Forkhead box Q1: A key player in the pathogenesis of tumors (Review)

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Abstract. As a member of the Forkhead box protein family, Forkhead box Q1 (FOXQ1) is a transcription factor that functions to regulate cell differentiation. Recently, an increasing number of studies have demonstrated that FOXQ1 is significantly associated with the pathogenesis of tumors. This review aims to predominantly discuss the relationship between FOXQ1 and various types of tumor. The FOXQ1 gene is located at human chromosome 6p25.3 and encodes a functional 403 amino acid protein, which has many physiological functions, including promoting epithelial differentiation, inhibiting smooth muscle differentiation, activating T cells and autoimmunity, and controlling mucin gene expression and granule content in stomach surface mucous cells. There are several modes of regulation of FOXQ1 expression that have been demonstrated in normal and tumor cells, such as microRNA and the Wnt signaling pathway. The activation of FOXQ1 affects downstream genes promoting the initiation, proliferation and invasion, in addition to the metastasis of tumor cells. Amongst these, the regulation of invasion and metastasis by FOXQ1 is the most extensively studied. The detailed mechanism involves angiogenesis, tumor re-initiation, alterations in the tumor microenvironment and epithelial-mesenchymal transition. In a number of studies, the expression of FOXQ1 has been reported to be upregulated in breast, colorectal, pancreatic, bladder and ovarian cancer, and glioma, amongst other tumor types. Together, these studies contribute to cancer diagnostics, prognostics and therapeutics. In conclusion, the application prospect of FOXQ1 in tumors is hopeful.

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Contents

1. Introduction

The Forkhead box (FOX) transcription factor was a mutant that was first identified in *Drosophila* melanogaster in 1989, and was initially called *Drosophila* homeotic forkhead (fkh) protein (1). At that time, all that was known was that FOX was a nuclear protein and was associated with transcriptional regulation; however, little was known regarding the detailed structure and function. The subsequent discovery of the rat gene, hepatocyte nuclear factor-3 (HNF-3), brought to light a previously unknown family of transcription factors carrying the 'Forkhead' motif (2). Since then, studies have increased regarding the presence of FOX transcription factors (3-5).

Currently, the FOX transcription factor family is known to consist of >100 members, which are classified into 19 subfamilies, called FOXA-S (2,6). The 19 subfamilies are involved in cell differentiation, proliferation and apoptosis, embryonic development, ageing, glucose and lipid metabolism, and immune regulation, which serves an important role in human health and disease, particularly in cancer (7). Recently, a number of studies have demonstrated that FOX transcription factors are associated with the initiation, progression and metastasis of human cancers (8-12). As a member of the FOX transcription factor family, FOXQ1 is a major oncogenic transcription factor, similar to other subsets of FOX transcription factors, including FOXA1, FOXC1, FOXC2, FOXG1, FOXM1 and FOXO. Several lines of evidence reported in the literature demonstrate a key role for FOXQ1 in the progression of tumors (13-15). In this review, we discuss the FOXQ1 structure and the association between FOXQ1 and tumors, in addition to the application prospect of FOXQ1 in tumors.

2. FOXQ1 structure and physiological function

The FOXQ1 gene, in addition to the FOXF2 and FOXC1 genes, is located at human chromosome 6p25.3 and consists of 2319 base pairs (bp) (16). FOXQ1 is encoded by an open reading frame of 1029 bp, producing a functional 403 amino acid protein. The FOXQ1 transcription factor, also called HNF-3/fkh homolog-1 (HFH-1), is a DNA-binding protein that regulates the cell cycle. The FOXQ1 protein is divided into three domains: The alanine and glycine enrichment region, the forkhead box domain (FHD) containing 96-amino acids and the proline-enrichment region. The FHD, also known as the Winged-helix domain, is the DNA binding domain, while the other two are associated with transcriptional activators, transcriptional repressors or DNA repair complexes. The core of this polypeptide contains three α-helices (α1, α2 and α3) and two wing-like loops located side by side. Among these three helices, α3 is the most significant part as it contains the DNA binding sequence (7). In 1994, Overdier *et al* (3) reported a core FOXQ1 binding sequence of 5'-TGTTTA-3'. However, more recently, Abba *et al* (17) suggested that the precise binding sequence may be 5'-GTTT-3'. By searching Transfac (www.biobase-international.com) and the JASPER CORE database (www.jasper.cgb.ki.se), matrix motifs were identified in rat alone, with a core 'GTTT' motif. Compared with other genes, FOXQ1 is evolutionarily conserved, and the forkhead DNA binding domain of the human, mouse and rat FOXQ1 proteins share 100% sequence identity, suggesting that the 5'-GTTT-3' core may be valid across all species (Fig. 1) (17).

