activation are regulated by multiple oncogenic signals.

In addition to oncogenes and oncogenic signals, FOXM1 expression may be induced by a number of growth factors and oncogenic stimuli *via* different mechanisms (Fig. 2A) [6], suggesting that FOXM1 is an important link between oncogenic extracellular signals and downstream signaling genes. Like many other cancers, pancreatic cancer develops *via* accumulation of oncogenic extracellular signals. Presumably, FOXM1 may participate in the integration of various oncogenic extracellular signals, regulate the expression of downstream signal genes, and contribute to pancreatic cancer development and progression.

3.2.2. Tumor suppressor genes inhibit FOXM1 expression in human cancer cells

P53 is a major tumor suppressor gene that is altered by point mutations in 50% of human cancer cases and functionally inactivated in the other 50% [50]. P53 mutation or inactivation is often found in patients with pancreatic cancer, and loss of its regulatory functions may be one of the differences between pancreatic cancer and chronic pancreatitis [51,52]. Mechanistically, p53 acts as a transcription factor, and it executes its tumor-suppressive activity mainly *via* positive transcriptional regulation of its target genes, such as Puma and p21, resulting in either apoptosis or growth arrest [50]. In addition, p53 negatively regulates a number of transcription factors, such as c-Myc [53], *via* various mechanisms.

Recently, Pandit et al. [54] found that blockade of p53 activity led to increased FOXM1 mRNA expression and, conversely, that induction of p53 expression by DNA damage suppressed mRNA expression in different cell lines. They suggested that p53 negatively regulates the oncogenic transcription factor FOXM1. Barsotti et al. [55] described a more detailed study of the downregulation of FOXM1 expression by p53. They demonstrated that p53 facilitates the downregulation of FOXM1 mRNA expression, which is accompanied by decreased FOXM1 protein expression. In addition, p53-mediated inhibition of FOXM1 expression is partially dependent on the activity of p21 and retinoblastoma (Rb) protein family members, although they observed p21-independent repression of FOXM1 in some cases. Furthermore,

their results indicated a potential contribution of p53-mediated repression of FOXM1 expression to maintenance of stable cell-cycle arrest at G2 phase. Determining whether overexpression of FOXM1 correlates with p53 mutations in pancreatic cancer cell lines and tumors would be interesting and important to further substantiate that p53 executes its pancreatic tumor-suppressive activity at least in part *via* downregulation of FOXM1 expression.

Expression and transcriptional activity of FOXM1 are also regulated by other tumor suppressor genes (Fig. 2A). For example, expression of FOXM1 is negatively regulated by phosphorylated Rb (pRb). Researchers suggested that the tumor-suppressive function of pRb relies on inhibition of FOXM1 expression [6]. In addition, FOXM1 expression may be regulated by p19ARF, which binds to FOXM1 and relocalizes FOXM1 to the nucleolus, thereby inhibiting expression of FOXM1-activated genes and tumor progression [56].

3.2.3. Autoregulation of FOXM1 expression

Many transcription factors regulate the rate of transcription of their own genes. In fact, 59% of the transcription factors are known to autoregulate it, and the list of such factors is growing [57,58]. Negative autoregulation (autorepression) of FOXM1 expression occurs more frequently than does positive autoregulation (autoactivation), but both are very common. This suggests that autoregulation plays important roles in transcription factor regulation [59]. Recently, an investigation surprisingly demonstrated a novel mode of positive autoregulation of FOXM1 expression (Fig. 2B). The researchers found that induction of exogenous FOXM1 expression led to a fourfold increase in endogenous FOXM1 mRNA expression and resulted in strong induction of endogenous FOXM1 protein expression. Furthermore, they observed a correlation between endogenous FOXM1 mRNA and protein expression at different time points, indicating strict regulation of endogenous FOXM1 expression by exogenous FOXM1. In contrast, protein expression of other members of the FOX family was not induced by overexpression of FOXM1, suggesting that FOXM1 specifically induces expression of itself only. Collectively, these data clearly suggest that FOXM1 is involved in a positive autoregulatory loop in which FOXM1 activates its own mRNA and protein expression [60]. Although FOXM1

can contribute to its own expression, whether and, if so, how this autoregulation contributes to the invasive and metastatic behavior of pancreatic tumors is unknown.

3.2.4. Loss of KLF4 expression causes FOXM1 overexpression in human cancer cells

KLF4 is a transcription factor associated with tumor suppression and oncogenesis. KLF4 suppresses pancreatic tumorigenesis *via* unknown mechanisms. Recently, we found a putative KLF4-binding site in the FOXM1 promoter. A chromatin immunoprecipitation assay indicated that KLF4 could bind to this region of the FOXM1 promoter. Furthermore, transfection of PDAC cells with a KLF4 expression vector repressed the activity of the FOXM1 promoter, whereas knockdown of KLF4 expression by KLF4 small interfering RNA (siRNA) markedly increased its activity. These results demonstrated that KLF4 negatively regulates FOXM1 expression at the transcriptional level, suggesting that FOXM1 is an important downstream target of KLF4 [61].

To determine whether this regulation occurs in pancreatic cancer cases, we examined KLF4 and FOXM1 expression in human pancreatic tumor specimens. In most of the specimens, KLF4 expression was greatly decreased, whereas FOXM1 overexpression was strikingly evident. Analysis of FOXM1 and KLF4 expression in pancreatic tumors revealed a strong inverse correlation between FOXM1 expression and KLF4 expression. These findings also indicated that FOXM1 expression is negatively regulated by KLF4 in pancreatic cancer cells, suggesting that dysregulated signaling of this novel KLF4/FOXM1 pathway plays an important role in pancreatic cancer development and progression [62].

