What is the relevance of Foxp3 in the tumor microenvironment and cancer outcomes?

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What is the relevance of FoxP3 in the tumor microenvironment and cancer outcomes?

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Abstract

Introduction: Forkhead box P3 (FoxP3) transcription factor plays critical roles in controlling immune responses and cancer progression in different cancers. FoxP3 expression within the tumor microenvironment (TME) may influence clinical outcomes negatively or positively, and it could play dual roles in cancer, either by promoting or inhibiting tumor development and progression. Some studies reported that high levels of FoxP3 could be associated with tumor progression and worse prognosis, while others reported contradictory results.

Areas covered: In this special report, we present a brief account on the role and function of FoxP3 in the TME, and its contribution to the clinical outcomes of cancer patients. Importantly, we give insights on the potential factors that could contribute to different clinical outcomes in cancer patients.

Expert opinion: Different studies showed that FoxP3 expression can be associated with bad prognoses in cancer patients. However, FoxP3 could have opposing roles by enhancing cancer progression or regression. Location and expression of FoxP3 in T cells or tumor cells can have different impacts on cancer prognoses. Different factors should be considered to establish FoxP3 as a more robust prognostic biomarker and a potential therapeutic target for enhancing anti-tumor immunity and improving clinical outcomes of cancer patients.

Keywords: FoxP3; Prognosis; tumor microenvironment (TME); Cancer; T Regulatory cells; Tregs.

Article Highlights

1. High levels of FoxP3$^+$ Tregs in some cancers are associated with poor prognosis due to their immunosuppressive properties.
2. FoxP3 could be a potential biomarker for predicting prognosis in various cancers.
3. FoxP3$^+$ Tregs can reprogram metastatic tumor immune microenvironment and suppress anti-tumor immune response.
4. FoxP3 have different effects on cancer prognoses, depending on different factors

1. Introduction

The tumor microenvironment (TME) is comprised of different types of cellular and non-cellular components. These components include immune cells, stromal cells, adipocytes, surrounding blood vessels, cancer-associated fibroblasts, cancer cells, extracellular matrix (ECM), exosomes, and signaling molecules [1]. Clearly, all of these factors can interact with each other and influence the behavior of tumor cells. One of the critical components of the TME is the different populations of immune cells including T regulatory cells (Tregs), which interfere with the functions of different immune cells, including effector T cells, natural killer (NK) cells, dendritic cells (DCs) and others, resulting in impairing anti-tumor activities against tumor cells [2]. Forkhead box P3 (FoxP3) is a master regulatory transcription factor [3], which is upregulated in highly immunosuppressive Tregs. In addition to its expression in Tregs, FoxP3 can also be expressed in other lymphoid cells, myeloid cells, hematological malignancies as well as epithelial cells (ECs) of different types of cancer [4,5].

In general, there are two major subsets of $FoxP3^+$ Tregs; thymus-derived Tregs (tTregs) and peripherally-induced Tregs (pTregs) [6]. tTregs originate from the thymus and they mediate peripheral tolerance, which benefit the host by preventing autoimmunity and controlling inflammation. pTregs are induced in the periphery from naïve $CD4^+$ T cells under some conditions, such as T cell receptors (TCR) stimulation in the presence of cytokines including transforming growth factor-β (TGF-β), interleukin (IL)-2, and retinoic acid (RA) [7]. These cells hurt the host by inhibiting beneficial anti-tumor immune responses and mediating tumor-induced suppression. Tregs inhibit immune responses through different mechanisms, including secretion of immunosuppressive cytokines such as IL-10 and TGF-β, consumption of IL-2, cytolysis, and reducing co-stimulation/antigen presentation [8]. Furthermore, Tregs can inhibit effector T cell subsets via direct cell-cell contact, such as binding of cytotoxic T lymphocyte antigen-4 (CTLA-4), which is constitutively expressed on Tregs, with CD80/86 expressed on antigen-presenting cells (APCs), resulting in inhibition of T cell activation [8].

