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**Role of T-box transcription factor 3 in gastric cancers**

Asano N *et al*. TBX3 in gastric cancers

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**Abstract**

The expression of T-box transcription factor 3 (TBX3) has been identified in various cancers, including gastric cancers. Its role in breast cancers and melanomas has been intensively studied, and its contribution to the progression of cancers through suppressing senescence and promoting epithelial-mesenchymal transition has been reported. Recent reports on the role of TBX3 in gastric cancers have implied its involvement in gastric carcinogenesis. Considering its pivotal role in the initiation and progression of cancers, TBX3 could be a promising therapeutic target for gastric cancers.

**Key Words:** Aging; Wnt; β-catenin; Transforming growth factor-β; Stomach; Carcinogenesis

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**Core Tip:** Expression of T-box transcription factor 3 (TBX3) has been reported in a variety of cancers. Preceding reports have shown that TBX3 contributes to the progression of cancers by suppressing cellular senescence and promoting epithelial-mesenchymal transition. Recent reports on the role of TBX3 in gastric cancers have implied its involvement in aging-related gastric carcinogenesis.

**INTRODUCTION**

The T-box gene family is involved in embryonic development[1] and is conserved among species[2]. Currently, seventeen transcription factors have been identified as members of the T-box gene family in mammals. The T-box gene family consists of five subfamilies, namely, T, Tbx1, Tbx2, Tbx6, and Tbr1 (Table 1).

T-box transcription factor 3 (TBX3), which belongs to the Tbx2 subfamily, was initially reported as the gene responsible for ulnar-mammary syndrome, an autosomal dominant human development disorder that affects limb, apocrine gland, tooth, hair, and genital development[3]. Studies with genetically engineered mice revealed that Tbx3 homozygous mutant mice were embryonic lethal and exhibited yolk sac defects, lack of mammary glands, and limb defects[4]. Subsequent studies have revealed the involvement of TBX3 in the development of numerous organs, including the heart[5], retina[6], ureter[7], and inner ear[8].

The TBX3 protein consists of 723 amino acids and is encoded by 2169 bp nucleotides in 7 exons. Differential splicing of the second intron leads to the addition of the 2a exon, resulting in the production of the TBX3+2a isoform. Both TBX3 and TBX3+2a are widely expressed in humans and mice, and alternative splicing of *TBX3* was shown to be tissue- and species-specific[9]. TBX3 contains a DNA-binding T-domain[10], two repression domains, and an activation domain[11]. The protein is recruited to the T-box binding sites in the promoter regions of its downstream genes and acts both as a repressor and an activator. The functional similarity between TBX3 and its isoform is still controversial. Fan *et al*[9] reported that the TBX3+2a isoform lacked the ability to bind to the T-box binding site, and that while TBX3 immortalized mouse embryonic fibroblasts, the TBX3+2a isoform accelerated the senescence in those cells. On the other hand, Hoogaars *et al*[12] reported that both TBX3 and TBX3+2a were able to bind to the T-box binding site and inhibit cardiac chamber formation in mouse embryonic hearts. Another report from Zhao *et al*[13] showed that overexpression of either Tbx3 or Tbx3+2a induced the differentiation of mouse embryonic stem cells, but only Tbx3+2a was able to interact directly with Nanog. This discrepancy could be due to the difference in tissues and cells, and future studies are needed to elucidate this issue.

**The function and regulation of TBX3**

As expected from its broad expression, TBX3 has important functions. As mentioned earlier, it plays a crucial role in development. TBX3 binds to DNA through its T-domain, and functions as a repressor or an activator owing to its repression domains and an activation domain. Regarding cell cycle-related molecules, Tbx3 has been reported to repress p19ARF (p14ARF in humans) and inhibit cellular senescence[14,15]. This repression was either regulated through direct binding of TBX3 to the *p14ARF* promoter[15] or through interactions of TBX3 with histone deacetylases (HDAC) 1, 2, 3, and 5[16]. Tbx3 has also been shown to suppress p53[17], while another preceding report demonstrated that it repressed p21CIP1/WAF in a p53-independent manner[18]. In addition, Burgucu *et al*[19] reported that TBX3 suppressed phosphatase and tensin homolog by repressing its promoter activity, which led to augmented phosphatidylinositol-3-kinase activity. Collectively, these findings indicate that TBX3 possesses the ability to enhance cellular proliferation by regulating these molecules.