3.2.5. Loss of microRNA expression causes FOXM1 overexpression in human cancer cells

MicroRNAs (miRNAs), which comprise 19–25 nucleotides, are highly conserved small noncoding RNAs that regulate normal gene expression during development and cell proliferation and apoptosis by targeting mRNAs of protein-coding genes at the posttranscriptional level. A growing body of direct and indirect evidence suggests that altered miRNA expression is associated with cancer [63]. Although miRNAs contribute to tumorigenesis *via* unknown mechanisms, recent studies have provided evidence that some human miRNAs are deregulated in human cancer cells and behave as tumor suppressors by negatively regulating the expression of specific oncogenes. For example, investigators demonstrated that the miRNA miR-370 functions as a

tumor suppressor by targeting FOXM1. In that study, Zhang et al. [64] found that the FOXM1 gene has a 249-bp 3′-untranslated region (UTR) that has a 7-mer binding site for miR-370. They then generated luciferase reporter constructs containing the miR-370 recognition sequence from the 3′-UTR of FOXM1 inserted downstream from the luciferase gene and found that transfection with a miR-370 precursor decreased the reporter activity in acute myeloid leukemia cells, which strongly indicated that FOXM1 is a target for miR-370. Furthermore, they assessed the effect of miR-370 expression on FOXM1 expression and found that transfection of acute myeloid leukemia cells with the miR-370 precursor resulted in reduced FOXM1 expression.

3.2.6. Hypoxia causes FOXM1 overexpression in human cancer cells

Hypoxia appears to be a common phenomenon in patients with solid tumors [65]. Hypoxia results in a tumor microenvironment that is closely associated with activation as well as overexpression of hypoxia-inducible factor (HIF)-1α. Thus, overexpression of HIF-1α is regarded as a sign of hypoxia. HIF-1α acts as a master regulator of expression of numerous hypoxia-inducible genes related to tumor angiogenesis, metastasis, cell proliferation or survival, and glucose metabolism [66].

Recently, Xia et al. [67] found that FOXM1 mRNA and protein expression in cancer cells increased greatly when the cells were subjected to hypoxia. They also demonstrated that HIF-1α bound directly to the HIF-1α-binding sites in the FOXM1 promoter and that the binding was specific as revealed by HIF-1α binding/competition and chromatin immunoprecipitation assays. Lines of evidence demonstrated that HIF-1α was highly expressed in dozens of cancers, including human pancreatic cancer. Also, Akakura et al. [68] reported that pancreatic cancer cells constitutively expressed HIF-1α and thus gained additional survival and proliferative advantages under severe hypoxia. The main mechanisms of the effects of hypoxia on FOXM1 expression in pancreatic cancer cells are unknown. However, more relevant studies may provide new insight into how pancreatic cancer cells overcome hypoxic stress and survive and identify a new regulatory mechanism of FOXM1 expression in pancreatic cancer cells.

4. FOXM1 and pancreatic cancer biology

Researchers have identified numerous genes downstream from FOXM1 (Table 1). Altered expression and regulation of these genes
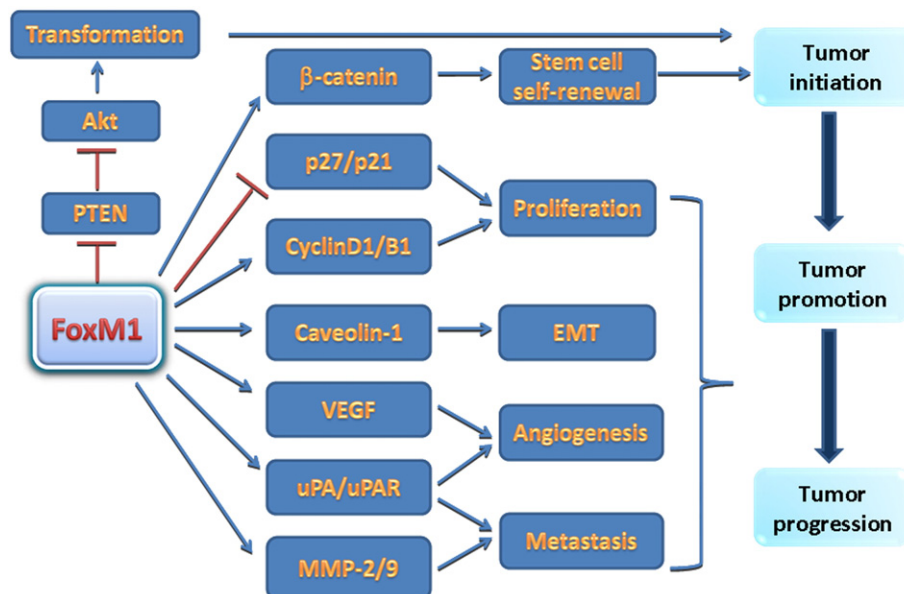

Fig. 3. Function of FOXM1 in cancer cells. FOXM1 stimulates the expression of genes involved in various steps of tumor development, including β-catenin, p27, p21, cyclin D1, cyclin B1, caveolin-1, VEGF, uPA, uPAR, MMP-2, and MMP-9.

play important roles in the pathogenesis of pancreatic cancer. The effects of FOXM1 on pancreatic cancer biology are mediated, at least in part, *via* the expression of FOXM1 target genes, which are essential to regulation of several aspects of tumor cell growth, proliferation, angiogenesis, EMT, and metastasis (Fig. 3).