A number of studies demonstrated that $CD8^+$ Tregs and gamma-delta T cells can express FoxP3. Some of FoxP3-expressing gamma-delta T cells have functions, similar to alpha beta Tregs, which may play a key role in inhibiting anti-tumor immune responses [9]. Furthermore, dysregulation of FoxP3 expression in these cells could be associated with immune evasion and tumor development in different tumors. A study showed that a high density of tumor-infiltrating $CD39^+FoxP3^+$ gamma-delta T cells was correlated with malignant

clinicopathological features and exhibited immunosuppressive effect through suppressing T cells in colorectal cancer (CRC) patients [9]. Another study in breast cancer (BC) showed that tumor-specific gamma-delta T cells were associated with advanced tumor stages and inhibition of activation of effector T cells and DCs [10,11]. A recent study reported that transient expression of FoxP3 by $CD8^+$ T cells in the TME impaired their anti-tumor activity [12]. Thus, inhibiting FoxP3 can potentially improve the role of $CD8^+$ T cells following TCR stimulation and enhance anti-tumor immune responses [12]. Overall, FoxP3 can be induced in $CD8^+$ T cells and/or gamma-delta T cells within the TME, which could contribute to the suppression of anti-tumor immune responses and consequently promoting cancer progression.

$FoxP3^+$ Tregs can impede anti-tumor immune responses by suppressing the activation of effector immune cells, resulting in worse clinical outcomes. In general, higher levels of $FoxP3^+$ Tregs within the TME showed positive correlations with poor prognoses in various cancers. However, FoxP3 in the TME could have a dual role by enhancing or inhibiting tumor progression and/or being associated with better or worse clinical outcomes. There are different factors that could contribute to these opposing roles, as will be discussed. This special report aims to further improve our understanding of the role of FoxP3, which is expressed in circulating or tumor-infiltrating Tregs (FoxP3-Treg) or tumor tissue (FoxP3-tumor), and its correlation with clinical outcomes in different cancers.

2. FoxP3 in the TME and factors contributing to opposing roles

2.1. FoxP3 expression in T cells or tumor cells

In this section, we discuss the role of FoxP3 expression in the TME and factors that could contribute to different outcomes in cancer patients. We have recently reviewed the prognostic significance of FoxP3-Tregs and their potential as a biomarker for predicting prognosis in various cancers [13]. Correlation between expression of FoxP3-Tregs and disease prognoses remains controversial in some cancers. Some studies reported that higher FoxP3-Tregs in the TME can be associated with poor clinical outcomes, while others reported contradictory results; some examples will be presented in this special report. A meta-analysis study analyzed data from 8666 BC patients reported that high levels of tumor-infiltrating $FoxP3^+$ Tregs were significantly associated with worse clinical outcomes [14]. Furthermore, other studies reported that high levels of tumor-infiltrating FoxP3-Tregs were associated with poor prognoses in

different types of cancer including glioblastoma (GBM), gastric cancer (GC) and non-small cell lung cancer (NSCLC) [15-17]. However, not all cancers with high density of FoxP3-Tregs are associated with worse prognoses, and FoxP3-Tregs could have beneficial effects in some cancers. A meta-analysis study reported that a high density of intra-tumoral FoxP3-Tregs in human CRC, esophageal cancer, and head and neck squamous cell carcinoma (HNSCC) was associated with a better prognosis and an improved overall survival (OS) rates [18]. Furthermore, a study on HNSCC showed that increased tumor infiltration with FoxP3-Tregs is positively correlated with better prognosis, and improved OS and recurrence-free survival (RFS) rates [19]. Similarly, high expression of FoxP3 in tumor-infiltrating Tregs of patients with mismatch repair (MMR)-proficient CRC has been associated with better prognosis [20].