Several studies have reported that TBX3 suppresses apoptosis in addition to cellular senescence. Huang *et al*[20] showed that knocking down TBX3 in hypopharyngeal cancer cells increased annexin V-positive cells and the level of cleaved caspase 3. Ito *et al*[21] demonstrated that transfection of anti-sense Tbx3 into a rat bladder cancer cell line increased annexin V-positive cells, and the floating cells in the transfected culture exhibited DNA ladders on gel electrophoresis. These two previous studies reported that suppressing TBX3 led to increased apoptosis. On the other hand, Wensing and Campos[22] showed that overexpressing TBX3 and TBX3+2a reduced apoptosis in mesangial cells as assessed by caspase 3 activity. Carlson *et al*[17] also reported the anti-apoptotic function of TBX3 in overexpression experiments showing that transfection of TBX3 rescued primary mouse embryonic fibroblasts from Myc-induced apoptosis. Taken together, these preceding studies demonstrated that TBX3 possesses an anti-apoptotic function.

Another reported function of TBX3 is the repression of E-cadherin, which contributes to the promotion of epithelial-mesenchymal transition (EMT). Rodriguez *et al*[23] showed that TBX3 bound to the T-box binding site in the promoter of the *E-cadherin* gene and repressed E-cadherin expression, which resulted in enhanced invasiveness of melanomas. Dong *et al*[24] also reported that TBX3 repressed E-cadherin expression in hepatocellular carcinomas (HCC), but the repression occurred through the interaction of TBX3 with HDAC5. Peres *et al*[25] demonstrated that phosphorylation of TBX3 by AKT serine/threonine kinase 3 (AKT3) stabilized and promoted the nuclear translocation of TBX3, which was essential for E-cadherin repression. Although the effects were exerted through different mechanisms, these reports demonstrated that TBX3 negatively regulates E-cadherin expression, which can promote tumor invasion and metastasis.

For the regulation of TBX3, TBX3 has been regarded as one of the target genes of the Wnt/β-catenin signaling pathway[7,26-28], and a preceding report showed that β-catenin directly bound to the Tcf binding site in the promoter region of *Tbx3* and induced Tbx3 expression[29]. However, the Wnt/β-catenin signaling pathway is not the only signaling pathway that regulates Tbx3 expression. Transforming growth factor-β (TGF-β) is another signaling molecule that has been shown to induce Tbx3. Li *et al*[30] showed that Smad3 and Smad4, downstream signaling molecules of TGF-β, bound to the Smad-binding element in the *TBX3* promoter together with JunB and enhanced *TBX3* promoter activity. Lee *et al*[31] also reported that TBX3 was upregulated by TGF-β, although they demonstrated that this induction was dependent on the MAPKK-like protein kinase TOPK. Notch signaling has been shown to facilitate the nuclear translocation of Smad3 and activate TGF-β signaling[32,33], and considering that Notch signaling activates Tbx5[34], another member of the Tbx2 subfamily, it is possible that Notch signaling also regulates Tbx3 expression, but further studies are needed to clarify whether Notch signaling regulates TBX3 expression (Figure 1).

**TBX3 in breast cancers and melanomas**

Overexpression of TBX3 has been reported in various cancers[35]. Among them, breast cancers and melanomas are the cancers in which the role of TBX3 has been intensively studied.

