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Review Article

TBL1XR1 in physiological and pathological states

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Abstract: Transducin (beta)-like 1X related protein 1 (TBL1XR1/TBLR1) is an integral subunit of the NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) repressor complexes. It is an evolutionally conserved protein that shares high similarity across all species. TBL1XR1 is essential for transcriptional repression mediated by unliganded nuclear receptors (NRs) and othe regulated transcription factors (TFs). However, it can also act as a transcription activator through the recruitment of the ubiquitin-conjugating/19S proteasome complex that mediates the exchange of corepressors for coactivators. TBL1XR1 is required for the activation of multiple intracellular signaling pathways. TBL1XR1 germline mutations and recurrent mutations are linked to intellectual disability. Upregulation of TBL1XR1 is observed in a variety of solid tumors, which is associated with advanced tumor stage, metastasis and poor prognosis. A variety of genomic alterations, such as translocation, deletion and mutation have been identified in many types of neoplasms. Loss of TBL1XR1 in B-lymphoblastic leukemia disrupts glucocorticoid receptor recruitment to chromatin and results in glucocorticoid resistance. However, the mechanisms of other types of genomic changes in tumorogenesis are still not clear. A pre-clinical study has shown that the disruption of the interaction between TBL1X and β-catenin using a small molecule can inhibit the growth of AML stem and blast cells both in vitro and in vivo. These findings shed light on the therapeutic potentials of targeting TBL1XR1 related proteins in cancer treatment.

Keywords: TBL1XR1, TBL1X, corepressor, coactivator, targeted therapy

Introduction

Transducin (beta)-like 1X related protein 1 (TBL1XR1), also known as TBLR1, is an F-box/WD40-repeat containing protein that was originally isolated as a gene transcript that is preferentially expressed in human $CD34^{+}CD38^{-}$ cells [1]. Biochemical studies identified TBL1XR1 as a core component of NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) repressor complexes that also contain HDAC3 (histone deacetylase 3), TBL1X (transducin (beta)-like 1X), and GPS2 (G-protein pathway suppressor 2) [2, 3]. TBL1XR1 is essential in targeting SMRT/NCoR corepressor complexes to the promoter of target genes by unliganded nuclear receptors (NRs) and other regulated transcription factors (TFs) to mediate repression [3-5]. On the other hand, TBL1XR1 is also required for transcriptional activation by liganded NRs and other TFs. It serves as a specific adaptor for the recruitment of the ubiquitin-conjugating/19S proteasome complex that mediates the exchange of corepressors for coactivators [6, 7]. TBL1XR1 is essential in the activation of Wnt-β-catenin and NF-κB signaling pathways [6-10]. De novo deletions and recurrent mutations have been identified in *TBL1XR1*gene, which are linked to intellectual disability (ID) [11-15]. Accumulating evidence suggests that TBL1XR1 may play an important role in tumorigenesis, invasion, metastasis, and developing resistance to therapies [16-22]. This review will focus on the genomic structure, expression, and functions of TBL1XR1 and the pathological states associated with its dysregulation. We will also discuss the therapeutic potentials of targeting TBL1XR1 and related pathways.

TBL1XR1 family

Human *TBL1XR1* (*hTBL1XR1/hTBLR1/IRA1*) gene is located on chromosome 3q26.32 and consists of 18 exons spanning 178, 119 base pairs [23]. Two closely related genes, *TBLR1X* and *TBL1Y*, have been cloned and mapped to

human chromosome X and Y, respectively [24, 25]. *TBL1XR1* mRNA is ubiquitously expressed in many human tissues with higher levels of expression observed in thyroid, prostate and breast tissues. The expression of *TBL1XR1* overlaps but is distinct from that of *TBL1X* and *TBL1Y*. In addition, an alternatively spliced form of *TBL1XR1* (*TBLR1β*) has been demonstrated in human material and it too has a unique pattern of expression [23]. The murine homologue is structurally similar and is also located on Chromosome 3. Only one isoform has been identified in *Drosophila* (*Ebi*) and *Xenopus (Xenopus TBL1XR1),* which are found more similar to *hTBL1XR1* than to *hTBL1X* [4, 26].

