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Journal
Clinical and Experimental Neuroimmunology, 5(2)

ISSN
1759-1961

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Publication Date
2014-06-01

DOI
10.1111/cen3.12125

Peer reviewed

Published in final edited form as:
Clin Exp Neuroimmunol. 2014 June 1; 5(2): 132–136. doi:10.1111/cen3.12125.

The role of antiproliferative gene Tob1 in the immune system

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Abstract

Tob1 (transducer of ERBB2-1, TOB1 is humans) is a member of the antiproliferative (APRO) family of proteins that controls cell cycle progression in several cell types. In addition, Tob1 has been implicated in diverse cellular mechanisms such as embryonic dorsal development, and T helper 17 (Th17) cell function. More recently, evidence linking Tob1 function to experimental and human immune related disorders has mounted, thus underscoring the potential of this molecule as a biomarker and as a therapeutic target. This article reviews these functions with an emphasis on their implications for human autoimmune diseases such as multiple sclerosis.

Keywords

Biomarker; cell proliferation; multiple sclerosis; quiescence; T lymphocyte.

Tob1 (Transducer of Erb-2, 1) is a member of the Tob/BTG antiproliferative (APRO) family of proteins, together with TOB2, BTG1, PC3/TIS21/BTG2, ANA/BTG3 and PC3/PC3K (1, 2). Like other members of this family, Tob1 plays important roles in suppressing tumor development. The common N-terminal domain characterizes all members of the APRO family. This domain (composed of ~120 residues), includes a nuclear localization signal (NLS) and a nuclear export signal (NES) and is presumably responsible for the antiproliferative effect of these genes. The NLS and NES enable translocation between nucleus and cytoplasm at different stages of the cell cycle. Indeed, Tob1 concentration is higher in the cytoplasm during G1/S phase, and then is reduced to enable cell division (3).

Tob1 is expressed in most cell types and in several species. Transcripts of zebrafish *tob1a* (4) and *tob1b* (5) and *Xenopus Tob2* (6) are ubiquitously distributed during blastula and gastrula stages of development, suggesting a role in early embryogenesis in these species. At later stages of embryonic development, the expression of *Tob* genes occurs in distinct domains, such as the notochord, hatching gland, blood islands, and gut, depending on the species. During segmentation, *Tob* genes are expressed in somites, which ultimately give rise to axial skeleton, skeletal muscle, and dermis (7), in amphioxus (*Tob*; (8), zebrafish (*tob1b*; (5)), *Xenopus* (*xTob2*; (6)), and mouse (*Tob2*; (9)). In adults, expression of *Tob1* (10) and *Tob2* (9) and human *TOB1* (2) and *TOB2* (11) has been demonstrated in skeletal muscle.

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Disclosure
No conflict of interest declared.

Some *Tob* genes are also expressed in the central nervous system (CNS). For example, *Tob* is expressed in the embryonic CNS of *Drosophila* (12), and amphioxus *Tob* is expressed in the nerve cord during the late neurula and larva stages (8). However, putative functions for *Tob* genes in neurogenesis remain unknown. *Tob* transcripts are also present in adult mouse (9, 10), rat (13), and human (2, 11) brain tissues. Specifically, Tob1 transcripts have been detected in the hippocampus, a region related to learning and memory. Long-term potentiation in the hippocampal CA1 region is thought to be a cellular model of memory formation (14, 15). When mice received intra-CA1 infusion of Tob antisense oligonucleotides, they performed poorly in the water maze, and exhibited a deficit in long-term contextual fear memory, leaving short-term memory intact (13)).

Tob1 has also been detected at relatively high levels in the cerebellum of rats. Tob1 expression is up-regulated in the cerebellum after animals received training on a rotarod-running task. Interestingly, rats infused with Tob1 antisense oligonucleotides into the 4th ventricle exhibited a severe deficit in running on a rotating rod or walking across a horizontally elevated beam (16).

