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Review

TBX2 and TBX3: The special value for anticancer drug targets

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\section*{A B S T R A C T}

TBX2 and TBX3 are members of the T-box family of transcription factors, which are implicated in embryonic development. Unlike most members of the T-box family, TBX2 and TBX3 are the only mammalian T-box factors which function as transcriptional repressors, mediated by the repression domain in the C-terminal. In addition to a role in development, recent evidence suggests that TBX2 and TBX3 are overexpressed in a number of cancers, including melanoma, breast, liver, lung, pancreas, ovarian, and cervical cancers. However, there is little information about the mechanisms for how these T-box genes contribute to tumorigenesis. Upregulation of TBX2 and TBX3 suppresses the expression of p14\textsuperscript{ARF} and p21\textsuperscript{CIP1} and promotes bypass of senescence through inactivation of p53 pathway. TBX2 functionally interacts with pRb, and pRb modulates TBX2 functional specificity. In addition, TBX2 is a player of Wnt signaling while TBX3 is a downstream target of the Wnt/beta-catenin pathway, and overexpression of TBX2 and TBX3 represses the expression of E-cadherin, which is demonstrated to be a prerequisite for epithelial tumor cell invasion. Moreover, TBX2 is shown to interact with EGR1 to block multiple downstream tumor suppressors. Here, we review the current knowledge on TBX2 and TBX3 in tumorigenesis and prospect their special value for development of target-based anticancer drugs.

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\section*{Contents}

1. Introduction .............................................................. 268
2. The T-box gene family ......................................................... 269
3. The T domain and the repression domain (RD).............................................. 269
4. TBX2/TBX3 and cancer ......................................................... 270
5. Roles of TBX2/TBX3 in tumorigenesis .................................................. 270
\hspace{1em} 5.1. p14\textsuperscript{ARF} (p19\textsuperscript{ARF})/MDM2/p53/p21\textsuperscript{CIP1} pathway .......................... 270
\hspace{1em} 5.2. pRb pathway ........................................................ 271
\hspace{1em} 5.3. Wnt/beta-catenin pathway .............................................. 271
\hspace{1em} 5.4. EGR1 signaling pathway ............................................. 272
6. Potential targets of cancer therapy. .............................................. 272
7. Concluding remarks and perspectives .................................................. 272
Acknowledgement .............................................................. 272
References ................................................................. 272

Abbreviations: CDKs, cyclin-dependent kinases; EGR1, early growth response 1; FGF, fibroblast growth factor; MEFs, mouse embryonic fibroblasts; RD, repression domain; RNAi, RNA interference; siRNA, small interfering RNA; TGFβ, transforming growth factor β; UMS, ulnar-mammary syndrome; CTCL, cutaneous T-cell lymphoma

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1. Introduction

The T-box gene family is an ancient gene family as indicated by phylogenetic analysis. This family has achieved great prominence in the field of developmental biology. Since the discovery of the first T-box factor, Brachyury, many studies have focused on the cloning of other family members and the determining their spatial and temporal expression patterns during development [1]. Later studies have
shown that the T-box family members are transcription factors, which are characterized by a highly conserved DNA binding domain. Unlike most members of the T-box family that function as transcriptional activators, TBX2 and TBX3 are the only mammalian T-box factors that function as transcriptional repressors [2,3]. Increasing efforts have been made to reveal the roles of TBX2 and TBX3 in cancer, and the data appear to suggest that they are potential targets in cancer therapy.

2. The T-box gene family

The T-box gene family encodes transcription factors which play a critical role in embryonic development. The first T-box member, Brachyury (T), has been shown to be involved in development since 1927 [4]. The prototypical mouse T (Brachyury) gene was cloned by Herrmann et al. in 1990 [5]. In 1992, the omb gene in Droso phila melanogaster was found to share homology with Brachyury in the DNA binding domain [6]. Since the discovery of mouse T gene and its orthologs, more than 20 TBX genes have been identified in species ranging from invertebrates such as Droso phila and C. elegans to vertebrates including Zebra fish, Xenopus, mouse, chicken and human. These T-box genes are highly conserved in evolution and achieve great prominence in the field of developmental biology.

Current phylogenetic analysis divides the T-box family into five subfamilies, namely T, TBX1, TBX2, TBX6 and TBX11 [7]. The TBX2 subfamily includes the closely related TBX2, TBX3, TBX4 and TBX5 genes. In human, there is a TBX2 and TBX4 cluster on chromosome 17q23 and a TBX3 and TBX5 cluster on chromosome 12q24 [8]. However, because of the unusual manner of tandem duplication and cluster dispersion, TBX2 on 17q23 is actually most closely related to TBX3 on 12q24, while TBX4 shares greatest homology with TBX5.

