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Review

The expression of FOXP3 and its role in human cancers

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ABSTRACT

FOXP3 is a transcription factor, which belongs to the family of FOX protein. FOXP3 was initially discovered in regulatory T cells and supposed to play a significant role in the process of regulatory T cell differentiation. Increasing evidence has shown that FOXP3 is also expressed in tumor cells. However, the results of tumor FOXP3 is inconsistent and even the opposite. In some types of human cancers, the expression of FOXP3 is upregulated, and it can promote the development of cancers, leading to a poor prognosis. While in some other types of cancers, it is a different story. The reason for the contradictory data is unknown. The discovery of FOXP3 isoforms, interaction between tumor cells and lymphocytes in the tumor microenvironment, subcellular location, and mutation of FOXP3 may provide some clues. In this review, we first summarize and analyze the recent development. The final section focuses on the regulation of FOXP3 expression.

1. Introduction

FOXP3 is a transcription factor which belongs to the family of forkhead box (FOX) protein. FOX protein was first identified in the Drosophila embryo by Weigel and Jackle and found to be localized in the nucleus to regulate gene expression [1]. All the family members have a forkhead (FKH) domain which acts as a transcriptional activator or repressor [2–4]. FKH domain of FOX can regulate the transcription of approximately 700 genes [5].

FOXP3 was first found to be a master regulator in the development and function of regulatory T cells (Tregs). Sakaguchi et al. found that a subset of CD4+ T cells with the potent suppressive activity could express CD25 and termed these CD4+ CD25+ cells as Tregs [6,7]. Later, they discovered that these cells expressed high levels of FOXP3 and that CD4+CD25− T cells could transform to Tregs phenotype after the forced expression of FOXP3 [8]. Tregs could be found in various tumors, including breast, lung, liver, pancreatic cancer, gastrointestinal cancer and melanoma [9]. Furthermore, Tregs could suppress many other antitumor immune cell subsets including NK cells, macrophages, dendritic cells, and B cells [10]. By producing immunosuppressive factors, suppressing cell-cell contact and cytolysis, Tregs could promote tumor growth. As a result, the depletion of FOXP3+Tregs could restore normal immunity and trigger the anticancer effect.

Recently, increasing evidence has shown that FOXP3 is also expressed in tumor cells. After the expression of FOXP3 was reported in pancreatic carcinoma cells [11], studies have shown the expression of FOXP3 in other tumors, including breast cancer, prostate cancer, lung cancer, gastric cancer, thyroid cancer and melanoma cells [12–16], which suggest that FOXP3 may play a broader role in tumorigenesis. However, the functional role of tumor FOXP3 is inconsistent and even the opposite. In some types of human cancers, the expression of FOXP3 is upregulated, and it can promote the development of cancers, leading to a poor prognosis. While in some other types of cancers, it is a different story. In this review, we will underscore the expression of tumor FOXP3 and its role in human cancers.

2. FOXP3 gene and protein

FOXP3 gene locate at the p arm of the X chromosome at Xp.11.23, which has 11 coding exons (E2–E12) and 1 non-coding exon (E1). There is still controversy over the number of FOXP3 exons. If non-coding exon E1 is excluded, the number of exons in FOXP3 should be 11 [17,18]. Full-length of human FOXP3 protein has 431 amino acids with a molecular weight of about 47.25 kDa [19]. Four main domains with different functions make up the FOXP3 protein. From the N terminus, it contains a repress domain, a zinc-finger, a leucine zipper motif, and finally the FKH domain (Fig. 1).

The proline-rich repressor domain serves a suppressive effect on the

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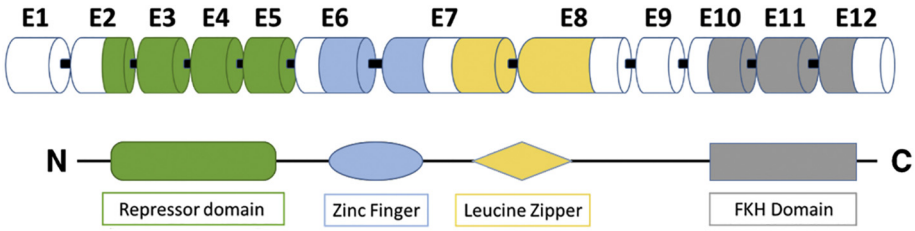

Fig. 1. Schematic representation of the structure of FOXP3. Full-length FOXP3 consists of 12 exons, 11 of which encode for the protein. Four main domains with the different functions make up FOXP3 protein.

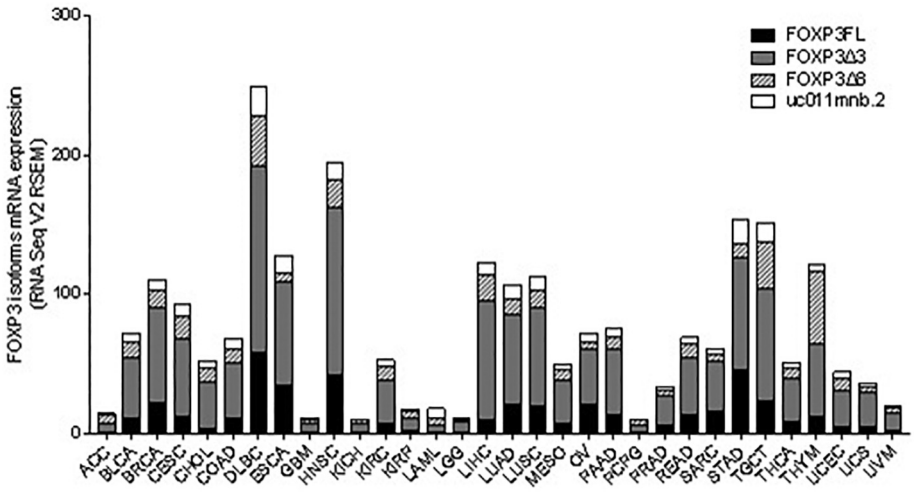

Fig. 2. Expression of FOXP3 isoforms in cancers. The FOXP3Δ3 is the most frequent FOXP3 isoform detected in almost all types of cancers according to TCGA databases. Distribution of the expression of transcript isoforms encoded by the FOXP3 gene analyzed using the RSEM method for 32 cancer types from TCGA databases. Details for tumor types, case number, abbreviations used and the definition of the isoforms are described in Supplementary Tables 1 and 2.