Tob genes are negative regulators of transcription and translation

In T lymphocytes Tob1 associates with Smad2 and Smad4 and enhances Smad4 DNA binding and Smad-dependent transcription (17). In contrast, in osteoblasts, Tob associates with Smads and enhances Smad DNA binding but inhibits Smad-mediated transcription (18). These data link Tob1 to the TGFβ family mediated signaling and regulation of transcription, which has a role in morphogenesis, as well as in cell survival, proliferation and differentiation

In zebrafish Tob1 acts upstream of B-catenin and competes with Lef/Tcf cofactors for binding to B-catenin, effectively blocking formation of a Lef1/Tcf/B-catenin protein complex that can stimulate the transcription of several genes. Additionally, Tob1a inhibits Smad3-induced embryonic dorsalization by physically interacting with and preventing Smad3 from binding to one of its cofactors, p300 (4).

In mammalian cells, mRNA decay begins with deadenylation. Regulation of the critical deadenylation step occurs at the RNA processing bodies (P-bodies), a site where poly (A)-shortened mRNA gets degraded. (19) TOB1 can simultaneously interact with the poly(A) nuclease complex CCR4-CAF1 (via its N-terminal domain) and the cytoplasmic poly(A)-binding protein PABPC1 and induced PABC (iPABP) (via its C-terminal domain), thus effectively enhancing mRNA decay and blocking translation of the target gene (20).

Tob1 in T cells

Tob1 was found to be constitutively expressed in unstimulated peripheral blood T lymphocytes but strongly down-regulated after both antigen-specific and unspecific stimulation. Indeed, down-regulation of Tob1 was required for T cell activation and expansion (17). When expressed, Tob1 inhibits T cell proliferation presumably by suppressing transcription of IL2, IL4 and IFNg, and other positive regulators of the cell cycle such as cyclin E and cyclin A. As these cyclins directly interact with CAF1, it is

possible that Tob1 regulates their expression through its interaction with CAF1-CCR4 complex (11, 21).

TGFβ inhibits T cell proliferation through a SMAD-dependent mechanism. TGFβ signal transduction is initiated by receptor phosphorylation of transcription factors Smad2 and Smad3. Then, phosphorylated Smad2 and Smad3 bind to Smad4 in the cytoplasm and translocate the nucleus where they exert their effect on transcription. Tob1 has been shown to interact with Smad2 and to enhance the ability of Smad4 to bind DNA, therefore increasing Smad-dependent gene transcription (17). However, since the IL-2 gene contains Smad-binding negative regulatory elements that block transcription when activated, the net effect of Tob1 in T cells is inhibition of IL-2 transcription and consequent blocking of cell division.

In addition to direct inhibition of IL-2 transcription, Tob1 also promotes transcription of the $p27^{kip1}$ Cdk inhibitor (*CDKN1B*), which also blocks cell cycle progression (17).

Lymphocyte quiescence has been shown to be dependent on the enzymatic activity of dipeptidyl peptidase 2 (DPP2), a serine protease with an amino terminal dipeptidase activity (22). Indeed, inhibition of DPP2 in resting T cells results in apoptosis (23). Interestingly, the *Dpp2* promoter contains a SMAD binding site, raising the possibility that SMADs are also involved in its transcriptional activation. Confirming this hypothesis, a recent article described the molecular mechanism by which TOB1 expression increases the *Dpp2* promoter activity, effectively positioning Tob1 upstream of Dpp2 in the regulation of T cell quiescence (24).

In addition to its effect on IL-2 transcription (negative) and Dpp2 (positive), Tob1 has been recently shown to suppress the expression of twisted gastrulation (Tsg), a secrete d protein that interacts with Drosophila decapentaplegic (Dpp) and its vertebrate orthologs BMP2/4 and regulates morphogenetic effects in embryos. *TSG* mRNA was found to be expressed at very low levels in unstimulated T cells but highly up-regulated after activation by TCR/CD3 and either CD28, IL-2, or PMA (25). Tsg inhibits proliferation of pre-activated T cells by enhancing TGF-β signaling through phosphorylation of Smad2 and facilitation of DNA binding of Smad3/4.