The *hTBL1XR1* gene encodes a 514 amino acid protein with an estimated molecular mass of 55.5 kDa. The β isoform of *hTBL1XR1* encodes a larger protein due to disruption of translation by alternative splicing. TBL1XR1 protein contains a LisH domain (Lis1 homology domain) and a F-box-like domain at the amino-terminus and seven WD40 repeats at the carboxy-terminus [23]. The LisH domain is required for oligomerization, transcriptional repression, and binding to hypoacetalyted H2B and H4 [27]. Deletion of LisH domain decreases the half life of TBL1XR1 protein and results in its translocation from nucleus to cytoplasm [28]. The F-box-like domain is essential for the recruitment of the ubiquitin/19S proteasome complex to the transcription units regulated by NRs and TFs for the degradation of SMRT/NCoR corepressor. It is also important for the ubiquitin-mediated degradation of other proteins [28]. The WD40 domains are important for self or heterodimer protein-protein interaction. The first WD repeat in TBL1XR1 is essential for its binding to the RD4 domain of NCoR [3].

The structure of TBL1XR1 family is evolutionarily conserved with high degrees of similarity from yeast to human at the amino acid level [29]. The N-terminal sequence is totally conserved between *Xenopus* TBL1XR1 and hTBL1XR1 and 94% identical to that of hTBL1X. The WD-40 repeat domain is 98% identical between *Xenopus* TBL1XR1 and hTBL1XR1 whereas only 90% identical between the two human proteins. The high degrees of similarity in TBL1XR1 family across all the species suggest similar biochemical and molecular functions [4, 23].

TBL1XR1 is also susceptible for posttranslational modifications, including phosphoration and sumoylation. It contains two putative phosphorylation sites (one for CK1/GSK3 and one for PKCδ) and one SUMO modification site (Lys497) [7, 9]. Multiple microRNAs (miRNAs) targeting TBL1XR1 have been identified by using computational prediction programs, of which hsa-miR-205 is found to be functional [30]. In addition, several potential long noncoding RNA (lncRNA) sequences are identified in the region encompassing the *hTBL1XR1* gene. Further studies are needed to understand the roles of these miRNAs and lncRNAs in the function and regulation of TBL1XR1.

TBL1XR1 is primarily localized in nucleus in tissues and many cell lines. However, we have shown that TBL1XR1 is mostly located in the cytosol of NIH-3T3 cells and translocates to the nucleus after induction of apoptosis or growth arrest. The cytoplasmic pattern is also observed in several prostate cancer cell lines and primary prostate cancer cells [21, 23].

Normal functions of TBL1XR1

TBL1XR1 acts as a transcriptional corepressor

Transcriptional repression mediated by unliganded NRs or other TFs requires a group of corepressors. Biochemical purifications have identified multiple corepressor complexes [2, 3, 31], which may be used combinatorially and recruited to chromatin in a sequential fasion [7]. The best characterized is the SMRT/N-CoR/HDAC3 complex that also contains TBL1X, TBL1XR1, and GPS2 [2, 3]. The corepressor complexes actively silence basal transcription through interaction with unliganded NRs. However, the interaction alone is not sufficient for targeting the corepressor complexes to target gene promoters and mediating transcriptional repression. As a subunit of the SMRT/NCoR repressor complexes, TBL1XR1 is found to be essential for repression by unliganded NRs in both human cell lines and *Xenopus* oocytes [3, 4]. A recent study using small interfering RNA (siRNA) revealed that TBL1XR1 and TBL1X are functionally redundant and essential for transcriptional repression by thyroid hormone receptor (THR). Both TBL1XR1 and TBL1X are able to directly bind to NCoR and SMRT through their N-terminal and first WD-40 domains. They do not have direct interaction with unliganded THR

and HDAC3. They are critical for targeting the SMRT/NCoR corepressor complexes to chromatin through binding hypoacetylated histones H2B and H4. Furthermore, this study proposes a two-interaction, feed-forward working model for targeting SMRT/N-CoR complexes to chromatin. In brief, the interaction between SMRT/NCoR and unliganded NRs initiates limited histone deacetylation at the target gene promoter sites. TBL1X/TBL1XR1 then stabilizes the association of the SMRT/NCoR complexes with chromatin by binding the deacetylated histones, resulting in deacetylation of additional histones. In the final step, the formation of stable complexes and extensive deacetylation lead to intiation and maintenance of repression. These studies implicate the critical roles of TBL1XR1 in the formation of stable corepressor complexes on chromatin for mediating repression [27, 32].

