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

Asano N *et al*. TBX3 in gastric cancers

Naoki Asano, Akira Imatani, Akio Takeuchi, Masashi Saito, Xiao-Yi Jin, Waku Hatta, Kaname Uno, Tomoyuki Koike, Atsushi Masamune

**Naoki Asano, Akira Imatani, Akio Takeuchi, Masashi Saito, Xiao-Yi Jin, Waku Hatta, Kaname Uno, Tomoyuki Koike, Atsushi Masamune,** Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan

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**Corresponding author: Naoki Asano, MD, PhD, Lecturer,** Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi Aoba-ku, Sendai 980-8574, Japan. asanon@med.tohoku.ac.jp

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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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**REFERENCES**

1 **Naiche LA**, Harrelson Z, Kelly RG, Papaioannou VE. T-box genes in vertebrate development. *Annu Rev Genet* 2005; **39**: 219-239 [PMID: 16285859 DOI: 10.1146/annurev.genet.39.073003.105925]

2 **Papaioannou VE**. T-box genes in development: from hydra to humans. *Int Rev Cytol* 2001; **207**: 1-70 [PMID: 11352264 DOI: 10.1016/s0074-7696(01)07002-4]

3 **Bamshad M**, Lin RC, Law DJ, Watkins WC, Krakowiak PA, Moore ME, Franceschini P, Lala R, Holmes LB, Gebuhr TC, Bruneau BG, Schinzel A, Seidman JG, Seidman CE, Jorde LB. Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nat Genet* 1997; **16**: 311-315 [PMID: 9207801 DOI: 10.1038/ng0797-311]

4 **Davenport TG**, Jerome-Majewska LA, Papaioannou VE. Mammary gland, limb and yolk sac defects in mice lacking Tbx3, the gene mutated in human ulnar mammary syndrome. *Development* 2003; **130**: 2263-2273 [PMID: 12668638 DOI: 10.1242/dev.00431]

5 **Singh R**, Hoogaars WM, Barnett P, Grieskamp T, Rana MS, Buermans H, Farin HF, Petry M, Heallen T, Martin JF, Moorman AF, 't Hoen PA, Kispert A, Christoffels VM. Tbx2 and Tbx3 induce atrioventricular myocardial development and endocardial cushion formation. *Cell Mol Life Sci* 2012; **69**: 1377-1389 [PMID: 22130515 DOI: 10.1007/s00018-011-0884-2]

6 **Motahari Z**, Martinez-De Luna RI, Viczian AS, Zuber ME. Tbx3 represses bmp4 expression and, with Pax6, is required and sufficient for retina formation. *Development* 2016; **143**: 3560-3572 [PMID: 27578778 DOI: 10.1242/dev.130955]

7 **Aydoğdu N**, Rudat C, Trowe MO, Kaiser M, Lüdtke TH, Taketo MM, Christoffels VM, Moon A, Kispert A. TBX2 and TBX3 act downstream of canonical WNT signaling in patterning and differentiation of the mouse ureteric mesenchyme. *Development* 2018; **145**:dev171827 [PMID: 30478225 DOI: 10.1242/dev.171827]

8 **Kaiser M**, Wojahn I, Rudat C, Lüdtke TH, Christoffels VM, Moon A, Kispert A, Trowe MO. Regulation of otocyst patterning by Tbx2 and Tbx3 is required for inner ear morphogenesis in the mouse. *Development* 2021; **148**: dev195651 [PMID: 33795231 DOI: 10.1242/dev.195651]

9 **Fan W**, Huang X, Chen C, Gray J, Huang T. TBX3 and its isoform TBX3+2a are functionally distinctive in inhibition of senescence and are overexpressed in a subset of breast cancer cell lines. *Cancer Res* 2004; **64**: 5132-5139 [PMID: 15289316 DOI: 10.1158/0008-5472.CAN-04-0615]

10 **Müller CW**, Herrmann BG. Crystallographic structure of the T domain-DNA complex of the Brachyury transcription factor. *Nature* 1997; **389**: 884-888 [PMID: 9349824 DOI: 10.1038/39929]

