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REVIEW

Multiple biological functions of transcription factor 21 in the development of various cancers

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Abstract: Transcription factor 21 (TCF21) is a basic helix–loop–helix transcription factor that binds to DNA and regulates cell differentiation and cell fate specification through mesenchymal–epithelial transition during development. The *TCF21* gene is epigenetically inactivated in many types of human cancers and exerts a wide variety of functions, including the regulation of epithelial–mesenchymal transition, invasion, metastasis, cell cycle, and autophagy. This review focuses on research progress in relation to the roles of TCF21 in tumor development. We systematically consider multiple pathological functions of TCF21 in various cancers, revealing the molecular bases of its diverse biological roles and providing new directions for future research.

Keywords: TCF21, cancer, DNA methylation, EMT, invasion, metastasis, cell cycle

Introduction

The helix–loop–helix (HLH) family of transcription factors includes key players in a wide array of developmental processes.[1–3] More than 240 HLH members have been identified to date in organisms ranging from yeast to human beings.[4–7] HLH proteins are characterized by the possession of highly conserved bipartite domains for DNA binding and protein–protein interactions.[8,9] A first motif of basic residues mainly permits HLH proteins to bind to a consensus hexanucleotide E-box (CANNTG),[10] whereas a second motif of primary hydrophobic residues, the HLH domain, allows these proteins to interact and form homo- and/or heterodimers.[9] HLH family members have been classified into seven families based on their tissue distribution, dimerization capacities, and DNA-binding specificities.[7] Class I factors are widely expressed and act as homodimers or heterodimers with class II proteins. The most representative HLH proteins, also known as E-proteins, belong to class I, and are encoded by the genes such as *TCF3* (E12 and E47 isoforms), *TCF4* (E2-2A and E2-2B isoforms), and *TCF12* (α/β isoforms). Class II factors are tissue-specific basic HLH (bHLH) proteins that always act as heterodimers with class I factors, including TWIST1 and TWIST2.

Transcription factor 21 (TCF21, also known as capsulin/pod1/epicardin), is encoded by a gene located on chromosome 6q23–q24 and is a cell-type-specific class II bHLH transcription factor. TCF21 can bind to the consensus E-box sequence to form a heterodimer with the widely expressed bHLH protein E12.[11] It mandates cell fate differentiation through mesenchymal–epithelial transition during development.[12] TCF21 was shown to be expressed at its highest levels during embryonic development in mice, and its expression levels then rapidly decreased in postnatal tissues, except in a subset of interstitial cells in organs including kidney, lung, and heart.[11–13] Antisense inhibition of *TCF21* disrupted epithelial differentiation and branching morphogenesis

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of the epithelium in mouse embryonic kidney, suggesting a role for TCF21 in epithelial–mesenchymal interactions.[13] *TCF21* knockout mice were born alive but died shortly after birth due to poor lung differentiation.[14] Perinatal lethality is a classic feature of tumor suppressor activity,[15] and all these features suggest that the *TCF21* gene plays a crucial role in maintaining life.

Using restriction landmark genomic scanning, Smith et al[16] identified *TCF21* as a candidate tumor suppressor that was epigenetically inactivated in lung cancer and head and neck cancer. This provided the first evidence of a role for TCF21 in human cancer, since when a growing number of studies have demonstrated that aberrant methylation and decreased expression of *TCF21* are tumor-specific and relatively common occurrences in human malignancies.[17–26] TCF21 exerts a wide variety of functions, including involvement in the regulation of invasion,[27,28] metastasis,[29] autophagy,[30] and cell cycle.[31,32] This review focuses on research progress in relation to the role of TCF21 in tumor development. These studies have furthered our understanding of the role of TCF21 in human cancers and provided new directions for future research.