expression of target genes. The zinc-finger domain locates centrally in the protein, and no mutations have been identified in the zinc-finger domain, suggesting that it may not play a vital role in FOXP3 function. To date, the function of the zinc-finger domain has not been elucidated clearly. The leucine zipper is notable for enabling FOXP3 homo- or heterodimerization. It has been found that murine FOX members can interact with each other. A mutation within the leucine zipper can result in an autoimmunity problem for the reason of preventing this interaction [20], which points out that the leucine zipper region is critical for homo- and heterodimerization. Besides, the leucine zipper is required for proper function of Tregs [19]. The FOX transcription factors that are characterized by a highly conserved FKH DNA-binding domain which is depicted as a winged helix. The FKH domain could act as a transcriptional activator or repressor [2–4], which can regulate the transcription of approximately 700 genes [5]. Moreover, it was also found that both FOXP3 translocating to the nucleus and its repressor functions are dependent on the presence of the FKH domain [21].

3. FOXP3 isoforms

Unlike mice, humans express multiple isoforms of FOXP3 [22]. To date, there are five isoforms of FOXP3 undrafted which are named FOXP3FL, FOXP3Δ3 [23], FOXP3Δ3Δ4 [16,24,25] FOXP3Δ8 [26], and FOXP3Δ3Δ8 [27] in human. Several reports refer to exon 3 as exon 2, because E1 non-coding exon is excluded as described above.

FOXP3Δ3, missing exon 3 which localizes in the repressor domain, was expressed at relatively similar ratios to FOXP3FL in Tregs [28]. Aarts-Riemens et al. investigated the cellular distribution and functional properties of FOXP3FL and FOXP3Δ3 isoforms in T cells. The study indicated that both FOXP3FL and FOXP3Δ3 were preferentially expressed in CD4 + CD25 + cells, probably in a simultaneous but independent fashion and demonstrate that the ectopic expression of both FOXP3 isoforms is capable of converting conventional CD4 + CD25– cells into Tregs [29]. Allan SE et al. also reported the two most abundant expressed FOXP3 isoforms could confer a suppressive ability to Tregs [23]. In contrast, FOXP3Δ3Δ8 lacking both exons 3 and 8 is unable to confer a suppressive ability to Tregs in mice [30]. FOXP3Δ8 lacks exon 8 that constitutes a part of a leucine zipper domain. Compared with abundant expression of FOXP3FL and FOXP3Δ3, FOXP3Δ8 is present at a low proportion of the total FOXP3 expression in Tregs [26]. FOXP3Δ3Δ4 missing both exons 3 and 4 was identified in tumor cell lines but was absent from Tregs [16,24,25].

Zhang H et al. reported that the FOXP3Δ3 was predominantly expressed in bladder cancer compared with FOXP3FL and FOXP3Δ8, which can promote larger and more aggressive tumors *in vivo* as well as chemotherapy resistance [18]. Shi JY et al. demonstrated that FOXP3Δ3Δ4 were detected in 13.3% (2/15 cases) of laser-captured microdissection of primary hepatocellular carcinoma (HCC) cells. FOXP3FL was found to inhibit the proliferation, migration, and invasion of HCC cells *in vitro* as well as reduce tumor growth *in vivo*, whereas FOXP3Δ3Δ4 showed a significant reduction in the tumor suppression function [25]. However, the expression and role of FOXP3 isoforms in cancers are still largely unknown and few studies have addressed their roles in human cancers.

To examine the expression of FOXP3 isoforms, we analyzed TCGA RNA-seq data from 9171 primary tumor tissues across 32 tumor types. Four FOXP3 isoforms including FOXP3FL, FOXP3Δ3, FOXP3Δ8, and an uncharacterized isoform (uc011mnb.2) were identified in the different types of tumors. FOXP3Δ3 is preferentially expressed in most cancer types excluding acute myeloid leukemia (Fig. 2). Current studies on FOXP3 isoforms in cancer appear to focus on FOXP3Δ3 mainly, since its predominant expression and missing exon 3 that is localized in the repressor domain. Our lab has recently investigated the role of FOXP3FL and FOXP3Δ3 in non-small cell lung cancer (NSCLC) and hepatocellular carcinoma (unpublished data). The result showed that tumor cells with FOXP3Δ3 were much more oncogenic than those without, though the ectopic expression of either FOXP3FL or FOXP3Δ3 could promote the proliferation, migration, and invasion of NSCLC cells *in vitro*. In contrast, in HCC FOXP3FL appears to play a suppressive role while FOXP3Δ3 seems to be less inhibitory than FOXP3FL.