11 **Carlson H**, Ota S, Campbell CE, Hurlin PJ. A dominant repression domain in Tbx3 mediates transcriptional repression and cell immortalization: relevance to mutations in Tbx3 that cause ulnar-mammary syndrome. *Hum Mol Genet* 2001; **10**: 2403-2413 [PMID: 11689487 DOI: 10.1093/hmg/10.21.2403]

12 **Hoogaars WM**, Barnett P, Rodriguez M, Clout DE, Moorman AF, Goding CR, Christoffels VM. TBX3 and its splice variant TBX3 + exon 2a are functionally similar. *Pigment Cell Melanoma Res* 2008; **21**: 379-387 [PMID: 18444963 DOI: 10.1111/j.1755-148X.2008.00461.x]

13 **Zhao D**, Wu Y, Chen K. Tbx3 isoforms are involved in pluripotency maintaining through distinct regulation of Nanog transcriptional activity. *Biochem Biophys Res Commun* 2014; **444**: 411-414 [PMID: 24472544 DOI: 10.1016/j.bbrc.2014.01.093]

14 **Brummelkamp TR**, Kortlever RM, Lingbeek M, Trettel F, MacDonald ME, van Lohuizen M, Bernards R. TBX-3, the gene mutated in Ulnar-Mammary Syndrome, is a negative regulator of p19ARF and inhibits senescence. *J Biol Chem* 2002; **277**: 6567-6572 [PMID: 11748239 DOI: 10.1074/jbc.M110492200]

15 **Lingbeek ME**, Jacobs JJ, van Lohuizen M. The T-box repressors TBX2 and TBX3 specifically regulate the tumor suppressor gene p14ARF via a variant T-site in the initiator. *J Biol Chem* 2002; **277**: 26120-26127 [PMID: 12000749 DOI: 10.1074/jbc.M200403200]

16 **Yarosh W**, Barrientos T, Esmailpour T, Lin L, Carpenter PM, Osann K, Anton-Culver H, Huang T. TBX3 is overexpressed in breast cancer and represses p14 ARF by interacting with histone deacetylases. *Cancer Res* 2008; **68**: 693-699 [PMID: 18245468 DOI: 10.1158/0008-5472.CAN-07-5012]

17 **Carlson H**, Ota S, Song Y, Chen Y, Hurlin PJ. Tbx3 impinges on the p53 pathway to suppress apoptosis, facilitate cell transformation and block myogenic differentiation. *Oncogene* 2002; **21**: 3827-3835 [PMID: 12032820 DOI: 10.1038/sj.onc.1205476]

18 **Platonova N**, Scotti M, Babich P, Bertoli G, Mento E, Meneghini V, Egeo A, Zucchi I, Merlo GR. TBX3, the gene mutated in ulnar-mammary syndrome, promotes growth of mammary epithelial cells via repression of p19ARF, independently of p53. *Cell Tissue Res* 2007; **328**: 301-316 [PMID: 17265068 DOI: 10.1007/s00441-006-0364-4]

19 **Burgucu D**, Guney K, Sahinturk D, Ozbudak IH, Ozel D, Ozbilim G, Yavuzer U. Tbx3 represses PTEN and is over-expressed in head and neck squamous cell carcinoma. *BMC Cancer* 2012; **12**: 481 [PMID: 23082988 DOI: 10.1186/1471-2407-12-481]

20 **Huang Y**, Zhu H, Ji X, Chen Y, Zhang Y, Huang R, Xie J, Dong P. TBX3 knockdown suppresses the proliferation of hypopharyngeal carcinoma FaDu cells by inducing G1/S cell cycle arrest and apoptosis. *Oncol Lett* 2020; **19**: 113-120 [PMID: 31897121 DOI: 10.3892/ol.2019.11089]