Regulation of TCF21 expression
TCF21 promoter hypermethylation

DNA methylation was the first epigenetic marker shown to be critically involved in tumorigenesis,[33] by providing a stable mechanism for gene silencing and thus playing an important role in regulating gene expression. Aberrant promoter hypermethylation represents a major mechanism leading to silencing of tumor suppressor genes in many kinds of human cancers.[34] Promoter hypermethylation involves methylation of CpG islands within or near the promoter region of certain genes, thus rendering them transcriptionally silent. This downregulation of gene expression has been shown to be important in terms of cancer progression and outcome.[35,36]

Many transcription factors and gene regulators responsible for early development have been shown to have promoters within DNA methylation valleys, and such genes frequently demonstrate aberrant methylation patterns in cancers during later life.[37] Notably, the *TCF21* promoter is located within a DNA methylation valley.[37] TCF21 is encoded by three exons, each of which is associated with a CpG island, and this feature has prompted extensive analysis of *TCF21* promoter hypermethylation in a wide variety of tumors. High rates of *TCF21* promoter hypermethylation have been identified in cancers of different origins, including lung,[16–19,38] kidney,[21,26] bladder,[17,21] prostate,[21] breast,[17] gastric,[25] colon and rectum,[27] and head and neck cancers.[16,22] *TCF21* methylation in all

these malignancies appears to have an excellent capacity for discriminating between cancerous and nonmalignant tissues with sensitivities for identifying bladder cancer, renal cell carcinoma, and prostate cancer of 92%, 67%, and 96%, respectively.[21] Consistent with its aberrant promoter hypermethylation, low TCF21 expression levels were also found in these cancers. Low TCF21 expression has been shown to be related to high-grade or invasive–aggressive cancers with lymph node or distant metastases indicating recurrence and with an inferior prognosis.[24,26] TCF21 may thus serve as an excellent diagnostic or prognostic biomarker predicting poor outcome, as demonstrated in gastric cancer,[25] melanoma,[29] colorectal cancer,[39] and lung cancer cases.[38,40] Aberrant methylation of *TCF21* can be detected not only in solid cancer tissues but also in sputum and urine samples from cancer patients.[17,24] Aberrant *TCF21* methylation has been demonstrated in cancer cell lines, animal models, and clinical tissues and biological fluids, indicating its potential value as a noninvasive marker for cancer detection; however, the detection of TCF21 is still far from suitable for clinical applications.
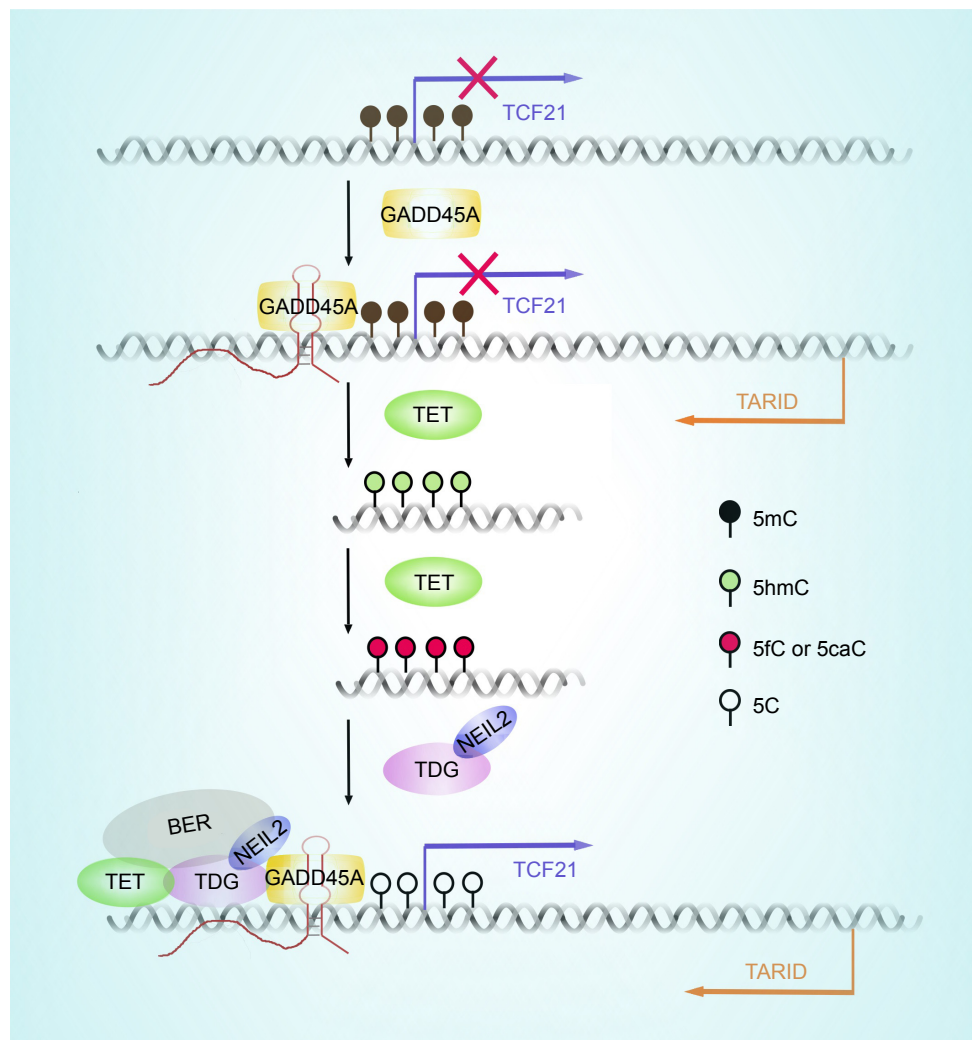
Sumoylation of TCF21