Sequencing of 100 primary breast cancers identified driver mutations in several genes, including *TBX3*[36], and another comprehensive study of 817 breast tumors identified that mutations in *TBX3* were enriched in invasive breast cancers[37]. In addition, genomic sequencing of 1918 breast cancers also indicated that alterations in *TBX3* were enriched in breast cancers[38]. These studies demonstrated that TBX3 is one of the key players in breast cancers. Recently, Kostecka *et al*[39] reported that sequencing of cancer-associated genes, including *TBX3,* in normal mammary glands of 52 patients with reportedly sporadic breast cancer revealed that subclonal somatic pathogenic variants of these genes were identified at considerable allelic frequencies. This suggests that TBX3 plays an important role in the initiation of breast cancers.

Functionally, TBX3 has been shown to promote the progression of breast cancers by suppressing cellular senescence and enhancing EMT, as described earlier in this review. However, although overexpression of TBX3 alone accelerated mammary epithelial cell proliferation and led to mammary gland hyperplasia, it did not lead to tumor development[40], which implies that overexpression of TBX3 alone is inadequate to initiate breast cancers.

Preceding studies have also reported the overexpression of TBX3 in melanomas[23,25,41], and interestingly, the constitutively active *B-RAF* mutation observed in melanomas was reported to induce TBX3[42]. Recently, a comprehensive study of 189 cohorts and 178 individual patients identified TBX3 as a marker of poorly differentiated melanomas[43]. Mechanistically, TBX3 was determined to promote tumor progression through inhibition of cellular senescence and promotion of EMT, similar to its role in breast cancers. However, in contrast to its role in breast cancers, the overexpression of TBX3 alone was sufficient to promote the formation and invasion of melanomas[44].

Taken together, the preceding studies suggested that TBX3 promotes tumor progression and invasion by suppressing senescence and enhancing EMT, but whether TBX3 can initiate cancers seems to depend on the type of cancer.

**TBX3 in colorectal cancers, pancreatic cancers, and liver cancers**

The involvement of TBX3 has also been reported in colorectal cancers. A genome-wide meta-analysis revealed the association of polymorphisms in *TBX3* with increased colorectal tumor risk[45]. Shan *et al*[46] reported that aberrant TBX3 expression was associated with a large tumor size, poor differentiation, invasion, lymph node metastasis, and advanced TNM stage in colorectal cancers, resulting in poor prognosis. They also showed through multivariate analysis that TBX3 can independently predict the outcome of colorectal cancer patients.

Similar to the findings in colorectal cancers, augmented TBX3 expression was associated with poor prognosis in pancreatic cancer patients and was reported to be an independent prognostic factor for overall survival[47]. Regarding the mechanism of TBX3, Perkhofer *et al*[48] demonstrated that TBX3 enhanced migration, invasion, and angiogenesis in pancreatic cancers through *in vitro* and *in vivo* studies.

TBX3 has also been reported to be associated with histological grade, tumor size, metastasis, and Ki-67 expression in HCC[49] and the expression of TBX3 in HCC was found to be induced by Wnt/β-catenin signaling[26,29,50]. Interestingly, Tbx3 in the hepatic microenvironment has been reported to play a crucial role in determining the fate of transformed hepatic cells and whether they develop HCC or intrahepatic cholangiocarcinoma[51].

Collectively, these studies demonstrated that TBX3 plays a major role in these cancers.

**TBX3 in gastric cancers**

Concerning gastric cancers, Miao *et al*[52] reported that TBX3 was overexpressed in 46 of 98 primary gastric cancer tissues, and its overexpression correlated with advanced TNM stage and with a higher relapse incidence. In vitro studies demonstrated that overexpression of TBX3 augmented cellular proliferation, whereas knockdown of TBX3 suppressed proliferation in gastric cancer cell lines. Regarding the mechanism involved in TBX3-induced accelerated proliferation, they showed that TBX3 overexpression led to a reduction in the percentage of cells in G1 phase and an increase in the percentage of cells in S and G2 phases in addition to augmented c-Myc and cyclin D1 expression, suggesting that TBX3 facilitated cell cycle progression. The *in vitro* studies also indicated that TBX3 downregulated E-cadherin and induced N-cadherin and vimentin expression, which suggested the enhancement of EMT. This enhancement of proliferation and EMT could be the reason why the expression of TBX3 is associated with advanced tumor stage in gastric cancers, similar to its correlation with poor prognosis in colorectal cancer patients[46].