21 **Ito A**, Asamoto M, Hokaiwado N, Takahashi S, Shirai T. Tbx3 expression is related to apoptosis and cell proliferation in rat bladder both hyperplastic epithelial cells and carcinoma cells. *Cancer Lett* 2005; **219**: 105-112 [PMID: 15694670 DOI: 10.1016/j.canlet.2004.07.051]

22 **Wensing LA**, Campos AH. TBX3, a downstream target of TGF-β1, inhibits mesangial cell apoptosis. *Exp Cell Res* 2014; **328**: 340-350 [PMID: 25158279 DOI: 10.1016/j.yexcr.2014.08.022]

23 **Rodriguez M**, Aladowicz E, Lanfrancone L, Goding CR. Tbx3 represses E-cadherin expression and enhances melanoma invasiveness. *Cancer Res* 2008; **68**: 7872-7881 [PMID: 18829543 DOI: 10.1158/0008-5472.CAN-08-0301]

24 **Dong L**, Lyu X, Faleti OD, He ML. The special stemness functions of Tbx3 in stem cells and cancer development. *Semin Cancer Biol* 2019; **57**: 105-110 [PMID: 30268432 DOI: 10.1016/j.semcancer.2018.09.010]

25 **Peres J**, Mowla S, Prince S. The T-box transcription factor, TBX3, is a key substrate of AKT3 in melanomagenesis. *Oncotarget* 2015; **6**: 1821-1833 [PMID: 25595898 DOI: 10.18632/oncotarget.2782]

26 **Liang B**, Zhou Y, Qian M, Xu M, Wang J, Zhang Y, Song X, Wang H, Lin S, Ren C, Monga SP, Wang B, Evert M, Chen Y, Chen X, Huang Z, Calvisi DF, Chen X. TBX3 functions as a tumor suppressor downstream of activated CTNNB1 mutants during hepatocarcinogenesis. *J Hepatol* 2021; **75**: 120-131 [PMID: 33577921 DOI: 10.1016/j.jhep.2021.01.044]

27 **Jin Y**, Anbarchian T, Wu P, Sarkar A, Fish M, Peng WC, Nusse R. Wnt signaling regulates hepatocyte cell division by a transcriptional repressor cascade. *Proc Natl Acad Sci U S A* 2022; **119**: e2203849119 [PMID: 35867815 DOI: 10.1073/pnas.2203849119]

28 **Asano N**, Takeuchi A, Imatani A, Saito M, Jin X, Hatta W, Uno K, Koike T, Masamune A. Wnt Signaling and Aging of the Gastrointestinal Tract. *Int J Mol Sci* 2022; **23**: 12210 [PMID: 36293064 DOI: 10.3390/ijms232012210]

29 **Renard CA**, Labalette C, Armengol C, Cougot D, Wei Y, Cairo S, Pineau P, Neuveut C, de Reyniès A, Dejean A, Perret C, Buendia MA. Tbx3 is a downstream target of the Wnt/beta-catenin pathway and a critical mediator of beta-catenin survival functions in liver cancer. *Cancer Res* 2007; **67**: 901-910 [PMID: 17283120 DOI: 10.1158/0008-5472.CAN-06-2344]

30 **Li J**, Weinberg MS, Zerbini L, Prince S. The oncogenic TBX3 is a downstream target and mediator of the TGF-β1 signaling pathway. *Mol Biol Cell* 2013; **24**: 3569-3576 [PMID: 24025717 DOI: 10.1091/mbc.E13-05-0273]

31 **Lee YJ**, Park JH, Oh SM. TOPK promotes epithelial-mesenchymal transition and invasion of breast cancer cells through upregulation of TBX3 in TGF-β1/Smad signaling. *Biochem Biophys Res Commun* 2020; **522**: 270-277 [PMID: 31757421 DOI: 10.1016/j.bbrc.2019.11.104]

32 **Asano N**, Watanabe T, Kitani A, Fuss IJ, Strober W. Notch1 signaling and regulatory T cell function. *J Immunol* 2008; **180**: 2796-2804 [PMID: 18292500 DOI: 10.4049/jimmunol.180.5.2796]