TBL1XR1 acts as an exchange factor of corepressors for coactivators

Transcriptional activation mediated by liganded NRs and other regulated TFs requires the dismissal of corepressors followed by the recruitment of a series of coactivator complexes harboring specific enzymatic activities. Although TBL1XR1 and TBL1X are intrinsic components of the SMRT/NCoR corepressor complexes, recent study has shown that they both are required for transcriptional activation by estrogen receptor (ER), androgen receptor (AR), THR, and peroxisome proliferator-activated receptor (PPAR). The activation by RAR (retinoid acid receptor) and AP-1 (activator protein-1) depends exclusively on TBL1XR1 [6, 7]. TBL1XR1 and TBL1X both serve as specific adaptors for the recruitment of the ubiquitin conjugating/19S proteasome complex to the promoter in a ligand-dependent fashion, mediating the exchange of corepressors for coactivatores [6, 7]. Unlike TBL1XR1, TBL1X is still required for the transcriptional activation by NRs in the absence of NCoR and SMRT. The data indicate that TBL1XR1 is specifically required for the ubiquitylation and degradation of NCoR and TBL1X may act on different corepressor complexes. Further study identified CtBP1/2 as a specific target of TBL1X [7]. The functions and the specificity of TBL1XR1 and TBL1X are regulated by signaling specific phosphoration events at target gene promoters [7]. This differential specificity is also observed in the NCoR clearance induced by the activation of Toll-Like Receptors (TLRs) [33]. Our early study showed that TBL1XR1 is also required for the degradation of SMRT [23]. Additionally, the phosphorytion of SMRT S2410 by IKKα is important for the dismissal of SMRT for the derepression of NF-κB target genes, which concomitantly correlates with the recruitment of TBL1XR1/TBL1X to the target gene promoters, suggesting that TBL1XR1 is also important in the dismissal of the SMRT complex for coactivator exchange [10]. Many signaling pathways in the higher eukaryotes are tightly regulated through a dual-repression "checkpoint" based on distinct corepressor complexes. The dedicated exchange factors such as TBL1XR1 and TBL1X serve as sensors for signal-specific dismissal of distinct corepressors, with specificity imposed by upstream signaling pathways [7].

TBL1XR1 in Wnt-β-catenin signaling pathway

The Wnt-β-catenin signaling pathway is critical for development, specification of cell fate, and adult stem cell proliferation. The aberrant activation of this pathway has been implicated in the tumorogenesis of several types of cancers [34]. The Wnt target genes are silenced by the T cell factor/lymphoid enhancing factor (TCF/LEF) family protein and Groucho/TLE1 corepressor complex in the absence of signaling. Upon activation of the Wnt-β-catenin signals, β-catenin enters the nucleus and binds to the TCF/LEF protein in displacing the corepressor complexes and recruiting other co-activator complexes to induce transcription [8, 9, 34]. Overexpression of TBL1XR1 or TBL1X in HK293T cells can lead to significantly enhanced $\beta\text{-cat}^{M}$ (a mutant form of β-catenin)-stimulated transcription. Depletion of TBL1XR1 or TBL1X by siRNAs abolishes the activation of β-catenin induced by lithium (LiCl) treatment or Wnt3a. Wnt signaling induces the formation of a complex between β-catenin and TBL1X-TBL1XR1, which is recruited to the Wnt target gene promoter. TBL1X can also bind to TCFs. The recruitment of TBL1X-TBL1XR1 and β-catenin to the Wnt target gene promoter is mutually dependent. Furthermore, TBL1X andTBL1XR1 do not affect the nuclear level of β-catenin and are not responsible for degradation of corepressor complexes. These findings indicate that unlike their roles as exchange factors in NR-mediated activation, TBL1X and TBL1XR1 are critical for the recruitment of β-catenin to Wnt target gene