4.1. Roles of FOXM1 expression in pancreatic cancer initiation

FOXM1 is one of the few genes whose expression is proven to be upregulated during early tumor initiation [6,13,69]. The involvement of FOXM1 in tumor initiation is largely correlated with its regulation of the cell cycle and cellular proliferation. Specifically, FOXM1 is a pivotal regulator of the G1/S and G2/M transitions and M-phase progression. Therefore, FOXM1-depleted cells are more prone than FOXM1-expressing cells to delays in G2 phase and severe mitotic abnormalities upon entry into mitosis [70]. In contrast, researchers found that cancer cell proliferation and tumor growth were highly induced in mice with the lung, prostate, or colon carcinoma and FOXM1 transgene expression when they were subjected to tumor induction by carcinogens [71–73]. The critical role of FOXM1 in tumor initiation was further supported by the finding that constitutive hepatic FOXM1b expression in transgenic mice had little effect on hepatocellular carcinoma (HCC) development but instead promoted cellular proliferation in preneoplastic and early neoplastic lesions. However, in conditional FOXM1-knockout mice, hepatocytes did not proliferate and were refractory to development of hepatic adenoma and HCC when induced by xenobiotic liver carcinogens [56,74]. Consistently, our unpublished studies have demonstrated that FOXM1 confers a proliferative advantage and stemness to pancreatic ductal epithelial cells and increases the susceptibility of the cells to transformation induced by oncogenes, supporting a critical role for FOXM1 in pancreatic cancer initiation.

4.2. Roles of FOXM1 expression in pancreatic cancer cell growth and proliferation

In addition to being a critical regulator during early tumor initiation, studies have demonstrated that FOXM1 promotes tumor cell proliferation and protects tumor cells against apoptosis by regulating the expression of genes encoding for proliferation-associated and antiapoptotic proteins, such as cyclin B, cyclin D, cyclin A, Cdk2, p21, p27, Cdc25, and survivin.

Cyclins are proteins that control cell-cycle progression by activating CDK enzymes [75]. FOXM1 signaling may contribute to cancer cell growth and proliferation *via* regulation of cyclin and CDK family genes. Constitutive activation of FOXM1 can promote expression of cyclins and CDKs by binding to their promoters and subsequently affecting cell-cycle progression in tumor cells. For instance, FOXM1 transgene expression correlates with increased expression of numerous mitosis-promoting genes such as cyclin A2, Cdc25B phosphatase, and cyclin B1, suggesting that these genes are somehow controlled by FOXM1 [76,77]. A more detailed study demonstrated that depletion of FOXM1 expression in the prostate cancer cell lines PC-3, LNCaP, and DU-145 by siRNA caused a marked reduction in their proliferation and anchorage-independent growth on soft agar. This phenotype was associated with diminished expression of S phase-promoting cyclin A2 and M phase-promoting cyclin B1 proteins, demonstrating that FOXM1 promotes prostate tumor cell proliferation by regulating the expression of cyclins [71]. Furthermore, several studies demonstrated that FOXM1 can regulate cyclin and CDK expression in human pancreatic cancer cells, as downregulation of FOXM1 expression inhibited pancreatic cancer cell growth by suppressing cyclin B, cyclin D1, Cdk2, and Cdc25a expression [78,79].

P21 and p27 are potent CDK inhibitors. In general, these two proteins bind to and inhibit the activity of cyclin–CDK complexes and thus function as negative regulators of cell-cycle progression [80,81]. Recently, results of a number of studies have suggested that FOXM1

regulates cancer cell proliferation by inhibiting p21 and p27 activity, contributing to accelerated cell-cycle progression [76,77]. In addition, blockade of FOXM1 expression in human prostate cancer cells can upregulate p27 protein expression and cause a reduction in and reduce cell proliferation and anchorage-independent growth on soft agar [71]. Moreover, Wang et al. [78] found that downregulation of FOXM1 expression decreased the pancreatic cancer cell population in S phase and inhibited the growth of the cells by increasing p21 and p27 expression.

Survivin is a member of the inhibitor of apoptosis family of proteins. This protein inhibits caspase activation, thereby leading to negative regulation of apoptosis [82]. Several studies have demonstrated that survivin is also a gene targeted by FOXM1 [83,84]. Moreover, treatment of pancreatic cancer cells with genistein may inhibit activation and expression of FOXM1 and induce apoptosis by downregulating survivin gene expression [79].

Consistent with these findings, we found that cyclin D1, cyclin B1, and survivin expression is significantly correlated with FOXM1 expression in human pancreatic tumors and cancer cell lines, and our preliminary studies demonstrated that inhibition of FOXM1 signaling by various means inhibited proliferation of pancreatic cancer cells and blocked the expression of cyclin D1, cyclin B1, and survivin in them [62,85]. Taken together, these findings are ample evidence that FOXM1 has role in pancreatic cancer cell proliferation and growth following the initial stages of tumorigenesis.