33 **Luo K**. Signaling Cross Talk between TGF-β/Smad and Other Signaling Pathways. *Cold Spring Harb Perspect Biol* 2017; **9**: a022137 [PMID: 27836834 DOI: 10.1101/cshperspect.a022137]

34 **Rentschler S**, Yen AH, Lu J, Petrenko NB, Lu MM, Manderfield LJ, Patel VV, Fishman GI, Epstein JA. Myocardial Notch signaling reprograms cardiomyocytes to a conduction-like phenotype. *Circulation* 2012; **126**: 1058-1066 [PMID: 22837163 DOI: 10.1161/CIRCULATIONAHA.112.103390]

35 **Wansleben S**, Peres J, Hare S, Goding CR, Prince S. T-box transcription factors in cancer biology. *Biochim Biophys Acta* 2014; **1846**: 380-391 [PMID: 25149433 DOI: 10.1016/j.bbcan.2014.08.004]

36 **Stephens PJ**, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, Nik-Zainal S, Martin S, Varela I, Bignell GR, Yates LR, Papaemmanuil E, Beare D, Butler A, Cheverton A, Gamble J, Hinton J, Jia M, Jayakumar A, Jones D, Latimer C, Lau KW, McLaren S, McBride DJ, Menzies A, Mudie L, Raine K, Rad R, Chapman MS, Teague J, Easton D, Langerød A; Oslo Breast Cancer Consortium (OSBREAC), Lee MT, Shen CY, Tee BT, Huimin BW, Broeks A, Vargas AC, Turashvili G, Martens J, Fatima A, Miron P, Chin SF, Thomas G, Boyault S, Mariani O, Lakhani SR, van de Vijver M, van 't Veer L, Foekens J, Desmedt C, Sotiriou C, Tutt A, Caldas C, Reis-Filho JS, Aparicio SA, Salomon AV, Børresen-Dale AL, Richardson AL, Campbell PJ, Futreal PA, Stratton MR. The landscape of cancer genes and mutational processes in breast cancer. *Nature* 2012; **486**: 400-404 [PMID: 22722201 DOI: 10.1038/nature11017]

37 **Ciriello G**, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, Bowlby R, Shen H, Hayat S, Fieldhouse R, Lester SC, Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB, Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW, Hoadley KA, King TA; TCGA Research Network, Perou CM. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell* 2015; **163**: 506-519 [PMID: 26451490 DOI: 10.1016/j.cell.2015.09.033]

38 **Razavi P**, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, Cai Y, Bielski CM, Donoghue MTA, Jonsson P, Penson A, Shen R, Pareja F, Kundra R, Middha S, Cheng ML, Zehir A, Kandoth C, Patel R, Huberman K, Smyth LM, Jhaveri K, Modi S, Traina TA, Dang C, Zhang W, Weigelt B, Li BT, Ladanyi M, Hyman DM, Schultz N, Robson ME, Hudis C, Brogi E, Viale A, Norton L, Dickler MN, Berger MF, Iacobuzio-Donahue CA, Chandarlapaty S, Scaltriti M, Reis-Filho JS, Solit DB, Taylor BS, Baselga J. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. *Cancer Cell* 2018; **34**: 427-438.e6 [PMID: 30205045 DOI: 10.1016/j.ccell.2018.08.008]

39 **Kostecka A**, Nowikiewicz T, Olszewski P, Koczkowska M, Horbacz M, Heinzl M, Andreou M, Salazar R, Mair T, Madanecki P, Gucwa M, Davies H, Skokowski J, Buckley PG, Pęksa R, Śrutek E, Szylberg Ł, Hartman J, Jankowski M, Zegarski W, Tiemann-Boege I, Dumanski JP, Piotrowski A. High prevalence of somatic PIK3CA and TP53 pathogenic variants in the normal mammary gland tissue of sporadic breast cancer patients revealed by duplex sequencing. *NPJ Breast Cancer* 2022; **8**: 76 [PMID: 35768433 DOI: 10.1038/s41523-022-00443-9]