In addition to its expression in T cells, several studies reported that FoxP3 can be expressed by some types of cancer cells, and it could have dual role in tumor progression and disease prognoses, as discussed below. For instance, over-expression of FoxP3 in tumor cells was positively related to $p16^{INK4a}$ expression in cervical cancer (CC) [21], which may play a main role in CC development. Upregulation of both FoxP3 and $p16^{INK4a}$ expression potentially contributes to promoting tumor proliferation and metastasis and may influence the prognosis of CC [21]. By inhibiting FoxP3 in CC cells, $p16^{INK4a}$ expression was significantly reduced, indicating a potential regulatory relationship between FoxP3 and $p16^{INK4a}$ proteins [21]. This suggests that both proteins could be potential biomarker targets in CC. Interestingly, FoxP3 expression in CRC, but not in T cells, was associated with disease progression [22]. In this study, it was shown that overexpression of FoxP3-tumor in late stages of CRC was associated with a worse prognosis, compared to higher or lower density of tumor-infiltrating FoxP3-Tregs, which did not show any impact on survival rates [22]. Furthermore, expression of FoxP3 in CRC was positively associated with VEGF-C expression and the formation of lymphatic vessel, which is one of key players in tumor progression and worse prognosis in cancer patients [23]. Another study found that overexpression of FoxP3 in tumor tissues induced epithelial-mesenchymal transition (EMT) and metastasis in NSCLC via regulation of VEGF expression and Notch1/Hes1 pathway [24]. Moreover, overexpression of FoxP3 upregulates the expression of matrix metalloproteinase-2 (MMP-2) and MMP-9, which are involved in tumor metastasis [24]. In NSCLC, FoxP3 can act as a co-activator to modulate the Wnt/β-catenin signaling pathway [25]. Overall, increased expression of FoxP3 in cancer cells can be positively associated with tumor progression and reducing OS and RFS rates in NSCLC [25]. In contrast, overexpression of FoxP3 in GC was positively correlated with cancer inhibition

and improved disease prognosis [17]. Inducing expression of FoxP3 in epithelial ovarian cancer cell lines resulted in inhibition of cell proliferation, and reduction in cell migration and invasion [26]. Cells upregulating FoxP3 showed decreased expression of the proliferation marker Ki-67, and the cell cycle-associated protein cyclin-dependent kinases (CDKs) [26]. In addition, expression of MMP-2 and urokinase-type plasminogen activator (uPA) were decreased in the FoxP3 transfected cell line, leading to inhibition of cell migration and invasion [26]. Similarly, a study in GC revealed that overexpression of FoxP3 in tumor cells predicts better survival [17]. A recent study showed the association between metastatic cancer and FoxP3$^+$ Tregs in BC [27]. The presence of FoxP3$^+$ Tregs and other immunosuppressive cells, within brain and liver metastasis of BC, may contribute to establishing an immunosuppressive microenvironment within the metastatic niches [27]. FoxP3$^+$ Tregs and other immunosuppressive cells could reprogram the metastatic ecosystem, which may play a crucial role in inhibiting anti-tumor immunity and enhancing the aggravation of disease outcomes [27]. Clearly, multiple factors interact with FoxP3 in the TME and influence its function, and contribute to tumor development and/or progression through evasion of immune surveillance.

2.2. Different FoxP3$^+$ T-cell subsets

Association of FoxP3 expression and clinical outcomes could also depend on the type and function of Treg subsets. Saito *et al.,* identified two subpopulations of FoxP3-Tregs that can infiltrate CRC tissues [28]. interestingly, a high density of tumor-infiltrating FoxP3^{low} Tregs was linked to favorable prognosis, compared to infiltration of FoxP3^{high} Tregs [28]. Therefore, both subpopulations can contribute differently to determining the clinical outcomes in CRC patients [28]. Of note, Tregs can co-express both FoxP3 and Helios transcription factors, suggesting a higher potential for immunosuppression, compared to cells expressing FoxP3 or Helios. We and others have shown that FoxP3$^+$Helios$^+$ Tregs are more stable and immunosuppressive cells than FoxP3$^+$Helios$^-$ Tregs [29-31]. Moreover, a recent study has proposed human Helios as a marker of Treg stability [32]. Expression of Helios regulates Treg stability by silencing IL-2 expression, while Tregs lacking Helios expression display more IL-2 expression, resulting in enhanced Treg proliferation, production of IL-2 following activation, and reduced suppressive activity [33]. Therefore, the stability and function of human FoxP3$^+$ Tregs could depend on Helios expression. Furthermore, we have shown that FoxP3$^+$Helios$^+$ Tregs could play important roles in cancer immunity by exerting higher immunosuppressive functions [34]. Interestingly, FoxP3$^+$Helios$^+$ Tregs co-express high levels of PD-1/CTLA-4 and PD-1/CD39, suggesting that they could contribute to tumor immune evasion and progression