Alternative splicing occurs in most human genes [31]. In this process, specific exons of a gene may be included or excluded in the final

processing of mRNA to produce a variety of different transcript isoforms, bringing enormous diversity to proteins, which has emerged as a vital mechanism in tumorigenesis by regulating the function of cancer-related genes [32]. In this regard, human FOXP3 is a good example. FOXP3Δ3 missing exon 3, which localizes in the repressor domain, may have an ineffective repression ability. Du J et al. demonstrated that FOXP3FL, but not FOXP3Δ3, could interact with and inhibit retinoic acid receptor-related orphan receptors (ROR)α because the region of FOXP3 involved in the interaction is encoded by exon 3 [28]. It is currently unknown whether this exon 3 can interact with other genes in the similar fashion. Nevertheless, the replacement of FOXP3FL with FOXP3Δ3 may fail to repress some target genes. If these target genes happen to be oncogenes, the increase of FOXP3Δ3 may favor cancer development or/and growth. Whereas, if these target genes are tumor suppressors, the appearance of this particular isoform will likely facilitate the tumor arrest. Furthermore, FOXP3Δ3 may have a more powerful transcriptional ability than FOXP3FL as FOXP3 fragment lacking the 198 N-terminal amino acids can significantly increase the transcriptional activity [19]. This feature may also enable FOXP3Δ3 to have differential effects on cancers depending on the target genes. Further investigation into the regulation of FOXP3 isoform expression and their downstream transcriptome will help to identify the role of FOXP3 in tumorigenesis and cancer treatments.

4. Expression of FOXP3 in cancer cells

It was previously believed that the expression of FOXP3 was restricted to T cells. As a result, the detection of the FOXP3 expression in surgical tumor samples was used as an indicator of tumors infiltrated by Tregs [33]. Recently, increasing evidence has shown that FOXP3 is also expressed in tumor cells.

Following the finding that FOXP3 can be expressed in pancreatic carcinoma cells [11] and normal breast epithelium [24], Karanikas et al. examined the expression of FOXP3 in 25 different cancer cell lines including lung cancer, colon cancer, breast cancer, melanoma, erythroid leukemia, and acute T-cell leukemia [34]. The expression of FOXP3 mRNA in all tumor cell lines examined was significantly higher than that in fibroblasts and EBV-transformed B cells. Among all the cell lines tested, MCF7 breast cancer cells expressed the highest level of FOXP3 mRNA, and at least half of these tested cancer cells were able to express FOXP3 at the similar level to the Treg clone which was used as a positive control. Ebert et al. have also reported the FOXP3 expression in colon cancer, lung cancer, breast cancer, prostate cancer, renal cell carcinoma, bladder cancer, glioblastoma cell lines [16]. Although it is now known that cancer cells can express FOXP3, the function of FOXP3 in cancer cells remains controversial because both inhibition and promotion have been reported.

5. Role of FOXP3 as a suppressor gene based on *in vitro* models

Table 1 summarized the role of FOXP3 as a tumor suppressor based on *in vitro* models. Zuo et al. found that FOXP3 could bind and repress the promoters of HER2 and SKP2 in the mouse model of breast cancer and human breast cancer cells [3,24]. HER2 is a well-known oncogene to promote cancer cell growth and survival while SKP2 is required for some cancer development [35]. Both HER2 and SKP2 are overexpressed in several types of human cancers and associated with the poor prognosis. Therefore, the finding of the repression of HER2 and SKP2 by FOXP3 may support a tumor inhibitory role of FOXP3 in certain types of cancers such as breast cancer.

In the same *in vitro* model, FOXP3 was found to be critical for the expression of p21(encoded by CDKN1A) and LATS2, both of which are tumor suppressor genes. By inhibiting the binding of HDAC2 and HDAC4 to the CDKN1A promoter, FOXP3 induced the expression of CDKN1A, which acted to arrest the cell cycle [2]. Furthermore, FOXP3 can bind to the promoter of LATS2 to upregulate its expression, which leads to the degradation of YAP [4], a molecule in the Hippo pathway to regulate cell proliferation and apoptosis [40]. Douglass et al. observed that the stable overexpression of FOXP3 could decrease the expression of CXCR4 [37]. Knocking down of FOXP3 in normal breast epithelial cells significantly increased the expression of CXCR4 and led to a significant enhancement of chemotactic response towards CXCL12, consistent with the suppressive role of FOXP3 in the regulation of cell migration.

Therefore, the above data suggest that FOXP3 may act on multiple molecules to inhibit the growth of breast cancer cells. A similar situation has also been demonstrated in some other types of human cancers. For example, in prostate epithelia, Wang et al. found that FOXP3 could repress the transcription of c-MYC, which was often overexpressed in prostate cancer and was influential in inhibiting cell cycle progression and apoptosis [2]. Zhang and Sun found that ectopic expression of FOXP3 in SKOV3 ovarian cancer cells could inhibit cell proliferation, migration, and invasion by downregulating the expression of MMP2 and urokinase-type plasminogen activator (uPA) [38]. Li et al. found that FOXP3 knockdown could stimulate DNA repairing. CHIP-Seq results revealed that BRCA1 and 12 others were direct targets under the transcriptional control of FOXP3 among 48 FOXP3-regulated DNA repairing genes [36]. Ma et al. examined the expression of FOXP3 in human gastric cancer cell lines, and found that the overexpression of FOXP3 could promote cell apoptosis and inhibit cell proliferation by upregulating pro-apoptotic molecules, including PARP, caspase-3, and caspase-9, and downregulating anti-apoptotic molecules, including c-IAP1 and Bcl-2 [39]. In hepatocellular carcinoma, Shi YJ et al. documented that FOXP3 could suppress tumor progression *via* TGF-β/Smad2/3 signaling pathway [25]. In summary, FOXP3 can exert its anti-tumor function in a variety of human cancers by regulating different molecules.

Table 1
FOXP3 acts as a tumor suppressor.