A recent report described a higher expression of Tob1 in the IL-17 producing pro-inflammatory CD4 T helper (Th17) population, compared to its expression in IFN-g producing Th1 cells (26). A significant positive correlation between IL-4 induced gene 1 (IL4I1) and Tob1 mRNA expression in human Th17 cells was found. These data suggest that IL4I1 upregulation in human Th17 cells limits their TCR-mediated expansion not only by blocking the molecular pathway involved in the activation of the IL-2 promoter, but also by maintaining high levels of Tob1, which impairs entry into the cell cycle

This article also shed some light on the possible mechanism of Tob1 degradation by identifying its interaction with Skp2, an inhibitor of the cyclin-dependent kinase inhibitor *CDKN1B*. Upon binding Tob1, Skp2 promotes its degradation via the ubiquitin-proteasome pathway (27). (Figure 1 summarizes the known processes of Tob1 in T cells)

Tob1 in human and experimental autoimmune demyelination

Using a genome-wide transcriptome analysis, we recently reported downregulation of TOB1 was associated with higher risk of disease activity in patients with multiple sclerosis (MS), an autoimmune disease of the CNS (28). While more than 900 transcripts were differentially expressed in $CD4^+$ T cells from subjects with early symptoms of MS and healthy controls, 108 genes were expressed at variable levels even within patients. This molecular signature obtained shortly after diagnosis defined 4 groups of patients, one of which was significantly associated with higher risk of disease activity in the subsequent 3 years of follow-up. The high-risk signature at baseline was characterized by 7-fold down-regulation of TOB1 (the largest differential expression of any transcript), down-regulation of the pro-apoptotic genes BRD7, PMAIP, SAT and CYCS and the cell cycle inhibitor CDKN1C, and up-regulation of the cell cycle inducer CDC34. This profile suggested that a significant fraction of $CD4^+$ T cells from subjects at high risk of future disease activity were poised to enter a proliferative state, triggering concomitant disease flares. These results suggested TOB1 could be a valuable biomarker in MS, thus relating this gene with a human disease for the first time.

In a follow-up study, we later demonstrated that Tob1-deficient ($Tob1^{-/-}$) mice experienced an earlier disease onset and a more aggressive disease when immunized with myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) to induce experimental autoimmune encephalomyelitis (EAE), a murine model of MS (29). These symptoms resembled the earlier results obtained in humans. T cells from $Tob1^{-/-}$ mice proliferated more than T cells from wild type (WT) mice in response to both antigen-specific and unspecific stimulation. Furthermore, when WT and $Tob1^{-/-}$ $CD4^+$ T cells were stimulated under polarizing conditions, higher proportions of Th1 and Th17 were induced from $Tob1^{-/-}$ cells. Concomitantly, a lower proportion of $CD4^+$ $CD25^+$ $FoxP3^+$ T regulatory (Treg) cells were seen in $Tob1^{-/-}$ mice. Taken together, these results indicate that absence of Tob1 results in a shift of immune responses towards a proinflammatory state.

In order to investigate whether Tob1 expression in T cells was sufficient to explain the symptoms, we reconstituted the $CD4^+$ T cell compartment of Rag1 knockout mice ($Rag^{-/-}$) with $CD4^+$ T cells from either $Tob1^{-/-}$ or WT mice and induced EAE in these animals. Transfer of $Tob1^{-/-}$ CD4+ T cells was sufficient to reproduce the EAE symptoms observed with the conventional $Tob1^{-/-}$ mice, thus highlighting the profound effect of Tob1 on the ability of these cells to proliferate and eventually, to cause damage to the host. Interestingly, when $Tob1^{-/-}$ were crossed to a transgenic mouse line (2D2) in which all T cell receptors (TCR) recognize MOG_{35-55} at least 50% of the resulting offspring develop spontaneous EAE within 120 days. This phenotype can be explained by hyper-proliferation (induced by the absence of Tob1) of encephalitogenic T cells (from the 2D2), which eventually cross the blood brain barrier and get access to the CNS where they cause inflammation and demyelination associated with the observed symptoms.