Takeuchi *et al*[53] recently reported the essential role of Tbx3 in aging-related gastric carcinogenesis. Analysis of gastric organoids established from young and aged mice revealed that cellular proliferation was enhanced in aged gastric organoids due to Tbx3-induced repression of cellular senescence. Aged gastric organoids exhibited suppressed expression of Dickkopf3 (Dkk3), a Wnt antagonist, due to methylation of the *Dkk3* gene, and consequently, the enhanced Wnt/β-catenin signaling induced Tbx3 expression. Epigenetic alterations, such as the methylation of the *Dkk3* gene, are considered as one of the hallmarks of aging[54]. The stochastic process that involves alterations of the methylation state over time is referred to as epigenetic drift and is considered to track biological tissue aging[55]. Indeed, Takeuchi *et al*[53] showed that DKK3 expression in human gastric tissues decreased as the patient aged, whereas TBX3 expression in human gastric tissues exhibited a positive correlation with patient age. Furthermore, they showed that gastric cancer tissues exhibited lower DKK3 expression and higher TBX3 expression than normal oxyntic glands, suggesting the central role of TBX3 in aging-related gastric carcinogenesis.

Another study of gastric precancerous lesions in 449 patients identified *TBX3*, along with *CDX2* and *MYC*, as one of the top 7 core genes that contributed to the progression from low-grade intraepithelial neoplasia to high-grade intraepithelial neoplasia[56], a finding that emphasizes the involvement of TBX3 in the early stage of gastric carcinogenesis.

Taken together, these studies imply that TBX3 plays a pivotal role in aging-related carcinogenesis and the progression of gastric cancers. Further studies are awaited to confirm the role of TBX3 in aging-related gastric carcinogenesis.

**TBX3 as a therapeutic target**

Since TBX3 is expressed in various cancers and possesses the ability to promote the progression of these tumors, it has been considered a therapeutic target in these cancers[57]. As TBX3 has been shown to promote cancer progression, its suppression will be required for therapies. Several microRNAs (miR) have been reported to inhibit TBX3. In adipocytes, miR-93 has been shown to inhibit TBX3 and negatively control adipogenesis[58]. On the other hand, miR-137 was reported to inhibit TBX3 in breast cancers[59] and melanomas[60]. In pancreatic cancers, members of the miR-17-92 cluster have been shown to inhibit TBX3 together with p21 and p57[61]. Furthermore, miR-183 was found to suppress TBX3 and enhanced sensitivity to chemotherapy in laryngeal cancers[62]. These miRNAs could be considered candidates for the treatment of TBX3-expressing cancers.

Concerning chemical reagents, an integrated computational approach indicated that two alkaloids, Jervine and Diflomotecan, can form stable complexes with TBX3 and suggested them as new effective drugs against breast cancers[63]. In another study, an aqueous extract of Fructus ligustri lucidi, a common Chinese herbal medicine, was reported to suppress TBX3 and enhance sensitivity to doxorubicin in colon cancer cells[64].

Recently, Willmer *et al*[65] reported that the multifunctional phosphoprotein nucleolin is required for TBX3 to function and that the nucleolin-targeting aptamer AS1411 exhibited an anticancer effect against sarcomas. These reagents could contribute to anticancer therapy against TBX3-overexpressing cancers.

In addition to its role as a therapeutic target, TBX3 can also contribute to treatment by aiding in the selection of medication for chemotherapy. Freeman *et al*[43] proposed using *TBX3* to predict the outcomes of immune checkpoint inhibitors against melanomas. They showed that patient stratification into risk groups regarding *TBX3* and *MAP4K1* expression was associated with overall survival; hence, evaluating the expression of these genes could enable individualized treatment for each patient. Similar findings may be found in other TBX3-expressing cancers, and further studies are warranted.