promoters. TBL1X and TBL1XR1 are also important for β-catenin mediated oncogenesis [8]. Recent study shows that both TBL1X and TBL1XR1 are subjected to sumoylation in a Wnt signaling-dependent manner, which dismisses TBL1X-TBL1XR1 from the NCoR complex and enhances the formation of TBL1X-TBL1XR1-β-catenin complex [9]. The depletion of TBL1X-TBL1XR1 or Inhibition of sumoylation may have important implications in developing new strategies for inhibiting Wnt-beta-catenin-mediated tumorigenesis.

TBL1XR1 in NF-κB pathway

NF-κB plays an important role in multiple cellular processes, including immune signaling, inflammation, development, proliferation and survival. Aberrant activation of NF-κB signaling pathway has been implicated in autoimmunity, chronic inflammation and cancer [35]. In unstimulated cells, NF-κB is sequested in cytoplasm as an inactive form by binding to a family of inhibitory proteins, IkB. Upon stimulation, IkB is phosphorylated by IkB kinase (IKK) complex and subject for subsequent proteasome-mediated degradation, allowing NF-κB to translocate to the nucleus to initiate transcription [35]. In unstimulated cells, p50 or p52 homodimers repress NF-κB regulated genes through BCL-3 dependent and BCL-3 independent mechanisms. The activation of NF-κB regulated genes is mediated by the exchange of BCL-3-associated NCoR corepressor for the Tip60 coactivator complex. This pathway only regulates a small subset of NF-κB regulated genes while the majority of NF-κB regulated genes are regulated by the classical NF-κB heterodimer complex [10]. Recent study reveals that both TBL1X and TBL1XR1 are required for the NF-κB mediated activation on canonical sites [6]. TBL1X is critical for the recruitment of p65 to NF-κB target genes to mediate activation. NF-κB transcription requires IKKalpha to phosphorylate SMRT on chromatin, stimulating the exchange of corepressor for coactivator complexes. The recruitment of TBL1X-TBL1XR1 to the target promoters coincides with SMRT phosphorylation [10]. Moreover, TBL1XR1 can directly interact with BCL-3 and is involved in BCL-3 degradation through a GSK3 independent pathway [36]. These results suggest that TBL1XR1 plays a critical role in NF-κB mediated activation through both canonical and non-canonical pathways.

TBL1XR1/HDAC3 corepressor complex is important for mitotic spindle formation

Studies have shown that altered HDAC3 level increases G(2)/M cells, suggesting a possible role of HDAC3 in cell proliferation. Ishii et al [37] showed that the HDAC3 complex, including N-CoR, TBL1X, and TBL1XR1, is localized on the mitotic spindle. Knockdown of HDAC3 or N-CoR or inhibition of HDAC3 activity with Trichostatin A results in a collapsed mitotic spindle independent of transcriptional regulation, which can be rescued by wild-type HDAC3. Inactivation of HDAC3 impairs kinetochore-microtubule attachments without affecting the kinetochores and the spindle assembly checkpoint. The studies suggest that acetylation-deacetylation of mitotic spindle components may be essential for mitotic spindle function.

TBL1XR1 in normal hematopoiesis

$CD34^+CD38^-$ cells are considered as the earliest human hematopoietic progenitors. They have the ability to initiate long term bone marrow culture in vitro and to reconstitute NOD/SCID mice in vivo [38, 39]. We have found that TBL1XR1 is expressed at a much higher level in $CD34^+CD38^-$ cells compared to their mature counterpart $CD34^+CD38^+$ cells. Northern blot analysis has shown that TBL1XR1 mRNA is expressed at a higher level in the tissues enriched for early hematopoietic cells such as fetal liver and bone marrow in comparison with peripheral blood [23]. TBL1XR1 has been implicated in multiple intacellular signaling pathways important for stem cell fate determination and proliferation such as Wnt pathway and NOTCH pathway. Ebi, the *Drosophila* homologue of TBL1XR1, is required for maintenance of photoreceptor neurons by suppressing pro-apoptotic gene expression [40]. This is confirmed by a recent report that TBL1XR1 mRNA is upregulated after the exposure of neural stem cells to ethanol [41]. We have shown that overexpression of TBL1XR1 is able to induce growth arrest [23]. These findings suggest that TBL1XR1 may be important in the maintenance of hematopoietic stem cells. Further studies in this regard may be helpful in our understanding its functions in hematopoiesis.