Cancer type	FOXP3 target	FOXP3-mediated biological effects	References
Breast cancer	SKP2 repression	Growth inhibition	Zuo et al. [3]
Breast cancer	HER2 repression	Growth inhibition	Zuo et al. [24]
Breast cancer	p21 induction	Growth inhibition	Liu et al. [2]
Breast cancer	LATS2 induction	Growth inhibition	Li et al. [4]
Breast and colon cancer	BRCA1 repression	Preventing repair of DNA Damage (chemotherapy and apoptosis)	Li et al. [36]
Breast cancer	CXCR4 repression	Migration inhibition	Douglass et al. [37]
Ovary cancer	MMP2 and uPA repression	Growth, migration, and invasion inhibition	Zhang and Sun [38]
Gastric cancer	PARP, caspase-3 and caspase-9 induction; c-IAP1 and Bcl-2 repression;	Growth inhibition, apoptosis induction	Ma et al. [39]
Hepatocellular carcinoma	Repressing TGF-β/Smad2/3 signaling pathway	Growth, migration, and invasion inhibition	Shi JY et al. [25]
Prostate	c-MYC induction	Growth inhibition, apoptosis induction	Wang L et al. [2]

Table 2
FOXP3 acts as an oncogene.

Cancer type	FOXP3 target	FOXP3-mediated biological effects	References
Cervical cancer	P16 induction	Promote growth, migration, and invasion	Luo et al. [35]
Lung adenocarcinoma	cyclin D1 induction	Promote growth, migration, and invasion	Li et al. [41]
NSCLC	Activating Wnt/β-catenin signaling pathway and EMT	Promote growth, migration, and invasion	Yang S et al. [13]
Thyroid	PPARγ and caspase-3 regression; NF-κB subunit p65 and cyclin D1 induction	Promote growth, migration, and apoptosis	Chu et al. [15]

6. Role of FOXP3 as an oncogene based on *in vitro* models

Recently, some reports have shown that FOXP3 might function as an oncogene in various tumors. Table 2 summarized FOXP3 as an oncogene in *in vitro* models. Luo et al. proved that FOXP3 could promote cervical cancer cell proliferation, invasiveness, and inhibit apoptosis, leading to the development, progression, and metastasis of cervical cancer [35]. They silenced FOXP3 expression in human cervical cancer cells and discovered that it could downregulate the expression of p16INK4a. Li et al. found that silencing FOXP3 inhibited cell proliferation, migration, invasion, and secretion of inhibitory cytokines including TGF-β1, IL-35, and HMOX1, suggesting that FOXP3 functions positively in tumor development. Moreover, they also found that FOXP3 could enhance lung adenocarcinoma cell proliferation *via* upregulating the levels of CCND1, a gene of cell cycle G1/S checkpoint [41]. One study in thyroid cancer and NSCLC demonstrated that FOXP3 knockdown could inhibit the cell proliferation, migration, and increase apoptosis, suggesting that FOXP3 was an oncogene in thyroid cancer growth [15]. Besides, silencing FOXP3 could upregulate PPARγ and caspase-3 expression, but downregulate NF-κB subunit p65 and cyclin D1 [15]. Recently, the study in NSCLC showed that the expression of FOXP3 is oncogenic and FOXP3 could activate the canonical Wnt signaling pathway to promote cell proliferation, invasion, and EMT induction by physically interacting with β-catenin and TCF4 [13]. These studies have indicated that FOXP3 can work to facilitate the survival and growth of human cervical cancer, lung cancer, and thyroid cancer.

7. FOXP3 regulates genes expression in tumorigenesis

It can be certain that FOXP3 can be expressed in tumor cells and plays a broader role in tumorigenesis by regulating the expression of numerous genes. There was a significant correlation observed between the low expression of FOXP3 and the overexpression of HER-2, SKP2 and c-MYC in breast and prostate cancer cells [2,3,24].

7.1. SKP2

SKP2 gene encodes an essential protein component of the SKP1-Cul1-Fbox ligases complex, which is responsible for ubiquitination and consecutive degradation of its substrate proteins [42]. SKP2 overexpression is frequently observed in many human cancers [43] and plays a key role in tumorigeneses, such as cell cycle regulation, cell differentiation, cell proliferation, metastasis, and apoptosis [44]. The most well-known role of SKP2 is a positive regulator of cell cycle progression by targeting the cell cycle inhibitor CDKN1B (p27). Increased levels of SKP2 and reduced levels of p27 are observed in many types of cancer [44], and inhibition of SKP2 functions is emerging as a promising and novel anti-cancer strategy [43].

Zou et al. investigated the relationship between FOXP3 and SKP2 in breast cancer [3]. The analysis of 206 clinical samples revealed that the expression of FOXP3 was negatively correlated with SKP2 expression [3]. The study also showed that MCF-7 cells with inducible FOXP3 expression had more than 8-fold reduction of SKP2 transcripts [3]. Besides, the study also demonstrated that FOXP3 could significantly repress the promoter activity of SKP2. Moreover, ChIP assay revealed that FOXP3 could bind to specific regions of SKP2 [3].

7.2. HER2/ErbB2

The HER2 oncogene is a member of the erbB-like oncogene family encoding transmembrane receptors for growth factors [45]. Overexpression of HER2 can transform normal cells into a malignant phenotype and accelerate carcinogenesis, and amplification of the HER2 gene is a significant predictor of the worse prognosis [45].

FOXP3 could bind and repress the promoters of HER2/ErbB2 acting as a transcriptional repressor [24]. ErbB2, the murine homolog of HER2, is overexpressed in mouse breast cancer TSA cell line. Transfection of FOXP3 cDNA significantly repressed ErbB2 levels on the TSA cell line, which suggests that FOXP3 is a repressor of the ErbB2. Furthermore, it has been verified that the FKH domain of FOXP3 can bind to motifs in the ErbB2 promoter [24]. In human breast cancer, the down-regulation of the FOXP3 gene were commonly found in cancer samples and cell lines, which was significantly correlated with HER2 over-expression [24]. When FOXP3 gene was silenced using siRNA in human breast epithelial cells, the expression of FOXP3 was reduced by more than 100-fold while the expression of HER2 was increased by 7-fold, suggesting that FOXP3 is a repressor of HER2 in human breast epithelial cells [24].