40 **Liu J**, Esmailpour T, Shang X, Gulsen G, Liu A, Huang T. TBX3 over-expression causes mammary gland hyperplasia and increases mammary stem-like cells in an inducible transgenic mouse model. *BMC Dev Biol* 2011; **11**: 65 [PMID: 22039763 DOI: 10.1186/1471-213X-11-65]

41 **Peres J**, Damerell V, Chauhan J, Popovic A, Desprez PY, Galibert MD, Goding CR, Prince S. TBX3 Promotes Melanoma Migration by Transcriptional Activation of ID1, which Prevents Activation of E-Cadherin by MITF. *J Invest Dermatol* 2021; **141**: 2250-2260.e2 [PMID: 33744299 DOI: 10.1016/j.jid.2021.02.740]

42 **Boyd SC**, Mijatov B, Pupo GM, Tran SL, Gowrishankar K, Shaw HM, Goding CR, Scolyer RA, Mann GJ, Kefford RF, Rizos H, Becker TM. Oncogenic B-RAF(V600E) signaling induces the T-Box3 transcriptional repressor to repress E-cadherin and enhance melanoma cell invasion. *J Invest Dermatol* 2013; **133**: 1269-1277 [PMID: 23190890 DOI: 10.1038/jid.2012.421]

43 **Freeman SS**, Sade-Feldman M, Kim J, Stewart C, Gonye ALK, Ravi A, Arniella MB, Gushterova I, LaSalle TJ, Blaum EM, Yizhak K, Frederick DT, Sharova T, Leshchiner I, Elagina L, Spiro OG, Livitz D, Rosebrock D, Aguet F, Carrot-Zhang J, Ha G, Lin Z, Chen JH, Barzily-Rokni M, Hammond MR, Vitzthum von Eckstaedt HC, Blackmon SM, Jiao YJ, Gabriel S, Lawrence DP, Duncan LM, Stemmer-Rachamimov AO, Wargo JA, Flaherty KT, Sullivan RJ, Boland GM, Meyerson M, Getz G, Hacohen N. Combined tumor and immune signals from genomes or transcriptomes predict outcomes of checkpoint inhibition in melanoma. *Cell Rep Med* 2022; **3**: 100500 [PMID: 35243413 DOI: 10.1016/j.xcrm.2021.100500]

44 **Peres J**, Prince S. The T-box transcription factor, TBX3, is sufficient to promote melanoma formation and invasion. *Mol Cancer* 2013; **12**: 117 [PMID: 24098938 DOI: 10.1186/1476-4598-12-117]

45 **Peters U**, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, Berndt SI, Bézieau S, Brenner H, Butterbach K, Caan BJ, Campbell PT, Carlson CS, Casey G, Chan AT, Chang-Claude J, Chanock SJ, Chen LS, Coetzee GA, Coetzee SG, Conti DV, Curtis KR, Duggan D, Edwards T, Fuchs CS, Gallinger S, Giovannucci EL, Gogarten SM, Gruber SB, Haile RW, Harrison TA, Hayes RB, Henderson BE, Hoffmeister M, Hopper JL, Hudson TJ, Hunter DJ, Jackson RD, Jee SH, Jenkins MA, Jia WH, Kolonel LN, Kooperberg C, Küry S, Lacroix AZ, Laurie CC, Laurie CA, Le Marchand L, Lemire M, Levine D, Lindor NM, Liu Y, Ma J, Makar KW, Matsuo K, Newcomb PA, Potter JD, Prentice RL, Qu C, Rohan T, Rosse SA, Schoen RE, Seminara D, Shrubsole M, Shu XO, Slattery ML, Taverna D, Thibodeau SN, Ulrich CM, White E, Xiang Y, Zanke BW, Zeng YX, Zhang B, Zheng W, Hsu L; Colon Cancer Family Registry and the Genetics and Epidemiology of Colorectal Cancer Consortium. Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. *Gastroenterology* 2013; **144**: 799-807.e24 [PMID: 23266556 DOI: 10.1053/j.gastro.2012.12.020]