4.3. Roles of FOXM1 expression in pancreatic tumor angiogenesis

Angiogenesis, also known as neovascularization, is a key step in cancer development and pathogenesis and involves the development of new blood vessels necessary for continued tumor growth and metastatic spread [86]. A large body of literature suggests that angiogenesis regulators and activation of oncogenes are essential for the maintenance of an angiogenic phenotype that contributes to oncogenesis [87]. Although numerous angiogenesis regulators and oncogenes are involved in tumor angiogenesis, FOXM1 in particular plays important roles in it [41,88].

Vascular endothelial growth factor (VEGF), which is produced and secreted by cancer cells, is the principal angiogenic activator in tumor angiogenesis [89]. FOXM1 may contribute to human tumor angiogenesis by regulating VEGF expression. Zhang et al. [41] found that FOXM1 overexpression enhanced and inhibition of FOXM1 expression suppressed the angiogenic ability of glioma cells *via* regulation of VEGF expression. Furthermore, they identified two FOXM1-binding sites in the VEGF promoter that specifically bound to the FOXM1 protein. Mutation of these sites attenuated VEGF promoter activity, suggesting that VEGF is a direct transcriptional target of FOXM1. Correspondingly, other studies demonstrated a strong correlation between FOXM1 overexpression and elevated VEGF expression in gastric and breast cancer cells. Moreover, FOXM1 overexpression increased VEGF expression in gastric and breast cancer cells, whereas blockade of FOXM1 expression did the opposite [88,90]. Recently, Wang et al. [78] found that downregulation of FOXM1 expression reduced VEGF expression in pancreatic cancer cells, resulting in inhibition of angiogenesis. Consistent with these findings, we demonstrated that promotion of pancreatic cancer angiogenesis by FOXM1 was directly and strongly correlated with transactivation of VEGF. Furthermore, FOXM1 overexpression in pancreatic cancer cells resulted in increased VEGF secretion into the culture media and promoted capillary tube formation by human umbilical vascular endothelial cells, whereas suppression of FOXM1 expression did the opposite [85].

Another important aspect of regulation of angiogenesis in tumors involves urokinase-type plasminogen activator (uPA) and its membrane-bound receptor uPAR, which can modulate tumor angiogenesis and growth *via* regulation of vascular endothelial cell proliferation, adhesion, migration, and microvascular tube formation [91,92]. A number

of lines of experimental evidence from *in vitro* and *in vivo* as well as clinical studies indicate that the uPA/uPAR systems have critical roles in human tumors, including pancreatic cancer [93–95]. These systems may be regulated and activated by numerous angiogenesis regulators and/or transcriptional activators. Recently, a study demonstrated that downregulation of FOXM1 expression by siRNA reduced uPA expression in human HCC cells [96]. In addition, Ahmad et al. [97] found that inhibition of FOXM1 expression in breast cancer cells resulted in decreased cell proliferation and migration. Moreover, downregulation of FOXM1 expression inhibited the expression of many factors involved in degradation of the extracellular matrix and angiogenesis, such as uPA and uPAR. Consistent with these findings, our studies indicated that uPA/uPAR system activation in human pancreatic tumors and cancer cell lines correlated with FOXM1 expression and that blockade of FOXM1 expression *via* siRNA greatly suppressed uPA and uPAR expression, angiogenesis, and tumor growth both *in vitro* and *in vivo*.

4.4. Roles of FOXM1 expression in pancreatic cancer cell EMT

Epithelial and mesenchymal cells are two of the main cell types in mammals. Epithelial cells are defined as surface barrier cells with secretory functions that exhibit distinct apical *versus* basolateral polarity established by adherent and tight junctions. Mesenchymal cells have scaffolding and anchoring functions and multifunctional roles in tissue repair and wound healing. During oncogenesis, certain cells can switch from epithelial to mesenchymal status by means of the tightly regulated process EMT, resulting in the formation of mesenchymal cells with migratory and invasive properties. Thus, EMT induction in cancer cells results in the acquisition of metastatic properties [98,99].

Recently, Bao et al. [100] found that forced overexpression of FOXM1 led to the acquisition of an EMT phenotype in pancreatic cancer cells *via* activation of the mesenchymal cell markers ZEB, Snail, and vimentin. Moreover, forced overexpression of FOXM1 decreased the expression of miR-200 in pancreatic cancer cells, whereas re-expression of miR-200b reversed the EMT phenotype and decreased the expression of FOXM1. Overall, the data reported by Bao and colleagues suggest that FOXM1 overexpression is responsible for the acquisition of EMT, which is in part mediated *via* regulation of miR-200b expression.

A previous study by our group demonstrated that the poorly metastatic pancreatic cancer cell lines COLO357 and Panc02 exhibited a typical epithelial morphology, whereas the highly metastatic pancreatic cancer cell lines L3.7 and Panc02-H7 exhibited a typical mesenchymal morphology [101]. Therefore, we used the murine pancreatic cancer cell lines Panc02 and Panc02-H7 and human pancreatic cancer cell lines COLO357 and L3.7, which are derived from biologically homogenic neoplasms but have different metastatic properties, to determine the potential role of FOXM1 in EMT of pancreatic cancer cells. We found that FOXM1 expression correlated directly with the metastatic potential and EMT phenotype of both the murine and human cell lines. In addition, FOXM1 overexpression contributed to metastasis of pancreatic tumors and EMT of pancreatic cancer cells, whereas knockdown of FOXM1 expression did the opposite. Moreover, we observed that caveolin-1 plays vital roles in pancreatic cancer cell EMT and is an important gene downstream from FOXM1, suggesting that FOXM1 indirectly contributes to EMT of pancreatic cancer cells by regulating caveolin-1 expression [101].