FOXQ1 was highly expressed in the mouse stomach, as reported by Bieller *et al* (18). In this study, the authors measured FOXQ1 expression levels in human gastric mucosa and muscle, which indicated that the transcripts were present in the gastric mucosa tissue but not in the gastric muscle tissue. Additionally, the authors investigated human FOXQ1 expression in different tissues, observing strong expression of FOXQ1 in the stomach, trachea, bladder and salivary glands, and significant expression in the duodenum, prostate and fetal liver tissue (18).

There are numerous physiological functions of FOXQ1, which have been reported in previous studies, including promoting epithelial differentiation, inhibiting smooth muscle differentiation, activating T cells and autoimmunity, controlling mucin gene expression and granule content in stomach surface mucous cells (19-21). Additionally, FOXQ1 has been demonstrated to regulate hair differentiation. Hong *et al* (22) reported that the hair of the satin mouse could been modified by FOXQ1. Further study indicated that a key role of FOXQ1 was as a transcription factor and target of homeobox C13 regulation; a member of the Homeobox gene family that is able to control hair follicle development and hair growth (23,24). Future investigations of FOXQ1 will provide valuable insight into its physiological function.

3. The regulation of FOXQ1 expression

There are several modes that have been demonstrated to regulate FOXQ1 expression in normal and tumor cells. Herein, we present some modes of FOXQ1 regulation in human health and disease, focusing on the modes of regulation of FOXQ1 in tumors in particular (Fig. 2).

The relationship between microRNA (miRNA or miR) and FOXQ1. miRNAs are small noncoding regulator RNAs comprised of 18-25 nucleotides, which are able to control the translation of mRNA involved in cellular processes, such as cell proliferation and apoptosis (25-27). miRNAs combine with other proteins to form the RNA-induced silencing complex (RISC), which is able to bind to the 3'-untranslated region (3'UTR) of a target mRNA, regulating the translation of the target gene (28). A number of studies have directly or indirectly indicated that miRNAs are associated with types of human cancer, including breast, colorectal and pancreatic cancer (29-31). Recently, studies have demonstrated that several miRNAs function as tumor regulators by targeting FOXQ1. In this review, we describe some of these miRNAs.

Peng *et al* (32) demonstrated that miR-124 was able to inhibit the proliferation, migration and invasion of nasopharyngeal carcinoma cells through directly targeting the FOXQ1 3'-UTR. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), it was observed that FOXQ1 is highly conserved among different species, with the 3'-UTR of the mRNA containing a complementary site for the seed region of miR-124. Subsequently, dual-luciferase reporter vectors containing the target region sequence of FOXQ1 3'-UTR (wt3'-UTR) or the mutant sequence (mut 3'-UTR) were generated. The results revealed that miR-124 was able to downregulate the luciferase activity of the FOXQ1 wt3'-UTR construct, but not the mut 3'-UTR, suggesting that FOXQ1 is a direct target of miR-124. Additionally, a rescue experiment indicated that the overexpression of FOXQ1 could partially rescue the suppression of miR-124. Similarly, miR-422a, miR-506 and miR-1271 have been found to regulate the expression of FOXQ1 (33-35).

Oncogenic signaling pathways activate FOXQ1 in cancer cells. Dysfunction in the signaling pathway may lead to the loss of cell cycle control, and as a result, common epithelial cells transform into cancer cells, and normal tissue become neoplastic (36). Consistent with this, studies have reported that the expression of FOXQ1 is mediated by certain signaling pathways.