46 **Shan ZZ**, Yan XB, Yan LL, Tian Y, Meng QC, Qiu WW, Zhang Z, Jin ZM. Overexpression of Tbx3 is correlated with Epithelial-Mesenchymal Transition phenotype and predicts poor prognosis of colorectal cancer. *Am J Cancer Res* 2015; **5**: 344-353 [PMID: 25628943]

47 **Wang HC**, Meng QC, Shan ZZ, Yuan Z, Huang XY. Overexpression of Tbx3 predicts poor prognosis of patients with resectable pancreatic carcinoma. *Asian Pac J Cancer Prev* 2015; **16**: 1397-1401 [PMID: 25743805 DOI: 10.7314/apjcp.2015.16.4.1397]

48 **Perkhofer L**, Walter K, Costa IG, Carrasco MC, Eiseler T, Hafner S, Genze F, Zenke M, Bergmann W, Illing A, Hohwieler M, Köhntop R, Lin Q, Holzmann KH, Seufferlein T, Wagner M, Liebau S, Hermann PC, Kleger A, Müller M. Tbx3 fosters pancreatic cancer growth by increased angiogenesis and activin/nodal-dependent induction of stemness. *Stem Cell Res* 2016; **17**: 367-378 [PMID: 27632063 DOI: 10.1016/j.scr.2016.08.007]

49 **Li Z**, Wang Y, Duan S, Shi Y, Li S, Zhang X, Ren J. Expression of TBX3 in Hepatocellular Carcinoma and Its Clinical Implication. *Med Sci Monit* 2018; **24**: 9324-9333 [PMID: 30578408 DOI: 10.12659/MSM.909378]

50 **Lachenmayer A**, Alsinet C, Savic R, Cabellos L, Toffanin S, Hoshida Y, Villanueva A, Minguez B, Newell P, Tsai HW, Barretina J, Thung S, Ward SC, Bruix J, Mazzaferro V, Schwartz M, Friedman SL, Llovet JM. Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. *Clin Cancer Res* 2012; **18**: 4997-5007 [PMID: 22811581 DOI: 10.1158/1078-0432.CCR-11-2322]

51 **Seehawer M**, Heinzmann F, D'Artista L, Harbig J, Roux PF, Hoenicke L, Dang H, Klotz S, Robinson L, Doré G, Rozenblum N, Kang TW, Chawla R, Buch T, Vucur M, Roth M, Zuber J, Luedde T, Sipos B, Longerich T, Heikenwälder M, Wang XW, Bischof O, Zender L. Necroptosis microenvironment directs lineage commitment in liver cancer. *Nature* 2018; **562**: 69-75 [PMID: 30209397 DOI: 10.1038/s41586-018-0519-y]

52 **Miao ZF**, Liu XY, Xu HM, Wang ZN, Zhao TT, Song YX, Xing YN, Huang JY, Zhang JY, Xu H, Xu YY. Tbx3 overexpression in human gastric cancer is correlated with advanced tumor stage and nodal status and promotes cancer cell growth and invasion. *Virchows Arch* 2016; **469**: 505-513 [PMID: 27553355 DOI: 10.1007/s00428-016-2007-9]

53 **Takeuchi A,** Asano N, Imatani A, Saito M, Jin X, Saito M, Kanno T, Hatta W, Uno K, Koike T, Masamune A. Suppressed cellular senescence mediated by T-box3 in aged gastric epithelial cells may contribute to aging-related carcinogenesis. *Cancer Res Commun* 2022; **2**: 772-783 [DOI: 10.1158/2767-9764.CRC-22-0084]

54 **López-Otín C**, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013; **153**: 1194-1217 [PMID: 23746838 DOI: 10.1016/j.cell.2013.05.039]

55 **Yu M**, Hazelton WD, Luebeck GE, Grady WM. Epigenetic Aging: More Than Just a Clock When It Comes to Cancer. *Cancer Res* 2020; **80**: 367-374 [PMID: 31694907 DOI: 10.1158/0008-5472.CAN-19-0924]