To date, the majority of information available concerning TBX2 and TBX3, like other T-box proteins, comes mainly from developmental biology. Several studies have focused on the spatial and temporal expression patterns of TBX2/TBX3 during development, and their functions ranging from the specification of early embryonic germ layers to the determination of cell fate during organogenesis [9]. Indeed, the critical role of T-box genes in development is underscored by the fact that mutations in several T-box genes are associated with a number of developmental defects. In human, mutations in TBX3 and TBX5 would lead to autosomal dominant or semi-dominant genetic syndromes. Mutations in TBX3 cause an autosomal dominant disorder, the ulnar-mammary syndrome (UMS) [10], and mutations in TBX5 lead to Holt-Oram’s syndrome, with characteristic defects in the heart and limb [11,12].

Very little is known about the signaling pathways which regulate the expression of T-box genes during early embryonic development, and even less is known about the complex regulation of their expression post-embryogenesis. However, over the last few years, it has been demonstrated that several signaling pathways are closely associated with the expression of TBX2/TBX3, including transforming growth factor β (TGFβ), fibroblast growth factor (FGF) and Wnt signaling pathways [13], which are key players in cell proliferation, differentiation, and patterning during early embryonic development. In a recent study, TBX2 has been shown to be specifically phosphorylated by the p38 mitogen-activated protein kinase in response to UV C irradiation [14].

3. The T domain and the repression domain (RD)

The hallmark feature of the T-box family is a highly conserved region of approximately 200 amino acid residues corresponding to the DNA binding domain known as the T-box. The T-box is located anywhere within the protein and has no apparent sequence similarity to any known DNA-binding motif of other transcription factors [15]. The first member of the T-box family, Brachyury (T), has been the most extensively studied by far and utilized as a model for elucidating the structure and function of other T-box genes. For Brachyury, sequence analysis revealed a palindromic sequence [TTT(G/C)ACACC-TAGGTGTGAAA] to which Brachyury could bind as a monomer or a dimer [16]. All members identified to date have been shown to bind to the core sequence GGTGTGA, referred to as the T-element, as a monomer [15]. However, some members have been shown to recognize other non-canonical sequences. In Holt-Oram’s syndrome TBX5 was found to bind to an 8-bp core sequence that was part of the Brachyury consensus-binding sites [17]. In addition, TBX2 was reported to bind to the TRP-1 MSEu and MSEi motif as a repressor [3]. Another study identified a variant T-site, matching 13 of 20 nucleotides of a consensus T-domain, as the essential TBX2/3-binding site in the human p14 promoter [18]. Although all T-box genes recognize similar DNA sequences, increasing evidence suggests that they regulate different target genes which may

Fig. 1. The highly conserved residues in TBX2 and TBX3 repression domain (RD). The identical residues in TBX2 or TBX3 are shown with red color; the conserved residues are shown with green color while variable residues are shown with black color.
be dependent on the promoter context and co-factors, such as different quaternary structures of T-box genes, which suggest different DNA binding modes and interactions with different target genes [19–21]. It is believed that the functions of T-box proteins are regulated by protein-protein interactions with other transcription factors, including TAZ, YAP, SMADs, EGR1 and Pitx homeoproteins, and many studies have indeed proven this to be the case [22–28].

Unlike most members of the T-box family that function as transcription activators, TBX2 and TBX3 are the only mammalian T-box factors that function as transcriptional repressors [2,3]. The study of He et al. showed that the Xenopus TBX3 ortholog, ET, had a strong RD from residues 558 to 647 in the C-terminal, and the repression activity of this domain was quite similar to that of the full-length ET in both basal and activated transcription in human cell lines and Xenopus embryos. They also found that this RD was a portable repressor of both basal and activated transcription in human cell lines and the activity of this domain was quite similar to that of the full-length ET in Xenopus.