4.5. Roles of FOXM1 expression in the pancreatic CSC phenotype

CSCs constitute only a very small proportion of malignant cells in tumors and are able to self-renew, giving rise to differentiated tumor cells [102]. The CSC theory has fundamental clinical implications, especially because investigators have identified CSCs in many malignant tumors, including pancreatic tumors, and CSCs are considered to be highly resistant to chemoradiation [103,104]. Recently reported evidence indicates that EMT of tumor cells not only increases metastasis

but also contributes to the formation of CSCs [105,106]. In addition, acquisition of the EMT phenotype and induction of CSC or cancer stem-like cell phenotypes are highly interrelated and contribute to drug resistance and tumor recurrence [107]. Further studies suggested that the expression of genes that are fundamental to the acquisition of EMT and the CSC phenotype are regulated by miRNAs [108,109].

Recently, Bao et al. [100] found that forced overexpression of FOXM1 led to the acquisition of increased sphere-forming (pancreatosphere) capacity and CSC surface marker (CD44 and EpCAM) expression. Moreover, forced overexpression of FOXM1 decreased the expression of miR-200 in pancreatic cancer cells, whereas re-expression of miR-200b reversed the CSC phenotype and decreased expression of FOXM1. Overall, these data suggest that FOXM1 overexpression is responsible for acquisition of the CSC phenotype, which is mediated in part *via* regulation of miR-200b expression. Determining whether forced FOXM1 expression enhances the CSC phenotype of pancreatic cancer cells by promoting β-catenin nuclear localization and transcriptional activity would be interesting and important.

4.6. Roles of FOXM1 expression in pancreatic tumor invasion and metastasis

Metastasis consists of the following steps: local invasion of tumor cells, growth of new blood or lymphatic vessels, transportation of tumor cells in the blood and/or lymphatic vessels, and proliferation of tumor cells at the secondary site [110]. Metastasis plays critical roles in the morbidity and mortality of most human cancers, including pancreatic cancer. However, the exact molecular mechanism that governs metastasis remains poorly understood. Generally, the molecular mechanisms that control the steps in metastasis are related to alterations of various oncogenes, tumor suppressor genes, metastasis suppressor genes, and growth factors and their receptors [111].

In a recent genome-wide expression study of prostate cancer, the researchers identified FOXM1 as one of the genes whose expression is commonly upregulated in metastatic prostate cancer cells [112]. Investigators also have suggested that FOXM1 overexpression in human tumor specimens is closely correlated with the presence of lymph node metastasis, incidence of liver metastasis, and advanced TNM stage, indicating that FOXM1 is functionally involved in tumor invasion and metastasis [113–115]. More detailed studies demonstrated that each step in the metastatic process – from the initial EMT to the ultimate organotropic colonization – can be regulated by FOXM1, suggesting critical roles for FOXM1 in metastasis [43].

During metastasis, degradation of the basement membrane and various components of the extracellular matrix is facilitated by a group of proteolysis-related genes. Of these genes, researchers have consistently implicated that the matrix metalloproteinase (MMP) and uPA/uPAR genes have roles in tumor invasion and metastasis. FOXM1 may contribute to human cancer metastasis *via* regulation of MMP expression. Recently, studies suggested that FOXM1 promotes human cancer cell invasion and metastasis by directly or indirectly regulating MMP-2, MMP-9, and MMP-7 expression [73,116,117]. Dai et al. [116] demonstrated that MMP-2 expression is regulated directly by FOXM1 at the transcriptional level *via* a Forkhead consensus site on the MMP-2 promoter. They identified a FOXM1-binding site in the MMP-2 promoter and demonstrated that FOXM1 protein bound directly to it. Mutation of this FOXM1-binding site markedly attenuated MMP-2 promoter activity. They also found that FOXM1 overexpression directly activated the MMP-2 promoter and increased MMP-2 expression in glioma cells, whereas blockade of FOXM1 expression suppressed MMP-2 activation and expression. Furthermore, FOXM1 overexpression increased the invasiveness of glioma cells, whereas inhibition of FOXM1 expression suppressed this invasiveness, suggesting that FOXM1 contributes to glioma progression by enhancing MMP-2 gene transcription and, thus, tumor cell invasion. Authors have documented a similar role for FOXM1 in the regulation of MMP-2, MMP-7, and MMP-9 expression

as well as cell migration and invasion in other malignancies, including colorectal and breast carcinoma [13,97]. Consistent with these findings, recent studies indicated that FOXM1 contributes to pancreatic cancer invasion by enhancing MMP-2 and MMP-9 gene expression and that suppression of FOXM1 expression leads to reduced MMP-2 and MMP-9 expression in pancreatic cancer cells, which are associated with an overall decrease in cancer cell migration, invasion, and angiogenesis [78,79].

In addition to regulation of the expression of proteolysis-related genes, several studies have demonstrated that FOXM1 promotes pancreatic tumor invasion and metastasis by contributing to angiogenesis and pancreatic cancer cell EMT [78]. In summary, these findings provide evidence that FOXM1 promotes pancreatic cancer metastasis *via* regulation of multiple steps in the process in tumor invasion and metastasis.