TBL1XR1 and intellectual disability

Intellectual Disability (ID) affects almost 3% of the population and increasingly becomes a

public health challenge [11]. It can be sporadic or associated with other congenital abnormalities. Pons et al. reported an eight-year-old girl with facial dysmorphism, speech delay, and mild to moderate ID. Her mother displayed similar physical and intellectual features. Using array-CGH (array comparative genomic hybridization) techniques, a 708 kb microdeletion at 3q26.32 region was identified in both patients, which affects only TBL1XR1gene. Family studies revealed that the mother is a founder and the pattern of inheritance is autosomal dominant [11]. The third case is a six-year-old girl and she also has facial dysmorphism and mild to moderate ID. In this patient, a 1.6 Mb deletion is identified in the 3q26.31q26.32 region encompassing only TBL1XR1 gene [12]. The three cases are very similar and possibly represent a new autosomal dominant syndrome associated with TBL1XR1 haploinsufficiency. This syndrome is characterized by facial dysmorphism (pointed chin, long philtrum, and thin vermilion), speech delay, mild to moderate ID, and lack of autistic behaviors.

Additionally, two cases are also identified in the DECIPHER database [11]. The first patient has autism, hearing impairment and spotty hyperpigmentation, who carries a 2.14 Mb deletion encompassing the *TBL1XR1* and *KCNMB2* genes. The second case is characterized by ID, ataxia, ASD (stereotypic behavior and self-mutilation) and dysmorphism (wide mouth, macrotia, prominent ears, hypertelorism, and downslanted palpebral fissures). This patient carries four copy number variation (CNV) affecting several genes in addition to a 150 kb de novo deletion encompassing only *TBL1XR1*.

Recently, a whole exome sequencing (WES) study identified recurrent mutations in patients with autistic spectrum disorder (ASD), in which two had de novo *TBL1XR1* mutations [14]. Both patients have ASD and severe ID, but neither of them have any obvious dysmorphism or recurrent comorbidities. The TBL1XR1 mutations occur at positions 282 (p.Leu282Pro) and 397 (p.Ile397Serfs*19), respectively. The third case is a Japanese girl who had a de novo TBL1XR1 mutation (p.Gly70Asp) located in the F-box-like domain [13]. She presented with West syndrome, Rett syndrome-like and autistic features. Based on the guidelines in the diagnostic manual (DSM-IV) of the American Psychiatric Association, this case meets the diagnostic criteria for ASD. The mutation data replicate the importance of a β-catenin-chromatin-remodeling network to ASD etiology [14].

TBL1XR1 in cancers

TBL1XR1 dysregulations in solid tumors

Liu et al. identified TBL1XR1 as a differentially expressed gene in human primary lung squamous cell carcinoma (LSCC) by comparing lung tumor tissues and normal bronchial epithelial tissues using suppression subtractive hybridization method [15]. Quantitative real-time PCR (q-PCR) analysis showed upregulation of TBL1XR1 mRNA in 75% of LSCC cases in the validation set. The overexpression of TBL1XR1 protein was confirmed by western blot analysis in 53.3% of LSCC tumor samples, 5 lung cancer cell lines, and 1 human immortalized bronchial epithelial cell line [15]. Deletions at human 3q26.32 encompassing TBL1XR1 gene have been previously detected by array-based comparative genomic hybridization (aCGH) in human immortalized bronchial cell lines.