TBX2 and TBX3 can suppress several genes to alter downstream pathways and many studies have indeed proven this to be the case [22–28]. A few years later, the study of Carlson et al. confirmed and extended these results. Their study revealed a key RD between amino acids 567 and 612 in human TBX3. Additionally, they showed that most UMS-associated C-terminal mutants lacked this RD and exhibited decreased or loss of transcriptional repression activity [29]. Moreover, recent evidence has shown that the unique RD, composed of about 90–110 residues among different species, is found only in TBX2 and TBX3 and does not share any conserved sequence or motif with other known RDs (Dong et al., unpublished). Following a multiple alignment of TBX2 and TBX3 sequences among different species (i.e. ranged from zebrafish to human), they find that only 56 residues (between amino acids 558–622) are highly conserved (Fig. 1). The study of Yarosh et al. have shown that TBX3 interacts with HDAC1, 2, and 3 via two distinct binding sites, and deletion of the RD (amino acids 566–624) of TBX3 completely abolishes its interaction with HDAC5 [30]. This study suggests that TBX3 may function by recruiting HDACs to the RD in the promoter region. Therefore, detailed analysis on RD would help to further understand the molecular mechanisms of transcriptional repression mediated by TBX2 and TBX3.

4. TBX2/TBX3 and cancer

In addition to its key roles in development, accumulating evidence suggests a role of TBX2/TBX3 in tumorigenesis. In Fig. 2, we summarized the current findings of TBX2 and TBX3 and their associated signaling pathways involved in cancer development. The abnormal expression of TBX2 and TBX3 has been reported in various cancers (Table 1), including melanoma, breast, pancreatic, liver, lung, ovarian, bladder and cervical cancers [28,30–47]. TBX2 is shown to be amplified and upregulated in a subset of breast cancer cell lines, and BRCA1 and BRCA2 mutant breast tumors [28,30–33]. Similarly, TBX3 is shown to be overexpressed in a subset of breast cancer cell lines [34] and to be upregulated in plasma from breast and ovarian cancer patients [35]. Both TBX2 and TBX3 are also closely associated with pancreatic cancer. While TBX2 is found to be upregulated in 50% of 31 pancreatic cancer cell lines tested [36,37], TBX3 is found to be overexpressed in metastatic pancreatic endocrine neoplasms [38]. Notably, TBX2 is located in chromosome 17q23 [39], a region frequently amplified in breast and pancreatic cancer cells [30,36,40,41]. Furthermore, both TBX2 and TBX3 are expressed in normal melanocytes and have been shown to be strongly overexpressed in a panel of melanoma cell lines [3,42,43]. Deregulated expression of TBX2 and TBX3 has also been reported in other cancers. TBX3 overexpression is closely associated with the mutational status of beta-catenin in murine liver tumors induced by Myc as well as in human hepatocellular carcinomas and hepatoblastomas [44]. Moreover, the upregulation of TBX3 is strongly associated with metastatic phenotypes of uterine cervical cancer [45]. TBX2, which is expressed in normal human fibroblasts, is recently shown to be reduced in several transformed fibroblast cell lines [46], while TBX3 is found to be upregulated in transformed lung fibroblast cells [47]. In addition, ectopic expression of TBX2 could result in polyplody and cispallin resistance in several human transformed lung fibroblast cell lines [48]. Although the above results suggest a strong association of both TBX2 and TBX3 with cancer, they do not provide the exact mechanism(s) for how these T-box genes contribute to tumorigenesis.

5. Roles of TBX2/TBX3 in tumorigenesis

5.1. p14ARF (p19ARF)/MDM2/p53/p21CIP1 pathway

A combination of cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors interact together to regulate proliferation, cell cycle arrest, apoptosis and senescence, and the abnormal expression of these key cell cycle regulators can contribute to cancer. Cellular senescence is a protective mechanism against cancer, and is broadly defined as a physiological program of irreversible growth arrest, which could be triggered by telomeres shortening (replicative senescence) or other stress signals (accelerated senescence) [49]. This “accelerated senescence,” which does not involve telomere shortening, is also triggered in normal cells by oncogenic mutations of Ras or Raf and by some other forms of supraphysiological mitogenic signal [50–52]. One of the best characterized mechanisms for cellular senescence is the ARF/MDM2/ p53/p21CIP1 pathway [53]. Expression of ARF can be induced by overexpression of a variety of oncogenes or mitogenic signals resulting in binding of MDM2, stabilization of p53, and a program of gene expression that leads to either cell cycle arrest or apoptosis. Growth arrest of senescent cells is initiated with the activation of p53. The activated p53 has multiple effects on gene expression, the most relevant of which in regard to

| Table 1 |
|---|
| **TBX2 and TBX3 expression in human cancers.** |
| **Name** | **Cancer** | **Ratio [%]** | **References** |
| TBX2 | Breast | 50%–80% | [28,31–33,39,40] |
| | Pancreas | 50%–60% | [36,37] |
| Melanoma | 63% (12/19 melanoma cell lines) | [34,21] |
| TBX3 | Breast | 70%–90% | [30,34,35] |
| | Ovarian | 69% | [35] |
| Pancreas | ND | [36,38] |
| Melanoma | 57% (8/14 melanoma cell lines) | [43] |
| Liver | 79%–87% | [44] |
| Cervical | ND | [45] |
| Lung | ND | [47] |