[34]. Interestingly, we found that tumor-infiltrating FoxP3$^+$Helios$^+$ Tregs are associated with improved disease-free survival (DFS) in CRC patients, while increased levels of FoxP3$^-$Helios$^-$ non-Tregs were associated with shorter DFS [35]. We propose that the immunosuppressive FoxP3$^+$Helios$^+$ Tregs are anti-inflammatory cells, which inhibit inflammation and contribute positively to improved CRC prognosis, while FoxP3$^-$Helios$^-$ cells are pro-inflammatory cells, which enhance inflammation and contribute negatively to disease prognosis. Additionally, a higher proportion of FoxP3$^+$Helios$^-$ Tregs was observed in NSCLC patients with advanced stages and shorter survival [36]. We also found that various immune checkpoints-expressing CD4$^+$ Treg/T cell subsets [35] or CD8$^+$ Treg/T cell subsets [37] have different roles in DFS of CRC patients. Of note, full characterization and accurate identification of the exact subpopulations contributing to clinical outcomes in cancer patients are critical factors for prognostic and therapeutic purposes.

2.3. Tumor-infiltrating versus circulating FoxP3$^+$ Tregs

Role of Tregs in clinical outcome could also depend on location of Tregs, e.g., in the TME, circulation or tumor-draining lymph nodes (TDLNs). Location of Tregs could be associated with favorable or unfavorable clinical outcomes in some cancers, especially inflammatory ones such as CRC. Gallimore's group reported that peripheral blood FoxP3$^+$ Tregs are efficient in suppressing tumor-specific immune responses, which results in tumor progression and poorer survival in CRC patients [38,39]. Similarly, we have recently shown that increased levels of circulating FoxP3$^+$ Tregs and FoxP3$^+$Helios$^+$ Treg subset were associated with worse DFS in CRC patients, while their increased levels in the TME were associated with improved DFS [35]. We speculate that these Treg subsets in circulation are highly immunosuppressive and they can inhibit anti-tumor immune responses and contribute negatively to disease prognosis, while they could be anti-inflammatory in the TME and contribute positively to improved prognosis.

2.4. Intra-tumoral versus stromal FoxP3$^+$ Tregs

The presence of FoxP3$^+$ Tregs within tumor compartments or stroma is critical for determining clinical outcomes. A meta-analysis study analyzed data of 3811 CRC patients revealed that high FoxP3$^+$ Tregs density in tumor tissues, especially at the tumor stroma, could be associated with better clinical outcomes [40]. Another study reported a strong inverse correlation between density of stromal FoxP3$^+$ Tregs and tumor regression after chemoradiotherapy in patients with rectal cancer; patients with low density of stromal FoxP3$^+$ Tregs showed better responses to

neoadjuvant CRT [41]. Another study showed that a high density of FoxP3$^+$ Tregs in intra-tumoral areas could provide a favorable TME to prevent cancer recurrence and improve relapse-free survival in CRC patients [42]. Furthermore, increased levels of FoxP3$^+$ Tregs in intra-tumoral compartments were positively associated with improved DFS of patients with vulvar squamous cell carcinoma (SCC) [43].

2.5. Tumor stage and pathological features

FoxP3$^+$ Tregs may have varying prognostic values based on the stage of disease and pathological features. Thus, the presence of FoxP3$^+$ Tregs within the TME or in other locations may represent a prognostic biomarker for identifying disease stages in different cancers. Recent studies have documented a strong association between expression of FoxP3 and disease stage, tumor grade, histological features, or the presence of specific molecular markers in several tumors. We have recently reported that correlations between different tumor-infiltrating FoxP3$^+$ Treg subsets with expression of inhibitory immune checkpoints were higher in CRC patients with early stages, suggesting the potential role for these highly immunosuppressive cells in inhibiting inflammatory responses, especially in early stages of this inflammatory cancer [44]. In a meta-analysis study, it was shown that gynecological cancer patients with advanced stages have a higher density of Tregs in the tumor tissues and peripheral blood, compared with early stages; however, no correlations were reported between the proportion of Tregs and survival rates [45]. Other studies in ovarian cancer revealed that an increased proportion of tumor-infiltrating FoxP3$^+$ Tregs in patients with advanced stages was correlated with good prognosis and clinical outcomes [46,47]. Furthermore, some studies have investigated the correlation between the expression of FoxP3 and pathological features. For instance, in cervical cancer, increased expression of FoxP3 protein has been linked to stage II tumor and vascular invasion, indicating a more aggressive phenotype [48]. Similarly, the associations between high density of FoxP3$^+$ Tregs and clinical outcomes has been reported in prostate cancer (PC) [49]. Increased expression of FoxP3$^+$ Tregs could be associated with advanced stages of PC and reducing RFS, as well as promoting cancer cell proliferation via upregulation of Ki-67 [49]. Another study on nasopharyngeal carcinoma (NPC) revealed that expression of FoxP3 is correlated with prognostic survival in different tumor stages [50]. A higher density of tumor-infiltrating FoxP3$^+$ Tregs was associated with improved OS and progression-free survival (PFS) in patients with advanced disease stages, but had no influence on prognostic survival in early disease stages [50].