Posttranslational modifications, such as phosphorylation, methylation, acetylation, ubiquitination, and sumoylation, are important mechanisms for regulating protein actions[41] and allow proteins to have multiple functions.[42] Sumoylation plays important roles in protein regulation by affecting subcellular protein localization, protein stability, protein–protein interactions, and the transcriptional activity of transcription factors.[43] Several members of the bHLH family, including circadian locomotor output cycles kaput and differentiated embryo-chondrocyte expressed gene 1, have been shown to be subject to sumoylation.[44,45] Ao et al[32] reported that TCF21 could be sumoylated at lysine residue 24 by the small ubiquitin-like modifier (SUMO) 1, and this modification could be reversed by SUMO-specific protease 1. They found that sumoylation stabilized TCF21 but did not affect its subcellular localization. In addition, sumoylation of TCF21 could repress the transcriptional activity of estrogen receptor-α (ERα) through promoting the recruitment of histone deacetylases 1/2, and this negative regulation of ERα led to a reduction in breast cancer cell proliferation.[32]

Regulation of TCF21 by noncoding RNAs (ncRNAs)

In addition to DNA methylation, multiple lines of evidence have demonstrated that various ncRNAs act as upstream regulators to modulate *TCF21* via multiple pathways.

Arab et al[46] found that the long ncRNA TCF21 antisense RNA-inducing demethylation (TARID) activated *TCF21* expression by inducing promoter demethylation. TARID accomplished this by interacting with both the *TCF21* promoter and the DNA demethylation regulator, GADD45A (growth arrest and DNA damage inducible, alpha). GADD45A in turn recruited thymine DNA glycosylase (TDG) for base excision repair (BER)-mediated demethylation, involving oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in the *TCF21* promoter by 10–11 translocation (TET) methylcytosine dioxygenase proteins (Figure 1).[46] TDG is an essential component of the BER pathway for DNA demethylation[47–49] and can efficiently excise the iterative oxidation products of 5mC and 5hmC, namely 5-formylcytosine and 5-carboxylcytosine and initiate

downstream BER to restore unmodified cytosines.[50–52] Further detailed studies revealed that NEIL2 (nei-like DNA glycosylase 2) is required for TET–TDG-mediated gene-specific demethylation of *TCF21*, which cooperates with TDG during base excision. TDG occupies the abasic site and is replaced by NEIL2, which further processes the baseless sugar, thereby stimulating TDG substrate turnover.[53] In another study, Gooskens et al[54] demonstrated that TARID expression was significantly lower in clear cell sarcoma of the kidney (CCSK) compared with Wilms' tumor tissues and was negatively correlated with the degree of *TCF21* promoter methylation. This suggests that *TCF21* hypermethylation in CCSK may be caused by decreased TARID expression. Recent research has shown that aberrantly expressed miR-21 might regulate the TCF21–KISS1-associated renal cell carcinoma