ND, not determined.
senescence is transcriptional activation of the CDK inhibitor p21CIP1, which is necessary for p53-mediated growth arrest [54,55]. In primary cells, this pathway can act as a monitor of oncogenic transformation and can induce p53-dependent or -independent cell cycle arrest, senescence and apoptosis. However, downregulation or inhibition of ARF can result in bypass of senescence, failure of apoptosis, promotion of cell proliferation and immortalization.

The study of Jacobs et al. [33] has shown that TBX2 is able to promote bypass of senescence by downregulating the expression of p19ARF in senescence-predisposed BMI−/− mouse embryonic fibroblasts (MEFs). Moreover, forced expression of p19ARF in TBX2 expressing MEFs recapitulates the senescence process, indicating that TBX2 could promote bypass of senescence by inhibiting p19ARF levels. In addition to TBX2, TBX3 is also involved in regulation of cell senescence [30,56]. This is confirmed when ectopic expression of TBX3 results in immortalization of MEFs through suppressing the expression of p19ARF, suggesting that the immortalization effect is partly due to repression of the ARF/MDM2/p53 pathway. Consistent with this hypothesis, point mutations in the T-box domain of TBX3 which are believed to result in loss of binding to DNA, impair the ability of TBX3 to inhibit senescence or suppress p14ARF expression. Furthermore, the overexpression of TBX3 is also shown to regulate the p53 pathway to both suppress apoptosis and facilitate transformation [57]. These effects of TBX3 are probably due to the suppression of p14ARF, which leads to MDM2-dependent sequestration, degradation and inactivation of p53.

In addition to the repression of p14ARF/p19ARF, several studies have shown that TBX2/TBX3 could directly transcriptionally repress the expression of p21CIP1 [58–60]. By using a combination of in vitro DNA-binding, transfection, and chromatin immunoprecipitation assays, Prince et al. have shown that TBX2 can bind and repress the p21CIP1 promoter in vitro and in vivo, and small interfering RNA (siRNA)-mediated downregulation of TBX2 leads to a robust activation of p21CIP1 expression [58]. Similarly, TBX3 is also shown to bind the consensus T-element, the p21CIP1 promoter and the Nppa cardiac target gene, and repress the expression of p21CIP1 [59]. Because p21CIP1 is implicated in the regulation of cellular senescence, the ability of TBX2/TBX3 to suppress the expression of both p14ARF and p21CIP1 may provide an explanation for a powerful and cooperative antisenescence signal. The possibility that TBX2/TBX3 contributes to tumorigenesis by inhibiting senescence is supported by a study, which showed that expression of an inducible dominant-negative TBX2 could result in displacement of histone deacetylase 1, up-regulation of p21CIP1 expression, and the induction of replicative senescence in CDKN2A-null B16 melanoma cells [42].

Because p14ARF could also influence p53-independent pathways which regulate cell proliferation and apoptosis, it is likely that the repression of p14ARF by TBX2/TBX3 has a significant effect on these cellular processes. Thus, overexpression of either TBX2 or TBX3 is expected to suppress p14ARF-dependent repression of E2F-1 activity and disrupt E2F-dependent apoptosis and cell cycle arrest [60]. Similarly, TBX2 and TBX3 might impair p14ARF-dependent inhibition of NF-κB induction and activation by oncogenic factors [61]. With other p53-independent targets of p14ARF characterized, roles of TBX2 and TBX3 in tumorigenesis will become more apparent.