7.3. c-MYC

c-MYC is considered one of the proto-oncogenes based on the fact that alterations in its structure and expression can contribute to the genesis of cancers [46]. Wang et al. documented that FOXP3 could transcriptionally repress c-MYC in prostate cancer [2]. A significant inverse correlation was observed between the expression of FOXP3 and c-MYC in prostate cancer samples [30]. Knockdown of the FOXP3 expression elevated the level of c-MYC transcripts and protein in prostate epithelial cell lines [2]. Besides, FOXP3 transfection almost abrogated the expression of c-MYC in prostate cancer cell lines [2].

7.4. Summary

Obviously, FOXP3 can regulate the expression of numerous genes that are directly or indirectly related to tumorigenesis. Some other well-known tumor-related genes regulated by FOXP3 such as the LAST2, p21 (encoded by CDKN1A) and BRCA have been described in previous sections. As the FOXP3 can transcriptionally repress oncogenes and increase the expression of tumor suppressor genes, it can be concluded that FOXP3 functions as a suppressor gene in certain types of cancers. However, in some other types of cancers, FOXP3 appears to be is oncogenic. In this aspect, NSCLC is a good example. FOXP3 can activate the canonical Wnt signaling pathway to promote cell proliferation, invasion, and EMT induction by physically interacting with β-catenin and TCF4 [13]. The HER2 signaling pathway was activated, and the expression of c-MYC was upregulated in FOXP3-overexpression NSCLC cells [13]. The reason for FOXP3 having inverse functions in human cancers is currently unknown though it seems to be dependent on the type of cancers.

8. Correlation between FOXP3 expression and prognosis

Emerging evidence from studies of FOXP3 in human cancer samples

Table 3
FOXP3 expression and prognostic values in human cancers.

Cancer type	NO. of Pos./Tot. (%)	FOXP3 localization	Prognosis (OS or RFS)	Independent prognostic factor	References
Breast cancer	261/397 (66%)	C and N	Poor (OS/DMFS)	Independent	Merlo et al. [47]
Breast cancer	59/103 (57%)	C	Good (RFS and OS)	Independent	Ladoire et al. [59]
Breast cancer	35/58 (60.3%)	C and N	Good RFS (Online databases)	N.A	Li X et al. [63]
Cervical cancer	111/148 (75%)	C and N	N.A.	N.A.	Luo et al. [35]
Esophageal cancer	80/112 (71%)	C and N	N.A.	N.A.	Xue et al. [54]
Gastric cancer	71/122 (58)	C and N	No prognostic	N.A.	Wang et al. [53]
Gastric cancer	91/197 (46.2%)	C	Good (OS)	N.A.	Ma et al. [58]
Gastric cancer	74/117 (63.2%)	C and N	Good (RFS and OS)	N.A.	Suh et al. [40]
Hepatocellular carcinoma	115/240 (47.9%)	C	Good (OS)	Independent	Shi JY et al. [25]
Urinary bladder cancer	17/37 (46%)	C and N	Poor (OS)	N.A.	Winerdal et al. [55]
Head and neck cancer	43/76 (56.6%), 42/80 (52.5%)	N.A.	Poor(OS)	N.A.	Weller et al. [49]
Lung adenocarcinoma	16/40 (40%)	C and N	N.A.	N.A.	Li et al. [41]
Melanoma	5/15 (33%)	C	N.A.	N.A.	Quaglino et al. [56]
Melanoma	18/146 (12%)	N	N.A.	N.A.	Tan et al. [57]
NSCLC	22/44 (50%)	N	N.A.	N.A.	Dimitrakopoulos et al. [52]
NSCLC	27/87 (31%)	C	Good OS and RFS (must combine with Treg counts)	N.A.	Tao et al. [60]
NSCLC	29/53 (54.7%)	C and N	N.A.	N.A.	Fu et al. [51]
NSCLC	41/106 (38.7%)	C and N	Poor (RFS and OS)	Independent	Yang S et al. [13]
Ovary cancer	0/27 (0)	N.A.	N.A.	N.A.	Zhang and Sun [38]
Prostate cancer	29/92 (31%)	N	N.A.	N.A.	Wang et al. [2]
Tongue cancer	48/81 (59%)	C and N	Poor (OS)	Independent	Liang et al. [50]

Pos., number of seropositive specimens; Tot., number of specimens; C, cytoplasm; N, nucleus; N.A., not available; RFS, recurrence-free survival; OS, overall survival.

suggests that FOXP3 may play a pivotal role in cancer prognosis (Table 3). Most of these results have indicated that the expression of FOXP3 is associated with metastasis, shorter survival or poor outcomes. In human breast cancer, Merlo et al. found positive FOXP3 expression in 66% of the patient samples [47]. It was an independent and strong prognostic factor for distant metastasis, but not for local recurrence risk. Moreover, multivariate analysis results showed a similar hazard ratio (HR) for FOXP3 expression and lymph node positivity. Similarly, a significant correlation between FOXP3 expression and lymph node metastasis has been observed in breast cancer [48]. In another female-related cancer, cervical cancer, the expression of FOXP3 was strong in the cytoplasm and nucleus as well as in cancer interstitium, weak in cervical intraepithelial neoplasia (CIN), but was not observed in normal cervical epithelium, suggesting that FOXP3 may not participate in the initiation of this malignancy but may facilitate its growth [35]. The finding that the expression of FOXP3 in metastatic lymph nodes was significantly higher than that in normal lymph nodes may indicate the correlation of FOXP3 with tumor metastasis [35].