The pathway predominantly involved is the Wnt/β-catenin signaling pathway, which was first described by Nusse and Varmus in 1982 (37). Increasingly, studies have reported the functions of the Wnt/β-catenin signaling pathway, particularly its role in the initiation, progression and metastasis of human carcinoma (38). Additionally, FOXQ1 is a downstream mediator in the Wnt/β-catenin signaling pathway. Christensen *et al* (39) reported that the upregulation of FOXQ1 led to the loss of the expression of caudal-related homeodomain transcription 2, a transcription factor associated with Wnt signaling pathway activity. In addition, glycogen synthase kinase 3, a small molecular inhibitor typical of the Wnt pathway, was demonstrated to result in increased levels of FOXQ1 mRNA and protein, similar to a constitutively active form of β catenin. Furthermore, the Wnt pathway was demonstrated to regulate FOXQ1 expression via the identified transcription factor-4 binding site (39).

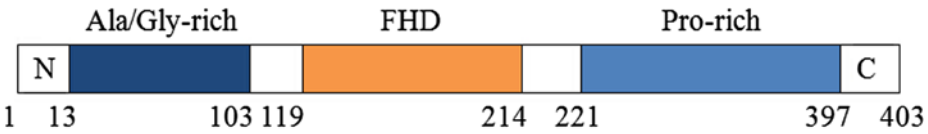

Figure 1. The structure of FOXQ1.

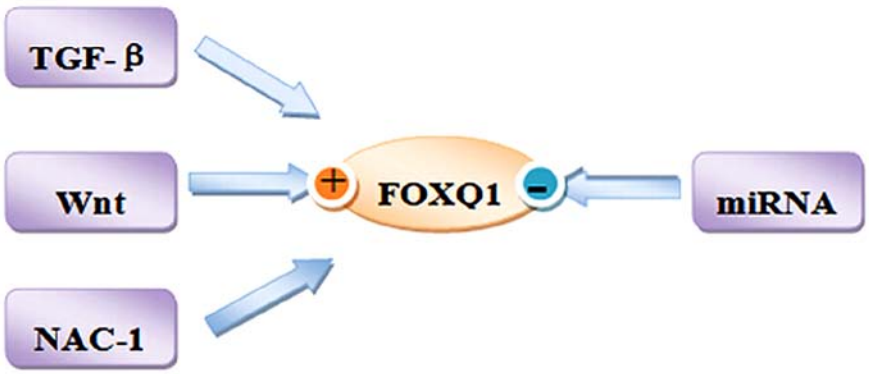

Figure 2. The regulation of FOXQ1 expression.