5.2. pRb pathway

The retinoblastoma protein (pRb) is a canonical tumor suppressor, negatively regulating different steps of cell-cycle progression [62]. Rb and its close homologs p107 and p130 physically interact with many proteins, but their binding to members of the E2F family of transcription factors is central to their role in cell cycle regulation, especially during G1 phase [63]. Complexes between pRb family members and various E2Fs block the activation of E2F responsive genes by recruiting HDACs and other chromatin remodeling factors to E2F responsive promoters [64–66]. Notably, E2Fs regulate genes necessary for DNA replication, including those enzymes required for DNA metabolism and synthesis, as well as cyclins E and A. Therefore, the active hypophosphorylated forms of Rb family members block entry into S phase by inhibiting the E2F transcriptional program. In addition to its role in E2F regulation, pRb family members also interact with transcription factors that govern cell differentiation, such as C/EBP, NFIL-6 and c-jun [67–69].

Recent studies have found that TBX2 is associated with pRb pathway [70,71]. Vance et al. has shown that TBX2 associates with active hypophosphorylated Rb1, but not with the Rb1-related proteins p107 or p130 in melanoma cells [70]. They find that TBX2 binds Rb1 in a LXCXE independent manner, and this interaction enhances TBX2 DNA binding and transcriptional repression. Microarray analysis of melanoma cells expressing inducible dominant-negative TBX2 shows that TBX2 regulates the expression of many genes involved in cell cycle control, and a mutation disrupting the Rb1–TBX2 interaction also affects TBX2 target gene selectivity. These data suggest that TBX2 plays an important role in cell cycle control through interaction with pRb. Vormer et al. has performed a cDNA screen and aimed to find oncopgenes which collaborate with the loss of pRb and RasV12 in transformation of MEFs. They identify the oncogene TBX2 as a cooperating factor in transformation of pRB-deficient MEFs [71].

5.3. Wnt/beta-catenin pathway

The canonical Wnt pathway has been identified as a critical regulator of stem cells, which is involved in virtually every aspect of embryonic development and controls homeostatic self-renewal in a number of adult tissues [72]. Germline mutations in the Wnt pathway may cause several hereditary diseases, and somatic mutations are closely associated with cancer of the intestine, liver and a variety of other tissues [73]. This has raised the possibility that the tightly regulated self-renewal mediated by Wnt pathway in stem and progenitor cells is disrupted in cancer cells to allow malignant proliferation.

A recent study has identified TBX3 as a downstream target gene of Wnt/beta-catenin pathway implicated in hepatocarcinogenesis and has shown that TBX3 is directly regulated by beta-catenin using chromatin immunoprecipitation and reporter assays [44]. TBX3 transcription is activated by overexpression of beta-catenin in mouse liver and in human tumor cell lines. Furthermore, it has also been shown that inhibition of TBX3 by specific siRNAs impairs beta-catenin-mediated cell survival and renders cells sensitive to doxorubicin-induced apoptosis. These results reveal a role of TBX3 as a mediator of beta-catenin activities on cell proliferation and survival and as an important player in hepatocarcinogenesis. Fong et al. has shown that TBX2 functions within the context of Wnt signaling to mediate cell migration in the early embryonic development [74], indicating that TBX2 may be also a target of Wnt signaling pathway. However, whether TBX2 is a direct target of Wnt signaling pathway in cancer cells should be further confirmed and investigated.

Beta-catenin functions in a dual manner in epithelial cells, depending on the intracellular localization. In the nucleus, beta-catenin can act as a main effector of the canonical Wnt pathway. However, at the plasma membrane, beta-catenin is a critical component of adherens junctions, acting in cell-cell adhesion by linking E-cadherin in conjunction with alpha-catenin to the actin cytoskeleton [75]. Loss of E-cadherin expression results in a reduced degradation and subsequent overexpression of free cytoplasmic beta-catenin, which will subsequently translocate the nucleus and activate the Wnt pathway. Rodriguez et al. has shown that TBX3 and TBX2 repress the expression of E-cadherin in melanoma cells, and depletion of TBX3 increases E-cadherin mRNA and protein levels and suppresses melanoma invasiveness in vitro [43]. These results suggest that TBX2 and TBX3 may play a dual role during the radial to vertical growth
phase transition by both inhibiting senescence via repression of p21\(^{CIP1}\), and enhancing melanoma invasiveness by reducing E-cadherin levels.

5.4. EGR1 signaling pathway

The early growth response (EGR) group of proteins is a family of zinc-finger transcription factors involved in cell proliferation and apoptosis. In the EGR family, EGR1 is the best characterized as a direct regulator of multiple downstream pathways. EGR1 has both activation and repression domains. Recent evidence has indicated that EGR1 has significant tumor suppressor properties, by participating in the regulation of a network of tumor suppressors including TGF\(\beta\)1, p53, PTEN, and fibronectin [76]. EGR1 can promote apoptosis in response to stress and DNA damage in a number of cancers.