Summary and conclusions

Several recent publications have significantly advanced our understanding of how the BTG/Tob protein family exerts controls over the cell cycle in health and disease. Elucidating

the mechanism by which Tob1 influences quiescence by controlling mRNA degradation and translation has provided conclusive evidence one how this APRO molecule exerts if cellular effects. On the other hand, still little is known about upstream regulators of Tob1 and no research has been reported on whether Tob1 could be targeted pharmacologically. Given the recent implications of Tob1 in MS and potentially other autoimmune diseases, this seems a goal worth pursuing.

Acknowledgments

This study was supported by a grant from the National Institutes of Health (R01NS26799) and National Multiple Sclerosis Society (NMSS) grant PP1959. SEB is a Harry Weaver Neuroscience fellow from the NMSS.

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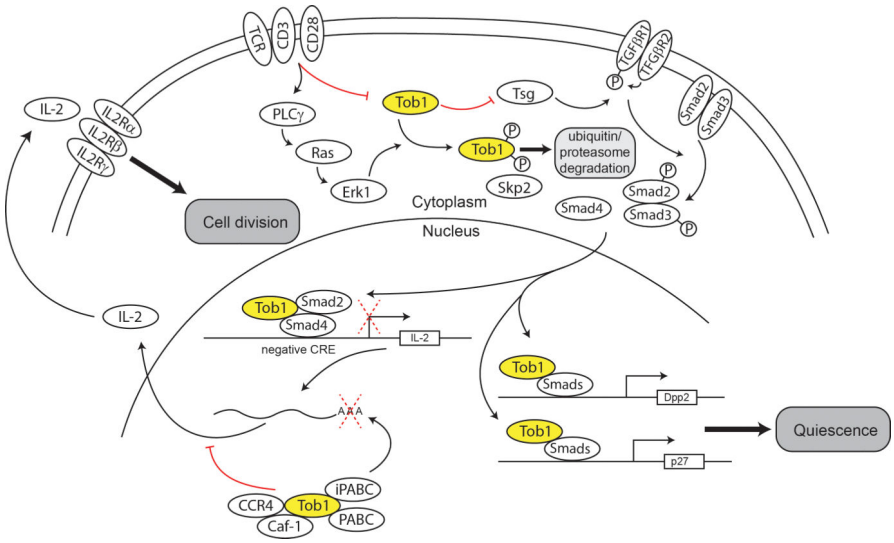

Figure. Tob1 in T cells

Tob1 gets downregulated following TCR signaling. In the nucleus Tob1 enhances binding of Smad4 to the negative *cis* regulatory element (CRE) of the IL-2 gene, thus negatively affecting IL2 expression. Concomitantly, the stability of IL2 mRNA is also regulated by the interaction of Tob1 and PABC and iPABC, a complex that favors deadenylation and subsequent mRNA decay. Through its interaction with CCR4 and Caf-1, Tob1 also inhibits IL2 mRNA translation. These coordinated actions by Tob1 result in quick IL2 downregulation and inhibition of cell division. In the cytoplasm, Tob1 also inhibits Tsg, which in turn, stimulates TGFb-mediated control of cell activation. TGFb acts through its heterodimeric receptor and phosphorylate Smad2 and Smad3, which when complexed with SMad4 translocate to the nucleus. Once there, Tob1 enhances Smad-mediated transcriptional activation of Dpp2 and p27, promoting T cell quiescence.