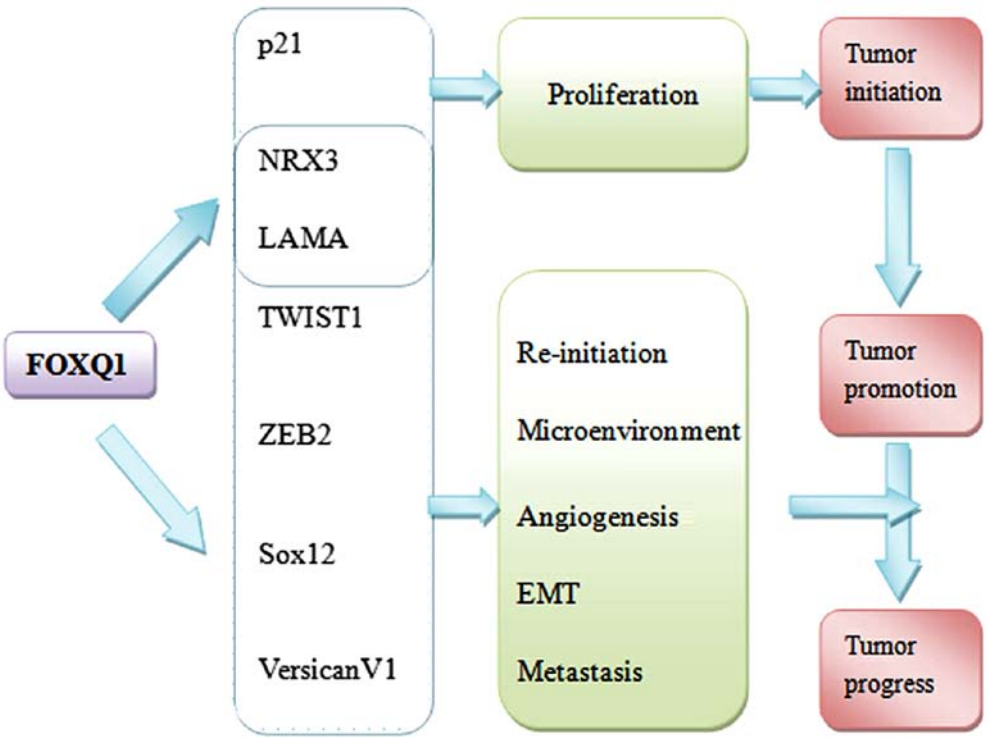

Figure 3. Function of FOXQ1 in cancer cells.

Similarly, it has been reported that FOXQ1 is upregulated by the transforming growth factor (TGF)-β signaling pathway (40). The TGF-β/mothers against decapentaplegic (SMAD) signaling pathway has been demonstrated to regulate expression in epithelial-mesenchymal transition (EMT) (41). Although the underlying mechanisms are unknown, treating cancer cells with TGF-β for 3 days markedly increased FOXQ1 mRNA and protein levels. Interestingly, further investigation of these cells indicated alterations in the Wnt pathway, including vascular endothelial growth factor (VEGF)-A, matrix metalloproteinase 2, vimentin, N-cadherin and E-cadherin, from which it may be inferred that FOXQ1 provides crosstalk between the Wnt and TGF-β signaling pathways (42).

Nucleus accumbens-associated protein 1 (NAC1) regulates FOXQ1 in cancer cells. NAC1 is a transcriptional coregulator, which participates in various biological processes. For instance, it is NAC1 that regulates bony patterning in the

murine vertebral column, is involved in acute psychomotor stimulant responses and contributes to tumor progression, tumor cell proliferation and survival (43-46). Gao *et al* (47) reported that FOXQ1 expression is transcriptionally regulated by NAC1. In addition, microarray analysis of gene expression and RT-qPCR indicated that Notch1 and Jagged1, two important factors in the Notch signaling pathway, were also regulated by NAC1. However, whether FOXQ1 is associated with the Notch signaling pathway remains unknown (47).

4. FOXQ1 and tumor biology

Tumor biology is a complex process involving cancer initiation, proliferation and invasion, in addition to metastasis. It is known that sustaining proliferative signaling, evading growth suppressors, resisting cell death, limitless replicative immortality and deregulated cellular energetics supports tumorigenesis (48). Several studies have reported that numerous genes downstream of FOXQ1 function at every stage of tumorigenesis (Table I and Fig. 3).

Roles of FOXQ1 expression in tumor initiation and proliferation. FOXQ1 belongs to the FOX gene family, which is involved in the regulation of the cell cycle (49). For example, FOXM1 is known to modify the G1/S and G2/M transitions and M-phase progression in the cell cycle (50). Similarly, FOXQ1 was able to promote epithelial differentiation and inhibit smooth muscle differentiation. However, with FOXQ1 overexpression, tumorigenesis is uncontrolled (51). Knockdown of FOXQ1 partially mimics miR-422a function, suppressing tumorigenesis in hepatocellular carcinoma (HCC) cells *in vitro*. In addition, tumor volume and weight in FOXQ1-knockdown mice were significantly increased. Thus, FOXQ1 serves a critical role in tumor initiation (33).

FOXQ1 may promote tumor cell proliferation, while the downregulation of FOXQ1 inhibits proliferation. Compared with the involvement of FOXQ1 in tumor initiation, studies have demonstrated that it may promote tumor cell proliferation by upregulating downstream genes encoding proliferation-associated proteins and downregulating apoptotic proteins.

