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REVIEW

TMBIM protein family: ancestral regulators of cell death

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The control of apoptosis in mammals has been historically associated with the activity of the BCL-2 family of proteins at the mitochondria. In the past years, a novel group of cell death regulators have emerged, known as the Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) protein family. This group of proteins is composed of at least six highly conserved members expressed in mammals, with homologs in insects, fish, plants, viruses and yeast. Different studies indicate that all TMBIM family members have inhibitory activities in different setting of apoptosis. Here, we overview and integrate possible mechanisms underlying the impact of the TMBIM protein family in the regulation of cell death, which include activities at diverse subcellular compartments, including death receptor regulation, modulation of endoplasmic reticulum (ER) calcium homeostasis, ER stress signaling, autophagy, reactive oxygen species production, among other effects. The possible intersection between the BCL-2 and TMBIM family in the control of cell death is also discussed, in addition to their implication in the progression of cancer.

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INTRODUCTION

Apoptosis is a conserved cell death mechanism essential for the development and physiologic maintenance of tissues in multicellular organisms. Apoptosis virtually participates in the development of most cell lineages, and its deregulation contributes to a variety of human diseases, including immunodeficiency, autoimmunity, neurodegenerative diseases, cancer, among other pathologies.[1,2] A delicate equilibrium between proliferation and apoptosis is necessary for maintaining homeostasis in healthy tissues. Although apoptosis has been defined in general as a caspase-dependent cell death pathway with well-defined morphological characteristics, two main initiation pathways control the activation of executor caspases.[2] The extrinsic pathway transduces proapoptotic signals from extracellular ligands through a variety of death receptors, whereas the intrinsic pathway is engaged by intracellular proapoptotic signals that converge into mitochondrial outer membrane permeabilization (MOMP) to release apoptogenic factors.[3,4] The occurrence of MOMP is associated with the loss of mitochondrial membrane potential ($\Delta\Psi_m$),[5] an increase in the levels of reactive oxygen species (ROS) and the release of cytochrome *c* to the cytosol,[6] among other factors. Although the nature of the signals involved in both extrinsic and intrinsic pathways is diverse, many examples depict a complex and dynamic crosstalk between different cell death pathways.

The BCL-2 family of proteins is a group of upstream regulators of MOMP that comprises both anti- and proapoptotic components.[1,2] At the structural level, BCL-2 family members have been defined by the presence of up to four small α-helical conserved *B*CL-2 *h*omology (BH) domains. Antiapoptotic components are characterized in most cases by the presence of four BH domains (BH1, BH2, BH3 and BH4).[7,8] Proapoptotic BCL-2 family members can be subdivided further into more highly conserved, 'multidomain' members displaying homology in the BH1, BH2 and BH3 domains (that is BAX, BAK and BOK), and the 'BH3-only' members that contain only one BH domain critical for apoptosis activation in mammals.[9]

Historically, the activity of the BCL-2 family of proteins has been attributed as the master regulator of apoptosis in mammals. However, it is important to mention that the degree of conservation of the family in invertebrates is low and apoptosis and program cell death (PCD) are observed in species where there is no clear implication of BCL-2-related proteins in the process such as flies or yeasts, or in species including plants where PCD is an important mechanism to eliminate cells, but no BCL-2 homologs have been identified (Table 1). Since the discovery of BCL-2 as a cause of human cancer in the late 1980s, most of the attention in the field has been focused in addressing the impact of the BCL-2 family of proteins to MOMP in mammals and its contribution to human disease. Nevertheless, the mechanisms explaining the regulation of MOMP by the BCL-2 family are still not fully understood, and include two main modes of action, or combination of both: antiapoptotic BCL-2 proteins can inhibit MOMP by sequestering activator BH3-only proteins and by inhibiting the downstream activation of BAX and BAK (reviewed in Martinou and Youle[10] and Tait and Green[11]). Then, sensitizer BH3-only proteins may repress this inhibitory interaction releasing activator BH3-only proteins.[12,13] Other models of action explaining the hierarchical organization of the BCL-2 family are also available.[2,14]

Saccahromyces cerevisiae is a simple eukaryote model widely used to study MOMP (see examples in refs Buttner *et al.*,[15] Carmona-Gutierrez *et al.*,[16] Eisenberg *et al.*,[17] Ludovico *et al.*,[18] Madeo *et al.*,[19] Madeo *et al.*,[20] Wissing *et al.*,[21]). In yeast, ectopic expression of mammalian BCL-2 family members is able to reconstitute MOMP.[22] Although overexpression of human BAX or BAK can induce apoptosis in yeast,[22,23] it was not clear in those studies if endogenous components present in yeast are part of that regulatory network. Fifteen years ago a remarkable study by Xu and Reed[24] identified Transmembrane BAX Inhibitor Motif