56 **Jin XX**, Xie XL, Niu F, Yin KG, Ji CG, Cui JF, Liu L, Feng ZJ. A Single-Center Follow-Up Study of Low-Grade Gastric Intraepithelial Neoplasia and the Screening of Key Genes of Precancerous Lesions. *Front Oncol* 2022; **12**: 899055 [PMID: 35847930 DOI: 10.3389/fonc.2022.899055]

57 **Lu J**, Li XP, Dong Q, Kung HF, He ML. TBX2 and TBX3: the special value for anticancer drug targets. *Biochim Biophys Acta* 2010; **1806**: 268-274 [PMID: 20624445 DOI: 10.1016/j.bbcan.2010.07.001]

58 **Cioffi M**, Vallespinos-Serrano M, Trabulo SM, Fernandez-Marcos PJ, Firment AN, Vazquez BN, Vieira CR, Mulero F, Camara JA, Cronin UP, Perez M, Soriano J, G Galvez B, Castells-Garcia A, Haage V, Raj D, Megias D, Hahn S, Serrano L, Moon A, Aicher A, Heeschen C. MiR-93 Controls Adiposity via Inhibition of Sirt7 and Tbx3. *Cell Rep* 2015; **12**: 1594-1605 [PMID: 26321631 DOI: 10.1016/j.celrep.2015.08.006]

59 **Lee JM**, Cho KW, Kim EJ, Tang Q, Kim KS, Tickle C, Jung HS. A contrasting function for miR-137 in embryonic mammogenesis and adult breast carcinogenesis. *Oncotarget* 2015; **6**: 22048-22059 [PMID: 26215676 DOI: 10.18632/oncotarget.4218]

60 **Peres J**, Kwesi-Maliepaard EM, Rambow F, Larue L, Prince S. The tumour suppressor, miR-137, inhibits malignant melanoma migration by targetting the TBX3 transcription factor. *Cancer Lett* 2017; **405**: 111-119 [PMID: 28757416 DOI: 10.1016/j.canlet.2017.07.018]

61 **Cioffi M**, Trabulo SM, Sanchez-Ripoll Y, Miranda-Lorenzo I, Lonardo E, Dorado J, Reis Vieira C, Ramirez JC, Hidalgo M, Aicher A, Hahn S, Sainz B Jr, Heeschen C. The miR-17-92 cluster counteracts quiescence and chemoresistance in a distinct subpopulation of pancreatic cancer stem cells. *Gut* 2015; **64**: 1936-1948 [PMID: 25887381 DOI: 10.1136/gutjnl-2014-308470]

62 **Lou G**, Huang JCLWYYY. Biological functions of miR-183 on chemosensitivity of laryngeal cancer cells. *J BUON* 2021; **26**: 785-791 [PMID: 34268937]

63 **Paul A**, Limon BH, Hossain M, Raza T. An integrated computational approach to screening of alkaloids inhibitors of TBX3 in breast cancer cell lines. *J Biomol Struct Dyn* 2022: 1-17 [PMID: 35253621 DOI: 10.1080/07391102.2022.2046166]

64 **Zhang JF**, He ML, Qi Dong, Xie WD, Chen YC, Lin MC, Leung PC, Zhang YO, Kung HF. Aqueous extracts of Fructus Ligustri Lucidi enhance the sensitivity of human colorectal carcinoma DLD-1 cells to doxorubicin-induced apoptosis via Tbx3 suppression. *Integr Cancer Ther* 2011; **10**: 85-91 [PMID: 20702496 DOI: 10.1177/1534735410373921]

65 **Willmer T**, Damerell V, Smyly S, Sims D, Du Toit M, Ncube S, Sinkala M, Govender D, Sturrock E, Blackburn JM, Prince S. Targeting the oncogenic TBX3:nucleolin complex to treat multiple sarcoma subtypes. *Am J Cancer Res* 2021; **11**: 5680-5700 [PMID: 34873487]

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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 |  |  |  |