Neurexin (NRXN) is a polymorphic neuronal-specific cell surface protein, which serves an important role in cell adhesion and recognition (52). It is encoded by three genes, NRXN1, NRXN2 and NRXN3, which together form two functional proteins; the long form, α-NRXN, and the short form, β-NRXN (52-54). It has been reported that a polymorphic site in the NRXN3 gene was significantly associated with a higher risk of developing breast cancer (55). Recently, Sun *et al* (56) observed that FOXQ1 is overexpressed in human glioma, and is negatively correlated with NRXN3 expression in clinical tissue. Additionally, FOXQ1 was observed to regulate the activity of NRXN3, which enhanced cell proliferation. To further understand the associated mechanism, three mutant versions of the FOXQ1-binding sites in NRXN3 were generated: A FOXQ1-binding site 1 mutation only, a FoxQ1-binding site 2 mutation only and both site 1 and site 2 mutations. These demonstrated that disruption of one or both of the FOXQ1 binding sites significantly inhibited the NRXN3 promoter. Consequently, NRXN3 is a downstream gene regulated by FOXQ1, which can promote cancer proliferation.

Table I. Genes targeted by FOXQ1 in human tumor progress.

Tumor type	Target gene	Refs.
Glioma	NRX3	(56)
Colorectal cancer	p21	(60)
HCC	ZEB2, VersicanV1	(74)
	SOX12	(87)
Breast cancer	TWIST1	(51)
	LAMA4	(69)

p21 is a cyclin-dependent kinase inhibitor, which functions as a regulator of cell cycle progression (57). A number of studies have demonstrated the negative correlation between polymorphisms in p21 and the susceptibility of certain malignant tumors (58,59). Recently, at the molecular level, a large body of literature suggests that p21 has negative roles in tumor growth using FOXQ1-overexpressing cells in which p21 is knocked down. In addition, p21 induction by FOXQ1 is p53 independent. By contrast, FOXQ1 overexpression did not affect p21 *in vivo*, and the pathogenesis of FOXQ1 in mediating p21 change to control tumor antiapoptosis requires further in-depth study (17,60).

Roles of FOXQ1 expression in tumor angiogenesis. Sustained angiogenesis is a key step in cancer development and involves the development of new blood vessels necessary for continued tumor growth and metastatic spread. Increasingly, studies have shown that angiogenic regulators and the activation of oncogenes are essential for the maintenance of an angiogenic phenotype that contributes to oncogenesis (61-63). Studies have shown that FOXQ1 is a protein that promotes tumor angiogenesis (42). Christensen *et al* (39) observed that knockdown of FOXQ1 expression suppressed the angiogenic capacity of SW480 colorectal cancer cells via the regulation of VEGF, which is an activator of angiogenesis that is secreted by tumor cells (64). Microarray analysis revealed that VEGF-A expression was upregulated 4.4-fold, suggesting the possibility of enhanced angiogenesis. RT-qPCR in SW480 cells and VEGF staining of tumor specimens confirmed this result. Despite this, the detailed mechanisms of FOXQ1-mediated angiogenesis, for example whether FOXQ1 protein is able to bind to the 3'UTR of the VEGF gene, requires further study.

Roles of FOXQ1 expression in tumor re-initiation. Tumor re-initiation, which assesses the capacity of human cancer cells to proliferate once implanted into a secondary host, is another characteristic of malignant tumors (65,66). Laminin α4, encoded by LAMA4, is a major component of the extracellular matrix, and has been implicated in cancer pathophysiology (67,68). In a recent study, FOXQ1 was observed to positively regulate the expression of LAMA4, which promoted the development of micrometastasis and tumor re-initiation *in vivo* (69).