In head and neck cancer, Weller et al. investigated the role of FOXP3 in the larynx and orohypopharynx squamous cell carcinoma (LSCC and OHSCC) [49]. They found that the higher FOXP3 expression was significantly associated with the poorer survival in OHSCC but not in LSCC patients. However, they observed that the combination of FOXP3 and AHNAK (in LSCC) or FOXP3 and CORTACTIN (in OHSCC) had significantly stronger prognostic values than either marker analyzed individually. Also, the combination of FOXP3 and cyclooxygenase-2 could enhance the accuracy of prognosis in OHSCC [49]. These results suggest that the prognostic value of FOXP3 needs to be assessed with other biomarkers and that FOXP3 alone may not be a useful prognostic marker in head and neck cancers. However, in tongue squamous cell carcinoma, another type of the head and neck cancer, the expression of tumoral FOXP3, which was detected in 59% of patients, was an independent negative prognostic factor for patient survival, and was related to pathological differentiation and T stage but not associated with local recurrence [50]. In thyroid cancer, the inhibition of FOXP3 has been demonstrated to induce apoptosis *via* downregulating NF-kB subunit p65 but upregulating peroxisome proliferator-activated receptor gamma [15].

In NSCLC, the higher tumoral FOXP3 was, the lower the overall survival and recurrence-free survival occurred. In line with the finding of an association between FOXP3 and poorer outcome, the expression of FOXP3 was increased in cancerous tissues, compared with normal lung tissues [41,51] and the stronger nuclear FOXP3 staining was correlated with lymph node metastases or/and TNM staining [51,52]. Moreover, there was a significant positive co-relationship between FOXP3 and TLR4 expression [51]. The positive correlation between FOXP3 and TLR4 may not be surprising, as TLR4 is known to be associated with HIF1A [51] and HIF1A can bind to the promoter of FOXP3 to stimulate its expression. These findings are consistent with the result that FOXP3 may work to induce epithelial-mesenchymal transition (EMT) *via* stimulating Wnt-b-catenin signaling pathway [13].

In cancers of the digestive system, Wang et al. found that FOXP3 was detected in the nucleus or cytoplasm of some gastric cancer cells, and in the nucleus of adjacent normal epithelial cells [53]. The frequency of FOXP3-positive cancer cells was increased in primary tumors (58.2%) compared with chronic gastritis patient samples (26.7%) and was related to lymph node metastases. Same as the FOXP3 in gastric cancer, a correlation between FOXP3 expression and lymph node metastasis has been reported in esophageal squamous carcinoma [54]. And the higher expression of FOXP3 was discovered not only in tumors compared with that in normal mucosa, but also in Stage IIB and III compared with that in Stage I and IIA [54].

In other cancers such as melanoma and urinary bladder tumor, the expression of FOXP3 was also associated with metastasis or/and poorer survival [16,55]. However, it is strange when the expression of FOXP3 was classified into cellular fractions (cytoplasm and nucleus), there was no significant association between FOXP3 and patient survival in the urinary bladder tumor [55]. The increase of the sample number may solve the odd findings. Though in melanoma, FOXP3 expression was positive in melanoma and negative in normal melanocytes [16,56], Tan et al. reported that the frequency of FOXP3 positive cells ranged between 0.03% and 0.75% only [57].

On the contrary, some studies suggest that FOXP3 is not significant as a prognostic marker or even show that that the level of FOXP3 is related to longer survival or better outcomes. These contradictory results were reported not only in different types of cancers but even in the same type of cancers. For example, in colorectal cancer, Sun et al. observed that the high expression of FOXP3 was associated with longer

overall and disease-free survival [58]. And *in vivo* tests showed that the overexpression of FOXP3 inhibited tumor growth. Suh et al. found FOXP3 expression was correlated with smaller tumor size, lower T stage, lower histological grade, lower recurrence, tubular tumor type, less lymph node invasion, and neural invasion [40]. Therefore, the expression of FOXP3 is significantly related to better disease-free and overall survival. Similarly, one study showed that FOXP3 expression in cell cytoplasm was associated with better relapse-free and overall survival in HER2-overexpressing breast carcinoma [59]. In NSCLC, a study by Tao et al. showed that the cytoplasmic expression of FOXP3 was not associated with either lymph node positivity or overall survival, though the expression of FOXP3 was found in 31% of patient samples [60].

The reason for these visible contradictory results is unclear. However, several factors may need to be considered. Firstly, FOXP3 isoforms have been identified [30,61,62]. These isoforms may function differently in cancer cells, and their expression levels are varied. Secondly, FOXP3 is a transcription factor, and it requires to be localized in the nucleus to exert its function [19]. Recently, one study showed that nuclear FOXP3 expression was inversely correlated with clinical stage, lymph nodes metastasis and HER2 expression [63]. Therefore, the subcellular localization of FOXP3 may contribute to different results. As summarized in Table 3, A nuclear and predominant cytoplasmic of FOXP3 localization were reported in cancer cells. The reason for the variable localized expression remains unclear. Chen et al. reported that the T cell receptor (TCR) signaling-mediated post-translational modifications could induce a shift in the subcellular localization of FOXP3 from a predominant cytoplasmic expression to a dense nuclear expression [64]. This suggests that post-translational modifications may also be involved in tumor cell development and that the subcellular localization of FOXP3 in tumor cells may enable this molecule to have different roles. Thirdly, the interaction between tumor cells and lymphocytes in the tumor microenvironment should be considered. Won KY et al. demonstrated that FOXP3 expression in breast cancer was positively correlated with infiltrated Tregs which contribute to tumor immune eacape [65]. Ma GF et al. reported that the high expression of FOXP3 in gastric cancer correlates with a good prognosis, whereas high-density Tregs predict a poor prognosis [14]. Besides, the tumorigenesis of FOXP3 can be varied due to inflammation environment. Compared with the proliferation suppression observed in basal condition, FOXP3 is ineffective to suppress the cell proliferation under TNFα treatment [66]. Finally, FOXP3 mutations have been reported [67,68]. The mutated FOXP3 may change its location as well as its function. Thus, if the mutated FOXP3 is detected, the outcome or/and significance is very likely different from the non-mutated one.