To identify genetic events implicating in cancer progression, Kadota et al. conducted a comprehensive genetic evaluation of 161 primary breast tumors (mostly invasive breast carcinomas) for gene amplifications [16]. Their findings show that the level of TBL1XR1 protein is higher in the malignant cell lines MCF10CA1h and MCF10CA1a than that in MCF10A, a normal immortalized mammary epithelial cell line. Depletion of TBL1XR1 in MCF10CA1h using shRNA results in reduction of cell migration and invasion as well as suppression of tumorigenesis in mouse xenografts [16]. A different study confirms these findings by showing that TBL1XR1is significantly upregulated in breast cancer cell lines and 52.8% of primary breast cancers cases in comparison with normal breast tissues. This study also shows that the level of TBL1XR1 protein expression is positively correlated with aggressive clinical behavior. The patients with higher levels of TBL1XR1 expression have shorter overall survival compared to those with lower levels of expression. Multivariate analysis indicates that TBL1XR1 overexpression is an independent prognostic indicator for the survival of breast cancer patients. They demonstrate that TBL1XR1 promotes tumor cell proliferation and tumorigenicity in breast cancer through the activation of Wnt/β-catenin signaling pathway [17].

Similar findings are also observed in cervical cancer, nasopharyngeal carcinoma (NPC) and esophageal squamous cell carcinoma (ESCC) [18-20]. Upregulation of TBL1XR1 in NPC cells leads to resistance of the tumor cells to cisplatin by activating the NF-κB pathway, which is associated with poor prognosis [19]. In cervical cancer, upregulation of TBL1XR1 is significantly associated with recurrence. Forced expression of TBL1XR1 in cervical cancer cell lines (Hela and Siha) promotes invasion by inducing epithelial-mesenchymal transition through the activation of NF-κB and Wnt/β-catenin signalling pathways [18]. TBL1XR1 promotes lymphangiogenesis and lymphatic metastasis in ESCC by inducing the expression of VEGF-C. The association between the TBL1XR1 expression and VEGF-C expression is not only seen in ESCC but also observed in gastric, colonic and breast cancers based on the published database [20].

Studies have shown that depletion of TBL1XR1 by shRNA in HT-29 cells, a colon cancer cell line significantly reduces the recruitment of β-catenin to its target gene promoters, such as AXIN2 and c-MYC, and inhibits the endogenous expression of c-MYC, MMP7 and ITF2, leading to inhibition of tumor cell invasion through the Matrigel-coated membrane and suppression of tumor growth in nude mice [8].

Our studies have shown that TBL1XR1acts as a coactivator of Androgen receptor (AR) in prostate cancer cells and the activation is dependent on both phosphorylation and 19S proteasome machinery. We also show that TBL1XR1 physically interacts with AR and directly occupies the androgen-response elements of the affected AR target genes in an androgen-dependent manner. TBL1XR1 is primarily localized in the nucleus in benign prostate cells and nuclear expression is significantly reduced in prostate cancer cells in culture. Similarly, in human tumor samples, the expression of TBLR1 in the nucleus is significantly reduced in the malignant glands compared with the surrounding benign prostatic glands. Stable ectopic expression of nuclear TBL1XR1 leads to androgen-dependent growth suppression of prostate cancer cells in vitro and in vivo by selective activation of androgen-regulated genes associated with differentiation and growth suppression, but not cell proliferation of the prostate cancer [21, 42]. TBL1XR1 gene amplification has been identified in invasive prostate cancer, which correlates with disease progression. PTEN loss is the most frequent aberration in progressers (57%); followed by TBL1XR1 gain (29%) and MYC gain (14%). Based on this study, it would detect progressers with 86% sensitivity and 100% specificity using a probe set consisting of PTEN, MYC, and TBL1XR1 [43].

Four novel translocations involving the *TBL1-XR1* gene have been identified in an effort to study the landscape of kinase fusion in cancers [44]. They include one *TBL1XR1-RET* fusion and three *TBL1XR1-PIK3CA* fusions. Two of the *TBL1XR1-PIK3CA* fusions are found in 1,072 breast cancer (invasive) samples and one in 335 prostate adenocarcinoma samples. Both tissue types are hormone driven and ranked among the highest for *TBL1XR1* mRNA expression across all normal tissues. The LisH motif in *TBL1XR1* is capable of dimerization, therefore likely leading to RET or PIK3CA activation.