2.6. FoxP3 isoforms

Human FoxP3 mRNA encodes multiple FoxP3 transcript isoforms, including FoxP3-full-length (FoxP3-fl) and a number of FoxP3 deleting exons, which may have different biological activities [51]. These isoforms can regulate gene expression, interact with other proteins, and affect immunity; therefore, all of these factors could be correlated with tumor progression and survival in cancer patients. Gong *et al.,* detected two FoxP3 isoforms in hepatocellular carcinoma (HCC) tissues and normal adjacent tissues by using different methods, including immunohistochemistry assay (IHC) and nested polymerase chain reaction (nested PCR), which were confirmed by RNA-sequencing [52]. The result of quantitative real-time PCR (QPCR) showed that FoxP3-fl was more upregulated in tumor tissue than in adjacent normal tissue, while the FoxP3 deleting exon-3 (FoxP3Δ3) did not show any significant difference between these tissues [52]. However, IHC assay showed different results with overexpression of FoxP3-fl protein detected in the adjacent normal tissues. compared with tumor tissues, while FoxP3Δ3 did not show any significant difference between both tissues [52]. Furthermore, levels of these isoforms and disease prognosis in HCC patients were investigated [52]. Interestingly, high levels of FoxP3-fl expression in tumor tissues were associated with good prognosis and inhibition of tumor growth and [52]. Moreover, expression of FoxP3-fl inhibited tumor growth via downregulating c-Myc expression in an HCC animal model [52]. Furthermore, some studies investigated the correlation between expression of the major isoform of FoxP3 (FoxP3Δ3) and tumor development [53,54]. Increased expression of FoxP3Δ3 isoform in bladder cancer was associated with larger tumor formation and high tumor grade, indicating a more aggressive phenotype [53]. Furthermore, expression of FoxP3Δ3 isoform in bladder cancer cell lines mediated resistance to chemotherapy [53]. Overexpression of FoxP3Δ3 isoform was observed in tumor lung tissues, compared to adjacent non-tumor tissues [54]. Interestingly, FoxP3Δ3 overexpression promoted cell proliferation, migration, and invasion in NSCLC via down-regulating E-cadherin, and up-regulating MMP2, MMP7, N-cadherin, snail, and slug [54].

3. Expert opinion

FoxP3 could be a potential prognostic and predictive biomarker in cancer patients. However, FoxP3 in cancer patients could have opposing roles by enhancing cancer progression or regression. Location of FoxP3^{+} Tregs can have different impacts on cancer prognoses. For

example, high density of FoxP3$^+$ Tregs in circulation could be associated with bad prognoses in some tumors such as CRC, while their increased levels in the tumor tissues are associated with improved prognoses. Therefore, investigations of levels and functions of different FoxP3$^+$ Treg subsets within the TME, circulation and TDLNs are essential for establishing FoxP3 as a more robust prognostic biomarker and a potential target for improving therapeutic efficacy in cancer patients. Several factors should be considered when investigating the role of FoxP3 in cancer and correlations of FoxP3 expression with disease prognoses and clinical outcomes. These factors include determination of the exact Treg subsets in each cancer, location of FoxP3 expression, location of Tregs, tumor stage and pathological features and FoxP3 isoforms, as we have explained in this special report.