9. The regulation of FOXP3 expression

9.1. PI3K/AKT/mTOR/FOXP3 pathway

Up to now, there are only a small number of reports on the regulation of FOXP3 expression. According to the study by Merkenschlager and von Boehmer, FOXP3 could be induced by signals from the T cell receptor (TCR) and interleukin-2 (IL-2) with the presence of transforming growth factor TGF-β [69]. These signals are integrated by the network involving the PI3K/AKT/mTOR signaling pathway. It appears that the upregulation of FOXP3 requires the activation of PI3K induced by some proteins such as Cbl-b and PTEN [70,71]. On the other hand, the constitutive Akt could downregulate FOXP3 expression [70]. Since mTOR can activate Akt by phosphorylation of Ser473 [71], the loss or inhibition of mTOR may promote FOXP3 induction *via* inactivating Akt (Fig. 3).

9.2. EGFR/GSK-3β/FOXP3 pathway

Wang S. et al. reported that AREG/EGFR signaling downregulated GSK-3β protein activity, inducing the loss of FOXP3 protein

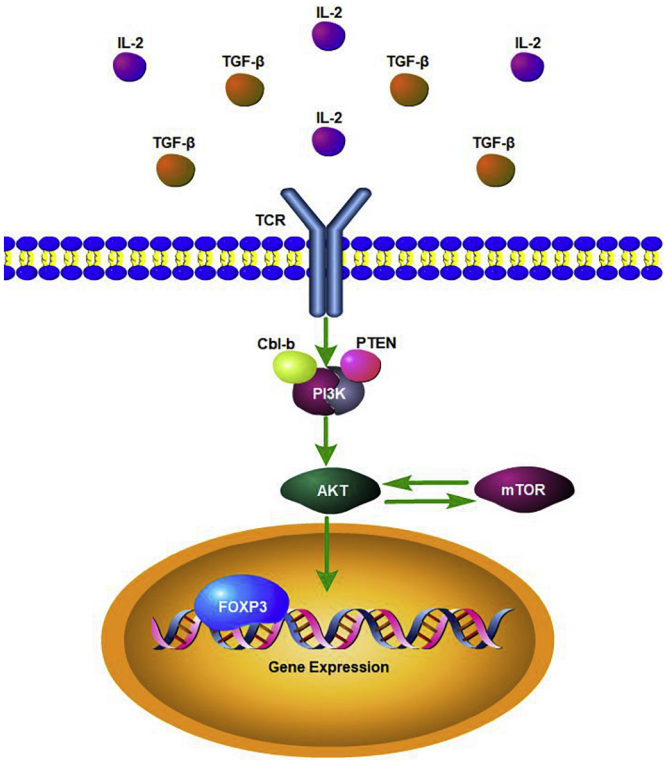

Fig. 3. The regulation of FOXP3 expression by PI3K/AKT/mTOR/FOXP3 pathway. PI3K/AKT/mTOR signaling pathway is involved in the expression of FOXP3 induced by the T cell receptor (TCR) in the presence of transforming growth factor (TGF)-β and interleukin-2 (IL-2).

phosphorylation and the suppression of Treg activity [72]. Therefore, it is suggested that the expression of FOXP3 can be regulated by EGFR and GSK-3β.

9.3. Hypoxia-inducible factors

Hypoxia-inducible factors, including hypoxia-inducible factor 1A (HIF1A) and hypoxia-inducible factor 2A (HIF2A), are transcription factors that are responsible for the induction of genes associated with cell survival under hypoxia [73]. The overexpression of HIF1A or HIF2A is associated with several types of solid cancers [74–76]. According to the study of Clambey et al. HIF1A could directly bind to the FOXP3 promoter, and then with the cooperation of TGF-β, upregulate the expression of FOXP3 in the cutaneous T cells [74]. However, in the inducible Tregs, HIF1A induced by IL-1b and mTOR were shown to reduce rather than enhancing the level of FOXP3, resulting in the decrease of the frequency of $FOXP3^+$ T cells of peripheral blood [77]. Moreover, in mycosis fungoides-cutaneous T-cell lymphoma (MF), the inhibition of HIF1A degradation increased the percentage of $FOXP3^+$ T cells in skin lesions, suggesting an inhibitory role of HIF1A in the regulation of FOXP3, since the level of HIF1A expression was determined by the balance between the rate of HIF1A protein synthesis and its degradation [78]. Thus, it seems that the regulatory role of HIF1A on FOXP3 can be entirely different in T cells of the inflammatory mucosa and T cells from peripheral blood or cutaneous T-cell lymphoma. The reason for the difference is currently unknown. To our best knowledge, there is no report on the impact of HIF1A on tumorous FOXP3. The relationship between HIF2A and FOXP3 also remains unknown in either lymphocytes or tumor cells.