TBL1XR1 dysregulation in lymphomas

Using WES approach, a novel TBL1XR1/TP63 gene fusion has been recently identified in diffuse large B-cell lymphoma (DLBCL) [45], peripheral T-cell lymphoma (PTCL) [46], and follicular lymphoma (FL) [45]. The gene fusions result from a chromosomal rearrangement between TBL1XR1 gene (3q26.32) and TP63 gene (3q28). In most cases, the exons 1-7 of TBL1XR1 fuse in frame to the exons 4-8 or 4-10 of TP63. The exon 14 or exon 4 of TBL1XR1 is involved in the remaining cases including two DLBCLs and one FL [45]. The TBL1XR1/TP63 fusion is reported in 5% of germinal center B-cell like (GCB) DLBCL and appears to be exclusive for the GCB type. It is also present in PTCL in a similar percentage, but this fusion is rare event in FL. P63 has two main classes of isoforms, TAp63 and ΔNp63. TAp63 has an N-terminal transactivation domain with tumor suppressor activity, whereas ΔNp63 contains a truncated N-terminal domain that has been proposed to confer oncogenic properties. The expression of the TBL1XR1/TP63 fusion was 5.2 fold higher than the wild-type [45]. The TBL1XR1/TP63 protein may function similarly to ΔN-TP63, antagonizing the action of TP53, TA-TP63, and TA-TP73. TP63 rearrangement in PTCLs is associated with significantly higher mean immunohistochemistry scores for p63,

ki67 and CD30. Patients with PTCLs carrying *TP63* rearrangements have significantly inferior overall survival compared with patients without *TP63* rearrangements. In one case, the fusion was retrospectively found in T cells before the clinical diagnosis of mycosis fungoides, suggesting that it is an initiating event in lymphomagenesis [47]. It has been speculated that this abnormality may not only provide a proliferative advantage but also resistance to the genotoxic stress induced by chemotherapy [45].

WES and array-based comparative genomic hybridization (aCGH) have been proven to be effective tools in identifying small genomic alterations in formalin-fixed paraffin-embedded (FFPE) specimens. Using WES analysis, recurrent mutations of *TBL1XR1* have been identified in 19% of primary central nervous system lymphoma (PCNSL) cases [48, 49]. The mutations are clustered in three main regions: LisH domain, F-box like domain and the C-terminus. Interestingly, one mutation at the C-terminus affects the potential phosphorylation site. *MYD88* mutations are also frequent in the same patient group. Moreover, a novel missense mutation in *TBL1XR1* (T229R) was identified in a patient with Sezary syndrome, which converts a neutral to a hydrophilic amino acid [50]. Since *MYD88* and *TBL1XR1* both are involved in NF-κB signaling pathway, it is possible that *TBL1XR1* mutations lead to aberrant activation of NF-κB pathway and subsequent lymphomagenesis. In one study, focal deletions affecting sole TBL1XR1 gene are identified in three out of nine PCNSL cases by aCGH analysis [51]. Similar genomic alterations are also reported in a small subset of de novo DLBCL cases. Like the B-lymphoblastic leukemia with deletions of *TBL1XR1*, these patients may develop resistance to steroid therapy due to loss of normal functions.

TBL1XR1 abnormalities in acute leukemias

Focal deletions of *TBL1XR1* have been identified in 3 to 10% of acute lymphoblastic leukemia (ALL) patients [52]. The ETV6-RUNX1 fusion is the molecular consequence of the t(12;21)(p13;q22), which is seen in approximately 25% of children with B-ALL. Focal deletions of *TBL1XR1* have been observed in 15% of these cases and appear to be exclusive for this subtype. A q-PCR analysis showed that the *TBL1XR1* mRNA is significantly under-expressed and deletion of *TBL1XR1* compromised the function of SMRT/N-CoR in the appropriate control of gene expression [52-54]. Deletions of *TBL1XR1* are significantly more common in B-ALL patients who relapse compared with those remaining in complete remission. Recently, a study has shown that knockdown of TBL1XR1 in B-ALL cell lines results in reduced glucocorticoid receptor recruitment to glucocorticoid responsive genes and ultimately decreased glucocorticoid signaling caused by increased levels of NCoR and HDAC3. The resistance is specific for glucocorticoid agonists. Treatment with the HDAC inhibitor SAHA restores sensitivity to prednisolone in TBL1XR1-depleted cells [22].