5. Clinical significance of FOXM1 expression in pancreatic cancer cells

Pancreatic cancer continues to be associated with very poor prognoses, with a 5-year survival rate of about 5%, which has not changed over recent decades. The molecular mechanism responsible for pancreatic cancer development remains unknown, and guidelines for prevention of it have yet to be established. Moreover, few clinical parameters predict outcome with acceptable sensitivity and specificity. Therefore, specific and sensitive biomarkers have attracted much attention from researchers with the hope that they can be used for prognosis and as therapeutic targets for pancreatic cancer. This would improve selection of pancreatic cancer patients for individualized treatment.

Several studies over the past several decades have identified numerous molecular markers that may have prognostic significance for pancreatic cancer. Although no currently used marker is perfect in predicting outcome of pancreatic cancer, an expression profiling based on FOXM1 expression may be helpful in guiding prognosis and therapy for it [113]. Numerous reports have indicated that FOXM1 is overexpressed in human pancreatic cancer cell lines and tumor specimens (Table 2). Specifically, pancreatic cancer cells and tumors have aberrant overexpression of FOXM1, whereas normal pancreatic tissue cells have very low expression of FOXM1 [16].

Pilarsky et al. [118] confirmed that FOXM1 is overexpressed in human pancreatic tumors by comparing microarray data of FOXM1 expression in tumor tissues and normal tissues. They also confirmed that FOXM1 is overexpressed in human pancreatic cancer cell lines. These data suggest that overexpression of FOXM1 not only promotes migration, invasion, and angiogenesis of pancreatic cancer cells but also leads to EMT and a CSC phenotype [78,79,108]. Recently, Xia and colleagues demonstrated that FOXM1 protein and mRNA expression was higher in pancreatic tumors than in paired adjacent normal pancreatic tissue specimens. They also found that a high level of expression of FOXM1 was highly correlated with clinical stage, lymph node metastasis, and histological differentiation. In addition, patients with high FOXM1 expression had much shorter survival durations than did patients with low FOXM1 expression. Furthermore, multivariate analysis revealed that FOXM1 expression is an independent factor for

Table 3
Modulation of FOXM1 functions for treatment of pancreatic cancer.

First author/year	Method	Targeted tumor biology	Reference
Wang/2007	RNAi	Tumor growth, migration, invasion, and angiogenesis	[78]
Wang/2010	Genistein	Tumor growth and invasion	[79]
Bao/2011	Genistein	EMT and CSC phenotype	[100]
Huang/2012	RNAi	Tumor growth, migration, invasion, and metastasis	[101]

poor prognosis, suggesting that FOXM1 is a novel prognostic marker and therapeutic target for pancreatic cancer [16].

Consistent with these findings, studies in our laboratory have demonstrated the prognostic importance of FOXM1 expression for pancreatic cancer at both the mRNA and protein level. In addition, high FOXM1 expression in pancreatic tumor specimens correlated with clinical phenotype, relapse rate, and survival duration. Moreover, we found that the three FOXM1 isoforms have different expression patterns in pancreatic tumors and cancer cell lines as well as different regulation mechanisms [62]. Therefore, further investigations of the expression and function of each FOXM1 isoform may assist in prognosis and individualized therapy decision-making for pancreatic cancer in the near future.

6. Modulation of FOXM1 functions for therapy for pancreatic cancer

FOXM1 is overexpressed in a number of different cancer cells, whereas its expression is turned off in terminally differentiated cells. FOXM1 is therefore an attractive therapeutic target for human cancers, including pancreatic cancer (Table 3). However, FOXM1 is a transcription factor that belongs to a group of traditionally undruggable molecules, and it is not easily targeted using traditional drug development approaches. Therefore, the therapeutic potential of FOXM1 inhibitors for pancreatic cancer has yet to be explored [119]. Efforts to develop inhibitors of FOXM1 are under way. Approaches to inhibition of FOXM1 activity in human cancer cells include the use of RNA interference (RNAi), thiazole antibiotics (siomycin A and thiostrepton), proteasome inhibitors, peptides, and small-molecule compounds.

6.1. FOXM1-targeting RNAi

RNAi is a process of sequence-specific posttranscriptional gene silencing initiated by double-stranded RNA [120]. Over the past 10 years, RNAi has become widely used as an experimental tool to analyze the function of mammalian genes both *in vitro* and *in vivo*. Compared with traditional gene therapy, RNAi has the advantages of exquisite precision and high efficacy in downregulating gene expression, thus providing a new approach to the treatment of diseases including cancer [121,122].

Several studies using experimental human tumor models have demonstrated the feasibility of RNAi in inhibition of expression of cancer-related genes, including FOXM1. For example, a study demonstrated that downregulation of FOXM1 expression by siRNA diminished

Table 2
Expression of FOXM1 in pancreatic cancer cell lines and tumor specimens.

First author/year	Expression of FOXM1		Method	Reference
	Cells	Tumors		
Pilarsky/2004		↑	Bioinformatic methods	[118]
Wang/2007	↑		Western blot/RT-PCR	[78]
Wang/2010	↑		Western blot/RT-PCR	[79]
Bhat/2011	↑		Western blot	[36]
Bao/2011	↑		Western blot	[100]
Xia/2012		↑	Western blot/IHC/RT-PCR	[16]
Huang/2012	↑		Western blot/IHC/RT-PCR	[101]

RT, reverse transcription; IHC, immunohistochemistry.

DNA replication and mitosis and reduced the proliferation and growth of lung cancer cells [9]. Another study demonstrated that silencing of FOXM1 by RNAi abolished estrogen-induced breast cancer cell proliferation and reversed acquired tamoxifen resistance [123]. Moreover, downregulation of FOXM1 expression *via* stable or transient knockdown using RNAi strongly sensitized human cancer cells of different origins to DNA damage-induced apoptosis through regulation of proapoptotic c-Jun N-terminal kinase and antiapoptotic Bcl-2 signaling [124].