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Table 1. Phylogenetic analysis of the expression of TMBIM family members in different species

Organism	*BCL-2 protein family*			*TMBIM protein family*					
	Antiapoptotic	*BH3-only protein*	*Proapoptotic multidomain*	*TMBIM1/ RECS1*	*TMBIM2/ LFG*	*TMBIM3/ GRINA*	*TMBIM4/ GAAP*	*TMBIM5/ GHITM*	*TMBIM6/ BI-1*
Homo sapiens	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yess	Yes
Danio rerio	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Drosophila melanogaster	*Buffy, Bok*	No	*Bok*	CG30379[a]	CG9722[a]/ CG3814[a]	CG3798	CG33673[a]	CG1287[a]/ CG2076[a]	CG7188
Caenorhabditis elegans	*Ced-9*	*Egl-1*	No	XBX-6[a]	Y42H9AR.2	Tag-120[a]	TMBI-4[a]	K11h1288[a]	No
Arabidopsis thaliana	No	No	No	AT4G14730.1[a], AT1G03070[a], AT4G15470[a], AtBI-1					
Saccharomyces cerevisiae	No	No	No	Ynl305cp/Bxip/Ybh3p					

Abbreviations: GAAP, Golgi antiapoptotic-associated protein; GHITM, growth hormone-inducible transmembrane protein; GRINA, glutamate receptor ionotropic NMDA protein 1; LFG, life guard; RECS1, responsive to centrifugal force and shear stress 1; TMBIM, Transmembrane BAX Inhibitor-1 Motif-containing. The presence of BCL-2 family member genes was analyzed in selected species and paralleled with the expression of putative TMBIM family homologs. The sequences were obtained from the National Center for Biotechnology Information (http: //www.ncbi.nlm.nih.gov/). [a]Indicate putative genes that were not yet assigned as a homolog.

containing (*TMBIM*)-6, also known as BAX Inhibitor-1 (BI-1), as a novel mammalian gene that negatively regulates apoptosis induced by BAX overexpression on a yeast screening. An important issue to clarify for the discussion in this review is that TMBIM6 was named BI-1 because it has a functional effect over BAX toxicity, but not because of a direct physical interaction.[24] Several bioinformatic studies have then demonstrated the presence of a consensus motif termed UPF0005 in TMBIM6/BI-1, which is expressed in homologs from several species across evolution.[25,26] The UPF0005 domain has an unknown function, and codifies for six or seven transmembrane-spanning regions.[24,27–29] Further protein sequence conservation analysis identified a putative family of at least five highly conserved orthologs of TMBIM6/BI-1 containing the UPF005 domain.[30,31] This group of proteins has also been classified as BI-1 family,[25,31,32] life guard (LFG) family[33] or TMBIM family of proteins.[27,32,34,35] In this review, we will use the definition of the TMBIM family of proteins based on the gene nomenclature of the founder member of this group of cell death regulators.

The TMBIM family of proteins includes TMBIM6/BI-1, TMBIM1/RECS1 (*re*sponsive to *c*entrifugal force and *s*hear stress gene 1 protein), TMBIM2/LFG (life guard), TMBIM3/GRINA (glutamate receptor ionotropic NMDA protein 1), TMBIM4/GAAP (Golgi antiapoptotic-associated protein) and TMBIM5/GHITM (growth hormone-inducible transmembrane protein). An interesting characteristic of the TMBIM family members is that they are highly conserved, where all human components have homologs in other mammals, in zebrafish and also have several homologs in flies. Moreover, this group of proteins is even present in species where no BCL-2 family members have been identified, including plants, yeast, some bacteria and viruses (Table 1). On the basis of their sequence homology, both BCL-2 and TMBIM family of proteins are apparently not related. Nevertheless, this concept is changing because of reports describing physical interactions between them (see below), in addition to the recent discovery of a putative BH3 domain in some TMBIM family members in mammals and in the yeast TMBIM6/BI-1 ortholog (known as Ynl305cp, Bxip or Ybh3p). So far, all studies available in mammals indicate that the TMBIM family of proteins has antiapoptotic activity. More importantly, recent findings also illustrate the contribution of this group of cell death regulators to diverse diseases such as cancer, diabetes and neurodegeneration. In this review, we discuss in detail the impact of the TMBIM family of proteins to the control of cell death in physiology and disease, and provide details on the association of this family with multiple stress pathways. Owing to the expression of TMBIM family members in diverse subcellular compartments, the concept of sentinels of organelle stress is also discussed.