A recent study has shown that TBX2 represses the putative breast tumor suppressor NDRG1 (N-myc downregulated gene 1) not through direct promoter interaction but as a co-repressor through recruitment of EGR1, and drives proliferation in breast cancer cells [28]. TBX2 interacts with EGR1 to target the NDRG1 proximal promoter and to drive cell proliferation. This is the first study to show that TBX2 is able to target genes independent of the T domain for DNA binding through EGR1. This ability would greatly enhance the oncogenic potential of TBX2, given the importance of EGR1 as an upstream modulator of multiple important tumor suppressors.

6. Potential targets of cancer therapy

The potential for using TBX2/TBX3 as targets of cancer therapy is now being explored. The theoretical rationale is based on the fact that TBX2/TBX3 is overexpressed and functions as a potential oncogene in various cancers. Such results offer the experimental bases for the use of TBX2/TBX3 as therapeutic targets. One strategy is to inhibit the function of TBX2/TBX3. HDAC inhibitors promote growth arrest, differentiation and apoptosis of cancer cells. The US FAD have approved vorinostate for the treatment of cutaneous T-cell lymphoma (CTCL). The other HDAC inhibitors, including FK228, PXD101, PCLI-24781, IFT2357, MGCD0103, MS-275, valproic acid and LBH589, have also demonstrated therapeutic potential as monotherapy or combination with other anti-tumor drugs in CTCL and other malignancies [77]. Inhibition of the functions of TBX2/TBX3 would be one of anticancer mechanisms. Given that TBX2/TBX3 is overexpressed in cancer, inhibition of its expression would reduce or eliminate its oncogenic properties. RNA interference (RNAi) is a strategy to specifically knockdown a target protein. The major challenge is to develop an effective siRNA delivery vector with a long half-life in circulation, low toxicity and low immunogenicity. Another concern is the potential off-targets effects of RNAi as silence of other members of T-box gene family or unpredicted important genes may lead to severe side-effects in vivo.

There is increasing interest in targeting the small repression domain of TBX2 and TBX3. As no any other protein has this special RD and the expression of TBX2/TBX3 is at low level in somatic cells, it is possible to design a drug targeting RD with high specificity. Unlike the current anticancer drugs, this drug would be very precious for the treatment of various cancers because of the low toxicity and low side-effects. The studies of the RD structure, especially the crystal structure of the interaction of RD with its counterparts, would help us to design specific compounds to block the functions of TBX2 and TBX3 in cancer cells. Based on the small RD, an alternative is to design synthetic peptides to block TBX2/TBX3 without the crystal structure information of the RD. For example, without the known crystal structure of spike protein of SARS-coronavirus, effective peptides were identified to block the interaction of spike protein and its receptor by peptide scan assays [78]. Currently, there is great interest in employing peptides or proteins as therapeutic agents for cancer treatment.

Several peptides derived from tumor suppressors or oncogenes have been developed as antitumorigenic drugs and proven to be effective in inhibition of tumor cell growth [79–82]. We prospect that antagonist peptides targeting the RD of TBX2/3 would be effective agents for the treatment of a number of human cancers in the future. We also prospect that computer-based drug design will help us to develop RD specific inhibitors after the crystal structure of RD is figured out in the future. Some of the RD inhibitors may be good for anticancer treatment.

7. Concluding remarks and perspectives

In conclusion, TBX2 and TBX3 have been shown to be amplified and overexpressed in a subset of cancers. Upregulation of TBX2 and TBX3 suppresses the expression of p14\(^{ARF}\) and p21\(^{CIP1}\) and promotes bypass of senescence through inactivation of p53 pathway. TBX2 functionally interacts with p70, and p70 modulates TBX2 functional specificity. In addition, TBX3 is shown to be a downstream target of the Wnt/beta-catenin pathway, and overexpression of TBX2 and TBX3 represses the expression of E-cadherin, which is demonstrated to be a prerequisite for epithelial tumor cell invasion and a negative regulator of the canonical Wnt pathway. Moreover, TBX2 is shown to interact with EGR1 and suppress multiple downstream tumor suppressors. Identification and characterization of target genes which are transcriptionally regulated by TBX2 and TBX3 are essential for an improved understanding of the roles of TBX2 and TBX3 in tumorigenesis. Drugs targeting the RD of TBX2/3 would be attractive for the treatment of a number of human cancers in the future.

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