Figure 1 Model of regulation of TCF21 by demethylation.
Notes: TARID binds to GADD45A and recruits TETs together with TDG to induce promoter demethylation. TET enzymes iteratively oxidize 5mC to form 5hmC, 5fC, and 5caC. TDG recognizes and excises higher oxidation products (5fC or 5caC) and initiates downstream BER to restore unmodified cytosines. NEIL2 cooperates with TDG during base excision.
Abbreviations: BER, base excision repair; 5C, 5-cytosine; 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; TARID, TCF21 antisense RNA-inducing demethylation; TCF21, transcription factor 21; TDG, thymine DNA glycosylase; TET, translocation.

cell invasion pathway,[55] whereas Wei et al[28] demonstrated that miR-205 inhibited *TCF21* expression and consequently attenuated the inhibitory effect of TCF21 on cell invasion in ovarian cancer cells.

Tumor suppressive signatures of TCF21

Numerous studies have shown that TCF21 acts as a transcription factor and is implicated in many aspects of carcinogenesis. TCF21 mediates various functions, such as epithelial–mesenchymal transition (EMT), invasion, metastasis, cell cycle, and autophagy, via interactions with different types of molecules (Figure 2).

Inhibition of EMT and tumor invasion and metastasis

Loss of chromosome 6q16–q24, including the *TCF21* locus, has been associated with metastasis.[56,57] An increasing number of studies have shown that TCF21 acts as a tumor suppressor