A novel t(3;17)(q26.32;q21) translocation has recently been reported in three patients with acute promyelocytic leukemia (APL). The rearrangement results in a fusion product between exons1-3 of TBL1XR1 gene and exons 6-8 of RAR gene. The TBL1XR1/*RARα* protein is predominantly localized in the nucleus and can form homodimers or heterodimers with retinoid X receptor α. The fusion protein can act as transcriptional activator in the presence of ligand, but the transactivity is much weaker than *RARα*. The TBLR1-RARα product exhibits diminished transcriptional activity as a result of the recruitment of more transcriptional corepressors compared with RARα. In the presence of pharmacologic doses of ATRA, TBLR1-RARα could be degraded, and its homodimerization was abrogated [55].

Therapeutic potentials of targeting TBL1XR1-related pathways

Disruption of interactions between TBL1XR1 and NCoR has been shown to inhibit gene repression by unliganded NRs [3]. Overexpression of the TBL1XR1-interacting domain of NCoR (DN-RD1) in frog oocytes can disrupt the interaction between endogeneous NCoR and TBL1XR1 and reverse the repression by unliganded RAR/RXR [4]. Knockdown of TBL1X or TBL1XR1 by shRNA in cell lines can block the binding of β-catenin to TCF4 and repress the transcription of WNT-β-catenin target genes, leading to inhibition of cell proliferation [8]. These research findings lay the foundation for development of therapies targeting these platforms. A recent pre-clinical study, testing

effects of BC2059 (β-Cat Pharmaceuticals), a small anthraquinone oxime-analog molecule on the growth of AML stem and blast progenitor cells (BPCs), provided promising results. Treatment with BC2059 disrupts the binding of β-catenin to TBL1X and promotes proteasomal degradation of β-catenin, inhibiting the expression of the genes of Wnt/β catennin pathway both in the cultured cell lines and in primary AML BPCs. It also shows dose dependent inhibition of growth and induces apoptosis of cultured and $CD34^+$ pAML BPCs expressing FLT3-ITD, but not of normal $CD34^+$ bone marrow progenitor cells. BC2059 enhances cell death induced by transient knockdown of TBL1X or β-catenin in MOLM13 cells and induces apoptosis in MOLM13-TKIR cells (a FLT3 antagonist-resistant cell line). More importantly, BC2059 treatment (by IV injection) exerts potent in vivo anti-AML activity and significantly improved the survival of immune depleted mice engrafted with cultured and patient-derived pAML BPCs. Furthermore, BC2059 and FLT3-antagonist quizartinib or ponatinib synergistically induces apoptosis of the FLT3-ITD expressing cultured cells (MOLM13 and MV4-11) and primary AML BPCs but not of normal $CD34^+$ progenitor cells. Collectively, these findings support that the targeted therapy is a promising approach in treating AML [56].

Summary

TBL1XR1 is an evolutionarily conserved protein that shares high structural and functional similarities from yeast to human. Three human homologues have been identified. TBL1XR1 is an intrinsic component of the SMRT/NCoR corepressor complexes, and it is also required for transcriptional activation by NRs and other regulated TFs. It is important for the activation of multiple intracellular signaling pathways, such as Wnt-β-catenin, NF-κB, and Notch. It is preferentially expressed in human hematopoietic stem cells and may be critical for the maintenance of stem cells. Germline mutations in TBL1XR1 or recurrent mutations are associated with intellectual disability. Dysregulation of TBL1XR1 has been observed in a variety of neoplastic conditions. TBL1XR1 appears to be multifunctional and its functions are cell type- and context- dependent. However, its exact functions are still not clear. A recent pre-clinical study shed light on the therapeutic potential of targeting TBL1XR1-related pathways in cancer treatment.

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