**CONCLUSION**

In this review, we discussed the role of TBX3 in cancers. TBX3 is expressed in various cancers and contributes to their progression mainly through the repression of senescence and the promotion of EMT. Given its crucial role in tumor progression, TBX3 could be a promising therapeutic target in malignant tumors, including gastric cancers.

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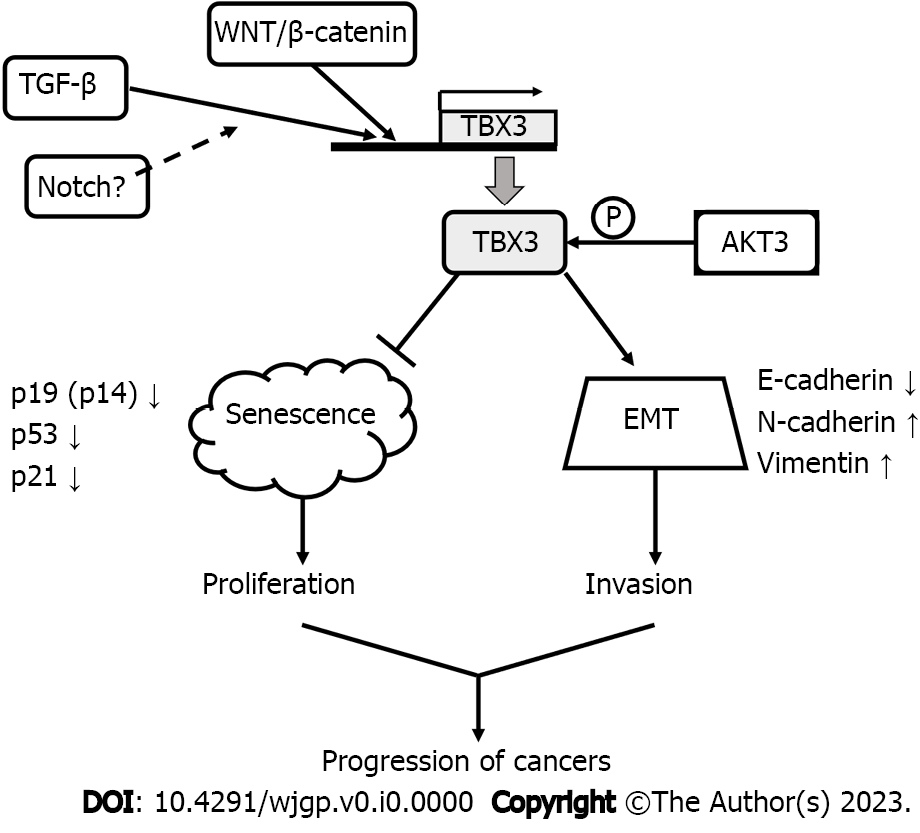
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**Figure Legends**



**Figure 1 The role of T-box transcription factor 3 in cancers.** A schema describing the regulation and function of T-box transcription factor 3 in cancers. P: Phosphorylation; EMT: Epithelial-mesenchymal transition; TGF-β: Transforming growth factor-β; TBX3: T-box transcription factor 3.

**Table 1 T-box gene family**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **T subfamily** | **Tbx1 subfamily** | **Tbx2 subfamily** | **Tbx6 subfamily** | **Tbr1 subfamily** |
| T | Tbx1 | Tbx2 | Tbx6 | Tbr1 |
| Tbx19 (Tpit) | Tbx10 | Tbx3 | Mga | Tbr2 (Eomes) |
|  | Tbx15 | Tbx4 |  | Tbx21 (Tbet) |
|  | Tbx18 | Tbx5 |  |  |
|  | Tbx20 |  |  |  |
|  | Tbx22 |  |  |  |