with a crucial role in regulating tumor cell invasion and metastasis. Local tumor invasion represents the first step in the metastatic cascade of carcinomas and requires profound changes in the adhesion and migration properties of tumor cells that are reminiscent of an important developmental process such as EMT.[58] A transition between epithelial and mesenchymal cells is known to occur during tumorigenesis.[59,60] Smith et al[16] first established a link between TCF21 and EMT in cancer cells and showed that TCF21 expression was highly associated with the epithelial phenotype. They showed that expression levels of the mesenchymal markers such as snail family transcriptional repressor 1 (SNAI1) and vimentin (VIM) were significantly downregulated, whereas the epithelial markers such as WNT4 and CDH1 were upregulated in lung cancer cells transfected with *TCF21*. The relationship between TCF21 and EMT has since been verified in a broad range of tumor types. Shivapurkar et al[17] observed significant expression of WNT4 in TCF21-positive lung cancer cells. Downregulation of SNAI1 was also observed in breast cancer
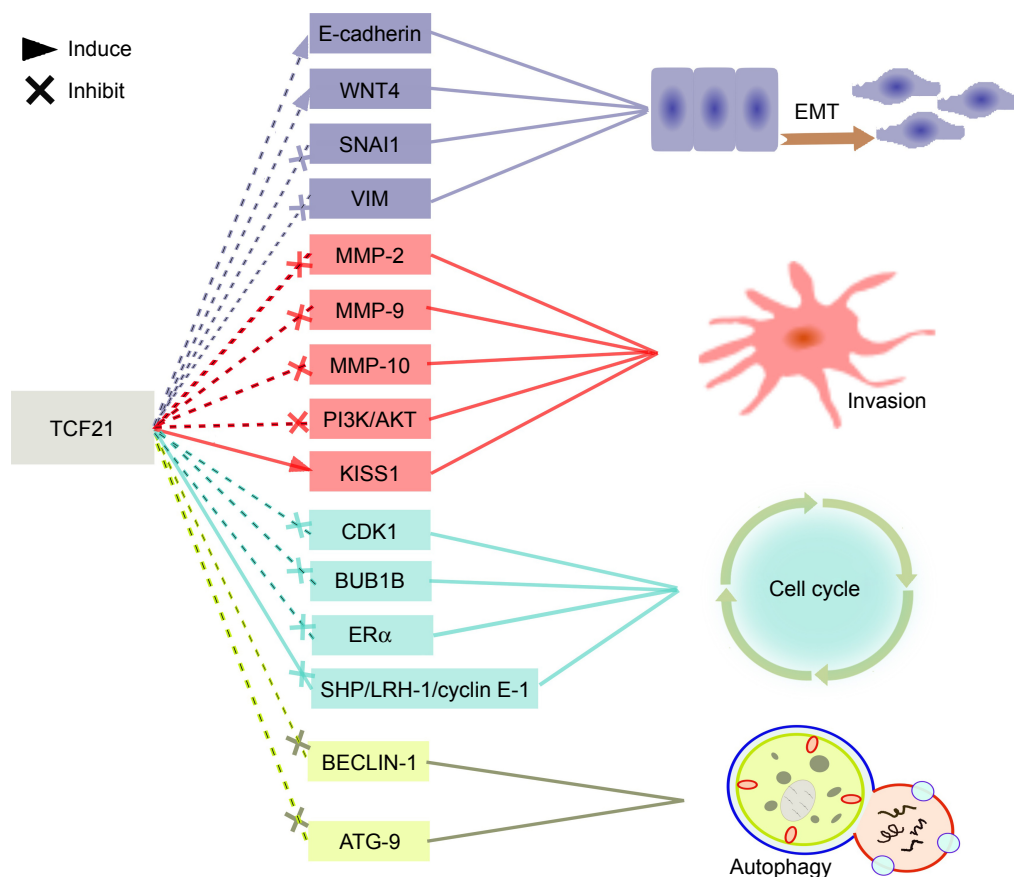
Figure 2 TCF21 regulates different functions by interacting with different partners.
Notes: TCF21 exerts multiple biological effects, including EMT, invasion, metastasis, cell cycle, and autophagy by interacting with different types of molecules. Solid line indicates direct effect; dashed line indicates unknown mechanism of action.
Abbreviations: BUB1B, budding uninhibited by benzimidazoles 1 homolog beta; CDK1, cyclin-dependent kinase; EMT, epithelial–mesenchymal transition; ERα, estrogen receptor α; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol 3-hydroxy kinase; SNAI1, snail family transcriptional repressor 1; TCF21, transcription factor 21; VIM, vimentin; PI3K, phosphatidylinositol 3-hydroxy kinase; SHP, small heterodimer partner; LRH-1, liver receptor homolog-1.

cells transfected with *TCF21*.[23] Dai et al[27] showed that restoration of TCF21 resulted in decreased expression of the mesenchymal marker such as VIM and increased expression of the epithelial marker E-cadherin in colorectal cancer cells. However, another study found no changes in mRNA levels of the mesenchymal markers such as VIM and SNAI1, but there was a significant upregulation of the epithelial marker E-cadherin at both the mRNA and the protein levels in renal tumor cells expressing ectopic *TCF21*.[61] These results also suggested that E-cadherin might be a proximate downstream target of TCF21. E-box elements of the E-cadherin promoter are involved in silencing E-cadherin promoter activity in cancer cells.[62] Although E-boxes act as recognition sites for bHLH transcription factors,[63] there is currently no evidence to show that bHLH factors bind to sequences in the E-cadherin promoter.[62] Further studies are therefore needed to clarify the mechanism whereby TCF21 regulates E-cadherin involvement in EMT.

In addition to the cardinal EMT pathways, TCF21 also regulates other aspects of the tumor invasion and metastasis processes via different signaling pathways. A recent study confirmed that upregulation of *TCF21* significantly increased the expression of KISS1 and decreased the expression of phosphatidylinositol 3-hydroxy kinase (PI3K) and phosphorylated AKT, indicating inactivation of PI3K/AKT signaling.[39] In addition, overexpression of *TCF21* also inhibited the expression of matrix metalloproteinase (MMP)-2 and MMP-9, which are involved in cancer cell invasion and metastasis.[39] Similarly, Wei et al[28] demonstrated that TCF21 inhibited MMP-2 and MMP-10 and decreased ovarian cancer cell invasion. In addition, Arab et al[29] reported that TCF21 was regulated epigenetically and had potential antimetastatic properties by directly activating the known metastasis suppressor KISS1 on chromosome 1q32 in melanoma.