9.4. Hedgehog pathway

In cancer cells, there are three pathways Notch, Wnt/β-Catenin and

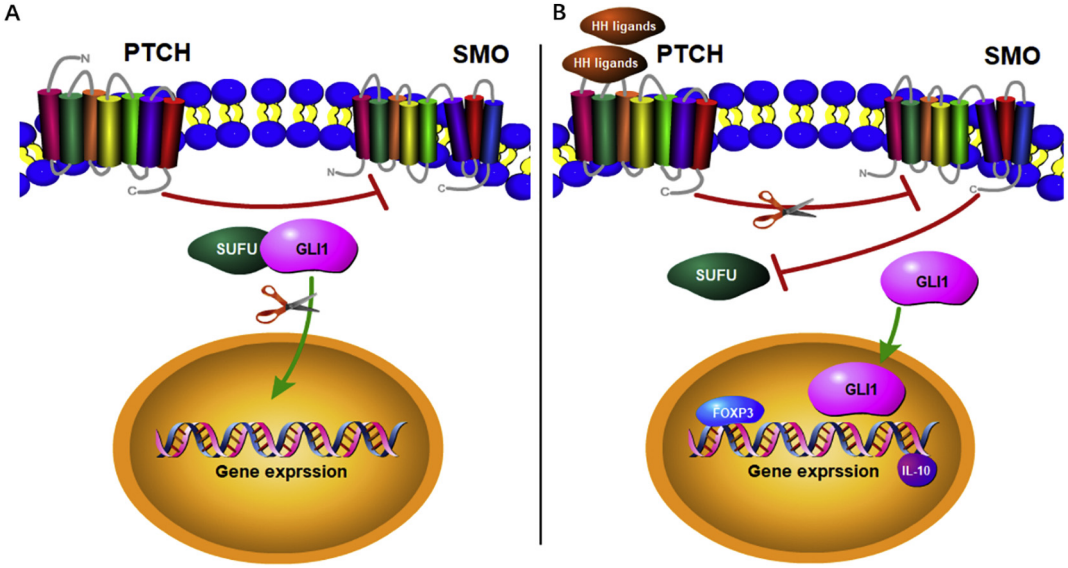

Fig. 4. The potential regulation of FOXP3 expression by the Hedgehog pathway. (A) In the absence of HH ligands, PTCH can inhibit SMO, resulting in SUFU to bind GLI1, which prevents GLI1 translocation to the nucleus to activate its target genes. (B) Once being activated by HH ligands, the suppression of PTCH on SMO is abrogated, leading to the GLI1 transcription factors to get rid of sequestration by SUFU and nuclear localization, which may enhance the level of FOXP3 in T cells.

Hedgehog, which are believed to be related to cancer stemness [79]. As shown in Fig. 4, in the absence of Hedgehog (HH) ligands, transmembrane receptors patched (PTCH) can inhibit factor smoothened (SMO), resulting in suppressor of fused (SUFU) to bind glioma-associated oncogene homolog transcription factor 1 (GLI1), which prevents GLI1 translocation to the nucleus to activate its target genes. Once being activated by HH ligands, the inhibition of PTCH on SMO is abrogated. This makes the GLI1 transcription factors get rid of sequestration by SUFU and the nuclear localization, leading to the activation of oncogenes [79]. According to the review by Katoh Y and Katoh M, Hedgehog signals upregulate the expression of FOXP3 for cell fate determination, but they have not detailed how Hedgehog stimulates FOXP3 expression in human cancers [80]. Recently it was reported in colitis that Hedgehog protein signals could increase the presence of CD4+ FOXP3+ Tregs by inducing expression of IL-10 in stromal cells [81]. The finding suggests that IL-10 may involve in the Hedgehog-mediated regulation of FOXP3 in T cells. However, whether this is the case in cancer cells is unknown.

9.5. Non-coding RNAs

MicroRNAs are known to have various impacts on the development, progression, growth, and treatment of cancers. Among the cancer-related miRNAs, a number of them have been found to participate in the regulation of FOXP3 in several types of cancers. Qin et al. reported that miR-126 could enhance the expression of FOXP3 in Tregs through altering the activation of PI3K/Akt pathway, resulting in increasing the antitumor effect of $CD8^+$ T cells in breast cancer [82]. In T-cell acute lymphoblastic leukemia (T-ALL), miR-146a could act as a tumor suppressor and increase the oncological immune response by up-regulating the expression of FOXP3 during the differentiation of Jurkat T-lymphoblasts [83]. The overexpression of miR-138 could suppress the expression of FOXP3 in human CD4+ T cells. And only with the presence of CD4+ or CD8+ T cells, could miR-138 exert anti-glioma efficacy by suppressing the expression of CTLA-4 and PD-1, which were deemed as immune checkpoints [84]. Some miRNAs were also found to be related to FOXP3 in non-cancer cells. For examples, by analyzing a microRNA profile of human CD8+ regulatory T cells from cord blood samples, Jebbawi et al. confirmed that FOXP3 was directly regulated by miR-335 [85]. In xenograft GVHD mouse model, the forced overexpression of miR-15a/16 in cord blood-derived regulatory T cells led to impairing FOXP3 expression, and luciferase-based assay showed that FOXP3 was determined to be a direct target of miR-15a and miR-16 [86].

In addition to miRNA, the expression of FOXP3 can also be regulated by some long noncoding RNAs (lncRNAs). LncEGFR appears to be the first lncRNA that was associated with the expression of FOXP3. It was reported that lncEGFR upregulation in Tregs correlated positively with the tumor size and the expression of FOXP3, and thus it could link an immunosuppressive state to cancer by promoting Treg cell differentiation [87]. The lncRNA FLICR (FOXP3 long intergenic noncoding RNA) is a negative regulator that influences FOXP3 expression, resulting in a subset of Tregs with the suppressive expression of FOXP3. In this way, it can modify chromatin accessibility in the conserved noncoding sequence 3 (CNS3)/accessible region 5 (AR5) region of FOXP3, enabling cells to escape from dominant Treg control during infection or cancer, at the cost of heightened autoimmunity [88].

10. Conclusion

It can now be certain that FOXP3 express not only in immune cells but also in tumor cells. The tumoral FOXP3 and its isoforms are functional and can interact with various molecules/pathways to impact the development, progression, growth and treatments of cancers. The majority of studies have suggested a suppressive role of FOXP3 in human cancers and the association of its expression with better survival and outcomes. However, some studies have reached opposite conclusions, suggesting an oncogenic role of FOXP3. The expression and role of FOXP3 isoforms, the interaction between tumor cells and lymphocytes in the tumor microenvironment, subcellular location, and mutations of FOXP3 may provide some clues for the contradictory data.

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Declaration of interest

The authors declared that there's no interest conflict concerning this article.

Appendix A. Supplementary data

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