To investigate the use of RNAi to block FOXM1 expression and its effect on the biology of human pancreatic cancer cells, Wang et al. [78] employed RNAi systems to knock down FOXM1 gene expression in pancreatic cancer cells. They found that RNAi silencing of the FOXM1 gene suppressed the expression of MMP-2, MMP-9, and VEGF, which was accompanied by marked inhibition of tumor cell migration, invasion, and metastasis. Also, downregulation of FOXM1 expression by FOXM1 siRNA inhibited cell growth *via* downregulation of cyclin B, cyclin D1, and Cdk2 expression and decreased the cell population at S phase *via* upregulation of p21 and p27 expression [78].

Recently, we reported that caveolin-1 is a novel target gene of FOXM1 and that RNAi silencing of the FOXM1 gene greatly suppressed caveolin-1 expression [101]. Moreover, we found that elimination of FOXM1 protein expression by RNAi in pancreatic cancer cells inhibited cellular proliferation, EMT, and migration and tumor angiogenesis, invasion, and metastasis *in vitro* and *in vivo* by decreasing uPA and uPAR expression. All of these results demonstrated that RNAi is capable of specific, highly stable functional silencing of FOXM1 gene expression in pancreatic cancer cells and that silencing of the gene by RNAi may be a novel strategy for pancreatic cancer treatment.

6.2. Thiazole antibiotics

Using a high-throughput cell-based assay system, Radhakrishnan et al. [84] discovered the first FOXM1-inhibiting thiazole antibiotic, siomycin A. They observed that this drug downregulated the transcriptional activity as well as the protein and mRNA expression of FOXM1. Consequently, they demonstrated that siomycin A can reduce anchorage-independent growth and induce apoptosis of cancer cells by repressing the activity of FOXM1's downstream target genes, such as Cdc25B, survivin, and CENPB. Next, Radhakrishnan and colleagues screened for and isolated a structurally similar antibiotic, the thiazole compound thiostrepton, identifying it as a new inhibitor of FOXM1 [125]. The investigators reported that thiostrepton specifically inhibited not only the expression but also the transcriptional activity of FOXM1. In addition, they observed that thiostrepton did not inhibit the transcriptional activity of other members of the Forkhead family or some unrelated transcription factors. Furthermore, thiostrepton inhibited the growth and induced potent apoptosis of human cancer cell lines of different origins. Taken together, these data suggest that thiazole antibiotics specifically target FOXM1 to inhibit the growth and induce apoptosis of cancer cells and that these drugs represent a useful starting point for the development of anticancer therapeutics.

We have employed thiazole antibiotics to repress FOXM1 gene expression in pancreatic cancer cells. Both siomycin A and thiostrepton can inhibit FOXM1 DNA-binding activity and mRNA and protein expression *in vitro*, leading to impediment of multiple oncogenic processes such as proliferation, angiogenesis, migration, and invasion in pancreatic cancer cells (data not published). These effects highlight the efficacy of thiazole antibiotics in targeting pancreatic cancer cells exhibiting constitutive FOXM1 signaling. In the future, we expect thiazole antibiotics to be used as single agents or in combination with low-dose chemotherapy in pancreatic cancer patients with relapsed or refractory disease.

6.3. Proteasome inhibitors

The proteasome is a protein complex that is responsible for ubiquitin- and ATP-dependent proteolysis of cellular proteins. Recent studies indicated that certain types of cancer rely on a functional proteasome for growth and that proteasome inhibitors selectively kill cancer cells but not normal cells [126]. Thus, proteasome inhibitors are widely used in the clinic and in clinical trials for treatment of cancer, but the mechanism of their anticancer activity is not completely understood. Researchers have presented several explanations for the antitumor properties of proteasome inhibitors, such as nuclear factor-κB inhibition, p53 stabilization, and a shift in the balance between proapoptotic and antiapoptotic Bcl-2 proteins [127]. However, the latest proteasome studies demonstrated that FOXM1 is a target for proteasome inhibitors [128].

Bhat et al. [129] found that well-known proteasome inhibitors such as MG115 and MG132 inhibited FOXM1 transcriptional activity and expression in cancer cells. Importantly, bortezomib (Velcade) was the first proteasome inhibitor approved for the treatment of multiple myeloma, with the potential for efficacy against other types of cancer in the future. Bhat and colleagues also found that treatment with bortezomib not only antagonized the transactivation ability of FOXM1 but also inhibited its expression. In addition, they found that bortezomib induced apoptosis in a variety of human cancer cell lines, which correlated with the suppression of FOXM1 expression. Moreover, overexpression of FOXM1 specifically protected cancer cells against bortezomib-induced apoptosis. These data suggest that negative regulation of FOXM1 expression is a general feature of proteasome inhibitors and may contribute to their anticancer properties.