Roles of FOXQ1 expression in tumor microenvironment and tumor promotion inflammation. Pathologists have long recognized that inflammation can supply bioactive molecules

to the tumor microenvironment, including growth factors that sustain proliferative signaling and survival factors that limit cell death, thereby promoting cell proliferation, EMT, invasion and metastasis (70-72). Of note, FOXQ1 alters the tumor microenvironment through regulating versican V1. Researchers confirmed that versican V1, which promoted the metastasis of HCC cells and promotes the recruitment of macrophages, is a direct transcriptional target of FOXQ1. Versican V1 overexpression regulated FOXQ1 and induced HCC cells to secrete chemokine ligand 2 (CCL2), which was able to increase tumor-associated macrophages, whereas inhibiting versican V1 can significantly inhibit FOXQ1 expression. In addition, RT-qPCR assay indicated that the levels of CCL2, TNF-α, IL-6 and IL-8, all of which are implicated in macrophage recruitment and inflammation, were significantly upregulated in versican V1 overexpressing HCC tissues compared with the control HCC tissues (73,74).

Roles of FOXQ1 expression in tumor EMT. The transition of epithelial cells to mesenchymal cells in EMT, is an indicator of tumor metastasis (75). Epithelial cells are surface barrier cells, while mesenchymal cells have scaffolding and anchoring functions, and roles in tissue repair and wound healing (76,77). In the EMT process regulated by FOXQ1, mesenchymal cell markers, such as E-cadherin (E-cad), increase while epithelial cell markers, such as vimentin (VIM), are reduced. EMT-associated protein expression in the non-small cell lung cancer cell lines, SPC-A-1-SCR and NCI-H1395-SCR, indicated that the levels of E-cad and mucoprotein increase, whilst VIM and calcium-binding protein S100A4 expression is reduced (78). Similar results were observed in studies of bladder, ovarian and breast cancer cells (79-81).

Furthermore, an increasing number of studies have reported two tumor-specific patterns of regulating the downstream genes of FOXQ1, which contribute to the EMT process in tumors. A chromatin immunoprecipitation assay in breast cancer cells indicated that FOXQ1 is able to bind to the promoter of E-cad directly (81,82), while FOXQ1 enhanced genes in other tumors indirectly, such as Twist-related protein 1 (TWIST1) in colon cancer cells and NRXN3 in glioma (17,56).

Roles of FOXQ1 expression in tumor invasion and metastasis. Tumor metastasis is a complex multistep process including the following steps: Invasion of primary tumor cells, new blood or lymphatic vessel formation, transportation of tumor cells via the blood or lymphatic vessels, and the re-initiation of tumor cells at the secondary site (83). Metastasis serves a crucial role in the morbidity and mortality of cancer. Although studies regarding the capability of invasion and metastasis have increased over the past decade, the molecular mechanisms which control these steps remain poorly understood (84,85).

As powerful new research tools and refined experimental methods have become available, studies have identified FOXQ1 as one of the critical regulators in tumor invasion and metastasis. Recent studies have suggested that FOXQ1 is overexpressed in human tumor tissues and is associated with the incidence of tumor metastasis and poor tumor TNM stage. Additionally, detailed experiments found that FOXQ1 could mediate all steps of tumor metastasis, from the initial EMT to the ultimate organotropic colonization. To be a critical regulator of tumor invasion and metastasis, studies have demonstrated that FOXQ1 promotes these steps by regulating the expression of downstream genes, such as zinc finger E-box binding homeobox (ZEB2), TWIST1 and Sry (sex determining region Y)-box 12 (SOX12) (74,86,87).

Table II. Expression of FOXQ1 and its clinical significance.