It is evident that further investigations on the role of FoxP3 in tumor inhibition or promotion are critical to determining its exact function in the TME and correlations with clinical outcomes of cancer patients. In addition, there are different aspects that need further investigations and clarifications. Discovering more specific surface markers to define and isolate human FoxP3$^+$ Tregs are of paramount importance to enable researchers to further establish the role and function of these critical cells in different disease settings including cancer. Furthermore, in addition to its expression in CD4$^+$ T cells, different studies reported the expression of FoxP3 in CD8$^+$ T cells; however, its role in tumor development and clinical outcomes remains to be further confirmed. Furthermore, FoxP3 expression and its prognostic value in gamma-delta T cells is still limited. Recent research has reported that different isoforms of FoxP3 may have different effects on tumor development. Thus, understanding the function of these isoforms in different tumors is important for developing new targeted therapies that can enhance immune responses to effectively combat tumor cells, while reducing potential effects on healthy tissues. In addition, a recent study reported that neddylation enzymes E2s (Ube2m and Ube2f) and E3s (Rbx1 and Sag) could play important roles in controlling Treg fitness, while their deletion leads to severe autoimmune phenotypes [55]. Clearly, more investigations are required to further define the role of these regulators in anti-tumor immunity and tumorigenesis.

In conclusion, further understanding of the role of FoxP3 in cancer is essential to developing novel therapeutics for enhancing anti-tumor immunity and achieving better clinical outcomes in cancer patients.

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Table 1: Summary of studies showing FoxP3 expression and its prognostic value in different types of cancer.

Cancer type	Source of FoxP3	FoxP3 expression	Clinical outcome	Notes	Ref.
BC	Tumor cells (cytoplasm)	Positive	Poor OS.	Localization of FoxP3 in tumor cells is a key determinant of prognostic value.	[56]
BC	T cells	High	Worse OS.	$FoxP3^+$ T cells may play a key role in tumor progression.	[56]
BC	TILs	High	Poor OS.	Positive association with c-erbB-2 status and lymph node status, and negative correlation with ER status and PR status.	[14]
GBM	Tregs	High	Reduced survival rates.	Associated with recurrent GBM.	[16]
NSCLC	Tregs	Low	Favorable prognoses.	Correlated with better response to chemoradiotherapy.	[57]
NSCLC	Tumor cells	High	Poor prognosis.	Promoted tumor development and metastasis via enhancing Wnt/β-catenin pathway and EMT.	[25]
Triple-negative breast cancer (TNBC)	TILs	High	Favorable prognoses.	Favorable marker in high level of IL-33.	[58]
TNBC	Intra-tumoral Tregs	High	Better DFS and OS.	Localization of FoxP3 Tregs within intra-tumoral is a key determinant of prognostic value.	[59]
GC	Tumor cells (cytoplasm)	High	Longer survival time and better prognosis.	Same study showed that high FoxP3 Tregs associated with worse prognosis.	[17]
Non-metastatic TNBC	Tumor stroma	High $CD8^+$ to $FoxP3^+$ ratio	Improved survival rate.		[60]
CRC	Tregs in the	High	Favorable		[40]

	stromal compartment		clinical outcomes.		
MMR-proficient CRC	Tregs	High	Improve survival rate.	Associated with early T stage.	[20]
CRC	Tregs in intra-tumoral.	High	Good prognostic factor.	A potential biomarker in stage II CRC.	[61]
HNSCC	Tregs (in stromal and intra-tumoral)	High	Favorable OS and RFS.	Patients with early-stage tumors showed better OS than advanced stage tumors.	[19]
Diffuse large B cell lymphoma (DLBCL)	TILs	High density of $Tim3^{+}FoxP3^{+}$ Tregs.	Poor survival of patients.	This co-expression promoted tumor growth by producing IL-10 in the TME.	[62]
Gallbladder tumor	TILs	Lack of $FoxP3^{+}$ and high $CD8^{+}$ T cells.	Improved patient survival.	Patients with advanced-stage tumors showed better prognosis.	[63]
Oral Squamous cell carcinoma (OSCC)	Tumor cells	High	Shorter RFS and OS.	Associated with lymph node metastasis.	[64]
Uterine sarcomas	T cells	High	Favorable prognosis.	Associated with low ECM expression and reduced YAP activation.	[65]
Follicular lymphoma (FL)	Tregs	High	Improved OS.	May plays a role in inhibiting FL.	[66]
HCC	Tumor cells	High	Better survival and reduced recurrence.	Regulated TGF-β/Smad 2 and 3 signaling pathways.	[67]