Although whether proteasome inhibitors suppress FOXM1 expression in human pancreatic cancer cells is unknown, a recent study demonstrated that treatment with the proteasome inhibitor MG132 decreased the growth of the human pancreatic cancer cell line BxPC-3 in a dose- and time-dependent manner [130]. In addition, Matsuo et al. [131] showed that treatment with MG132 blocked pancreatic cancer-associated angiogenesis *via* inhibition of nuclear factor-κB, VEGF, and interleukin-8 expression. Because FOXM1 plays important roles in pancreatic cancer cell proliferation and apoptosis and tumor angiogenesis by regulating the expression of VEGF and other target genes, mediation of the anticancer properties of MG132, at least in part, by inhibition of FOXM1 expression is reasonable. Recently, a study demonstrated that bortezomib exhibited antitumor effects on pancreatic cancer *in vitro* and *in vivo* [132]. In addition, bortezomib strongly enhances the anticancer activity of cisplatin and gemcitabine and induces the expression of genes with diverse apoptotic effects [133–135]. Taken together, these data establish that bortezomib may be a promising target for pancreatic cancer treatment. As experience using proteasome inhibitors increases, oncologists will administer bortezomib in combination with gemcitabine or other targeted standard drugs for treatment of pancreatic cancer.

6.4. Other therapies

There are several compounds, which function as FOXM1 inhibitors, such as FOXM1-targeting siRNA, proteasome inhibitors and thiazole antibiotics. More FOXM1 inhibitors would exist, while we describe below an incomplete list of compounds, which have been shown to inhibit FOXM1 activity in human cancer cells, including Genistein, Docetaxel and Peptide inhibitors.

6.4.1. Genistein

Genistein, a natural isoflavonoid found in soybean products, is believed to be a chemopreventive agent because of its reported association with decreased incidence of pancreatic cancer [136]. However, the molecular mechanisms by which genistein elicits its preventive effects on pancreatic cancer cells has yet to be fully elucidated. Recently,

Wang et al. [79] found that treatment with genistein inhibited pancreatic cancer cell growth and invasion and concomitantly attenuated expression of FOXM1 and its downstream genes, such as survivin, Cdc25a, MMP-9, and VEGF. This was the first report of a molecular role for FOXM1 in mediating the biological effects of genistein on pancreatic cancer cells, suggesting that targeting FOXM1 with genistein is a potential preventive and therapeutic strategy for pancreatic cancer.

6.4.2. Docetaxel

Docetaxel is a clinically well-established antimitotic chemotherapeutic drug. It is used mainly for the treatment of locally advanced or metastatic breast, ovarian, prostate, or non-small cell lung cancer in patients who have undergone anthracycline-based chemotherapy that failed to stop cancer progression or prevent relapse [137–139]. Recently, Li et al. [140,141] found that treatment with docetaxel alone or in combination with estramustine downregulated the expression of FOXM1 in prostate cancer cells, leading to cell growth inhibition and apoptosis induction. These observations clearly suggested that the anticancer properties of docetaxel were mediated at least in part by inhibition of FOXM1 expression. Determining whether chemotherapeutic drugs such as docetaxel and gemcitabine inhibit FOXM1 activity in pancreatic cancer cells, leading to cell-cycle arrest and apoptosis, is important, as it would suggest that these drugs are useful for inhibition of FOXM1 expression and will have beneficial effects and would be effective in pancreatic cancer therapy.

6.4.3. Peptide inhibitors

The first identified anti-FOXM1 peptide inhibitor is a 26–44 peptide of p19ARF. Expression of p19ARF proteins is induced during cancer initiation and exerts a cancer-inhibitory function by increasing the stability of the p53 tumor suppressor. The most efficient version of p19ARF is this 26–44 peptide containing nine D-Arg residues, which may inhibit FOXM1 activity and FOXM1-induced growth of cancer cells [56,142]. Gusarova et al. [74] performed a more detailed study of FOXM1 peptide inhibition. They subjected HCC-bearing mice to daily injections of a cell-penetrating alternative reading frame peptide inhibitor of FOXM1 expression. After 4 weeks of treatment, they observed reduced tumor cell proliferation and angiogenesis in the HCC regions but not in adjacent normal liver tissue. Furthermore, the treatment induced apoptosis of several distinct human hepatoma cell lines, which correlated with reduced protein expression of the mitotic regulatory genes encoding for Plk1, Aurora B kinase, and survivin, all of which are transcriptional targets of FOXM1 that are highly expressed in cancer cells and prevent apoptosis. These studies indicated that treatment with FOXM1 peptide inhibitors is an effective approach to limiting growth and inducing apoptosis of cancer cells. Although to our knowledge the literature contains little reported research on the use of peptide inhibitors of FOXM1 expression in pancreatic cancer cells, these studies make a compelling argument for examining the therapeutic benefits of these inhibitors for this purpose in the future.

7. Conclusions and future directions

Emerging evidence from experimental and clinical studies has demonstrated that FOXM1 plays key roles in pancreatic cancer pathogenesis and that abnormal FOXM1 expression promotes the growth, angiogenesis, and metastasis of pancreatic tumors *via* overexpression of many FOXM1 downstream genes. Therefore, FOXM1 has attracted much attention as a promising target for the prevention and treatment of pancreatic cancer. However, many questions in the field of FOXM1 signaling in pancreatic cancer cells remain to be answered. A better understanding of the complexity of the FOXM1 isoforms in the molecular biology of pancreatic cancer and determination of how the interaction and isoform switching between FOXM1a and FOXM1b or FOXM1c contribute to pancreatic carcinogenesis are urgently needed. Furthermore, future studies of the cross-talk between FOXM1 and other signaling pathways will provide valuable insight into the mechanisms of FOXM1 signaling in pancreatic cancer pathogenesis and the molecular basis for designing novel, effective strategies for pancreatic cancer prevention and treatment.

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