Modulatory role of TCF21 in cell cycle and autophagy

The basic biological characteristic of malignant tumors is unlimited proliferation, potentially related to cell cycle disorders. Accumulating evidence suggests that TCF21 is involved in regulating the cell cycle. França et al[64] demonstrated that TCF21 expression was inversely correlated with a panel of cell cycle genes in human adrenocortical tumor cells, including cyclin-dependent kinase and budding uninhibited by benzimidazoles 1 homolog beta. They also confirmed that TCF21 inhibited the expression of endogenous steroidogenic factor 1 (SF-1) through binding to the E-box sequence in the SF-1 promoter. SF-1 expression drives the

differentiation of the steroidogenic lineages during human and mouse development,[65] and increased SF-1 dosage can augment human adrenal cell proliferation, thus playing a critical role in adrenocortical tumorigenesis.[66] A subsequent study revealed that TCF21 reduced small heterodimer partner (SHP) expression via binding to its E-box element in hepatocarcinoma cells, resulting in increased expression of liver receptor homolog-1 (LRH-1), which binds to the cyclin E-1 promoter leading to G1-to-S phase transition of the cell cycle.[31] LRH-1 and SF-1 are members of the Fzt-F1 subfamily of nuclear receptors,[67] and both LRH-1 and its repressor SHP are involved in many types of cancers.[68–71] TCF21 binds directly to the E-box of the *SHP* promoter, inhibiting its activity, but does not bind to the *LRH-1* promoter E-box sequence. These results suggest that TCF21 has different effects on the cell cycle in tumor cells expressing LRH-1. A recent study also reported that sumoylation of TCF21 could inhibit the growth of ERα-positive breast cancer cells and decrease the proportion of S-phase cells in the cell cycle.[32] The relationship between epigenetic regulation and autophagy is currently a hot topic in cancer therapy. A recent study demonstrated a correlation between *TCF21* promoter methylation and autophagy, suggesting that TCF21 may inhibit autophagy by suppressing the autophagy-related proteins such as BECLIN-1 and ATG-9 in the progression of lung cancer.[30] Research into the role of TCF21 in the cell cycle and autophagy is currently limited, and further studies are therefore needed to confirm the significant roles of TCF21 in regulating the cell cycle and autophagy.

Summary and future prospects

In conclusion, the abovementioned studies demonstrate that the TCF21 is inactivated in a wide variety of carcinomas, largely as a result of aberrant DNA methylation. Reversal of DNA methylation by demethylating agents and DNA methyltransferase inhibitors restores *TCF21* activation, suggesting that the development of drugs targeting DNA methylation of *TCF21* may have vital clinical implications for the treatment or prevention of cancers.

TCF21 is implicated in many aspects of the carcinogenesis process, including tumor initiation, invasion, metastasis, cell cycle, and autophagy. Although, among these functions, the contribution of TCF21 to EMT has been verified in various cancers, the mechanisms underlying this association remain largely undefined. In addition to EMT, the involvement and molecular mechanisms of TCF21 in relation to apoptosis, autophagy, and drug resistance also require further investigation. Further detailed studies exploring how TCF21

participates in these processes will provide more comprehensive information about the crucial pathological functions of TCF21 in cancer. Moreover, in addition to studying these pathological effects of TCF21 independently, the correlations and interdependency among these multiple biological functions should also be explored.

In summary, the complex relationships between TCF21 and tumorigenesis remain to be clarified. However, a comprehensive understanding of the diverse roles of TCF21 in cancer biology will lay the foundations for the use of TCF21 as a diagnostic and prognostic marker and as a therapeutic target for cancer treatment in the clinic.

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Disclosure

The authors report no conflicts of interest in this work.

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