Tumor type	Expression of FOXQ1		TNM stage	Independent prognostic factor	Refs.
	Cell	Tumor tissue			
Breast cancer	Up				(97)
Colorectal cancer	Up	Up	Yes	No	(60)
Gastric cancer	Up	Up	Yes		(98)
Esophagus cancer	Up	Up			(86)
HCC	Up	Up	Yes	Yes	(99)
NSCLC	Up	Up	Yes	Yes	(100)
Pancreatic cancer	Up	Up	Yes	Yes	(101)
NPC	Up	Up	Yes		(32)
Bladder cancer	Up	Up			(79)
Glioma	Up	Up			(56)
Cervical cancer	Up				(40)
Ovarian cancer	Up				(47)

ZEB2, encoded by the ZEB2 gene, promotes tumor metastasis (88-90). Recently, it was identified that ZEB2 is essential for FOXQ1-mediated HCC metastasis, with FOXQ1 transactivating ZEB2 expression by directly binding to the ZEB2 promoter. Xia *et al* (74) reported that the downregulation of ZEB2 in HCC cells significantly reduced the capacity for FOXQ1-enhanced cell metastasis, whereas the upregulation of ZEB2 rescued the reduced metastatic abilities. Subsequently, a PCR array indicated that, when induced by FOXQ1 knockdown, the ZEB2 mRNA expression profiles of SMMC7721-FOXQ1 cells were upregulated 4.38-fold compared with the profiles of SMMC7721-control cells. Additionally, a FOXQ1-binding site was constructed in the ZEB2 promoter, which demonstrated that the FOXQ1 protein directly bound to it. However, in a similar experiment using colorectal cancer cells, opposing results were observed, with nearly all proteins associated with the Wnt signaling pathway unaltered, revealing that the underlying mechanisms remain unknown.

SOX12, at human chromosome 20p13, belongs to the SYR-related high mobility group box (SOX) family. SOX family proteins are critical for a number of physiological processes including the maintenance of stem cells and controlling terminal differentiation of a variety of cell types (91-93). SOX12 is enriched in human HCC tissues, in addition to the invasion and metastasis of HCC cells. Additionally, studies have indicated that SOX12 may transactivate TWIST1, which is able to directly bind to CDH1, which encodes E-cad, regulating cell migration (94). Fibroblast growth factor-binding protein 1 (FGFBP1) is a target of SOX12 and is secreted. Additionally, it has been shown to promote cancer growth, angiogenesis and metastasis (95,96).

5. Clinical significance of FOXQ1 expression

The identification of specific and sensitive biomarkers would improve the selection of tumor patients for individualized treatment. A number of *in vitro* and *in vivo* animal experiments have indicated a key role of FOXQ1 in the pathogenesis of various types of tumor. Additionally, studies have indicated that FOXQ1 is overexpressed in human tumor specimens and may be an indicator of poor prognosis (Table II). Therefore, FOXQ1 may have potential for the diagnosis and treatment of tumors.

6. FOXQ1 and tumor therapy

FOXQ1 is a transcription factor that is overexpressed in a number of different types of cancer cells (97). An increasing number of studies have indicated that the upregulation of FOXQ1 promotes resistance to chemotherapeutic drugs (42,51). However, the therapeutic potential of FOXQ1 inhibitors against tumor remains to be explored. The most efficient method is RNA interference, which has been widely investigated in chronic infectious arthritis, obesity and cancer (98,99). Studies have demonstrated that the downregulation of FOXM1 expression by small interfering RNA diminished the proliferation and metastasis of numerous cells (17,69,97,100). Following transfection of a short hairpin RNA (shRNA), eukaryotic expression vector (FOXQl-shRNA) targeting the human FOXQl gene, EMT of tumors reversed. Another compound is benzyl isothiocyanate (BITC) treatment which, prior to a carcinogen challenge, inhibited polycyclic aromatic hydrocarbon-induced mammary cancer in human (101). Anuradha *et al* (81) reported that the suppression of FOXQ1, at least in part, contributed to the BITC-mediated inhibition of cell migration.

7. Conclusion

FOXQ1 is closely associated with the occurrence of tumors, such as breast, non-small cell lung, colorectal and pancreatic cancer. Each step of tumorigenesis regulated by FOXQ1, including cell proliferation, invasion and apoptosis, has attracted significant research attention. Its function as a point of crosstalk between typical signaling pathways, including Wnt/β-catenin, TGF-β/SMAD, Hedgehog and Notch, will provide valuable insight into the mechanisms of FOXQ1 in tumor pathophysiology. FOXQ1 is predicted to become a key marker of cancer diagnosis and therapy.

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