ANTIAPOPTOTIC ACTIVITY OF THE TMBIM FAMILY OF PROTEINS

The TMBIM family is composed of proteins that share sequence homology within the six hydrophobic modules of the UPF005 domain,[24,27] which mediates the localization of the proteins in organelle membranes. TMBIM6/BI-1 is the best described member of the family[36] and because of this most of the field have assumed antiapoptotic activities for all the members of the family. Nevertheless, the presence of a BH3 domain in Bxip/Ybh3p,[37] and the conservation of this domain in other TMBIM family members open the need for a systematic characterization of all components.[37,38] In the next sections, we describe most relevant evidence relating each TMBIM family member with the regulation of extrinsic and intrinsic apoptosis.

TMBIM1/RECS1 AND TMBIM2/LFG: REGULATORS OF EXTRINSIC APOPTOSIS

In 2002, *TMBIM1/RECS1/PP1201* was identified as a gene *re*sponsive to centrifugal force and *s*hear stress (and thus termed RECS) in human endothelial cells of the umbilical vein.[39] TMBIM1/RECS1 has seven putative transmembrane domains[40] and it is located in membranous compartments including plasma membrane, lysosomes and endosomes.[41] However, Shukla *et al.*[40] also showed that endogenous TMBIM1/RECS1 is located at the Golgi apparatus. TMBIM1/RECS1 is expressed in many organs including brain, heart, lung, kidney, stomach, intestine, ovary, uterus, skeletal muscle, skin and adipose tissue, but it is not detected in thymus, liver, spleen or testis.[41] TMBIM1/RECS1 knockout (KO) mice are viable and do not develop spontaneous phenotypic abnormalities. However, aged TMBIM1/RECS1-deficient animals are more susceptible to cystic medial degeneration, a pathological condition associated with aortic aneurysms.[41] TMBIM1/RECS1 expression can specifically reduce Fas ligand (FasL)-induced apoptosis, but it does not affect apoptosis triggered by tumor necrosis factor (TNF) α or some intrinsic death stimuli.[40] At the molecular level, TMBIM1/RECS1 forms a complex with the death receptor Fas/CD95/Apo1 possibly at the Golgi apparatus.[40] TMBIM1/RECS1 expression also decreases the distribution of Fas to the plasma membrane without changing its global expression levels[40] (Figure 1).

TMBIM2/LFG is the closer ortholog of TMBIM1/RECS1.[32] TMBIM2/LFG was identified in a genetic screening to search

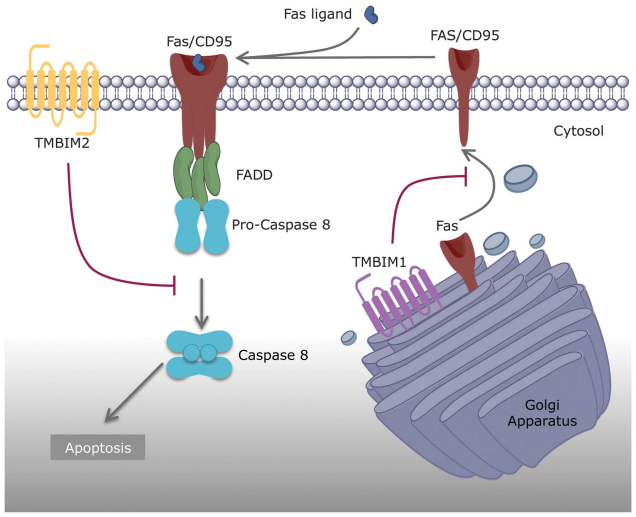

Figure 1. TMBIM family of protein and the control of extrinsic apoptosis. Both TMBIM1/RECS1 and TMBIM2/LFG interfere at different levels of apoptosis signaling induced by FasL. TMBIM1/RECS1 retains Fas/CD95 at the Golgi apparatus, decreasing its availability at the plasma membrane. TMBIM2/LFG blocks caspase-8 activation triggered by Fas/CD96.

genes that are able to block cell death induced by FasL but not by TNFα.[42] TMBIM2/LFG is predominantly expressed in the adult central nervous system depicting a neuronal expression pattern, enriched in dendrites of the pyramidal neurons,[43] Purkinje cells, cerebellar granule neurons and dep cerebellar neurons.[44] At the subcellular level, TMBIM2/LFG is expressed in lipid rafts of the plasma membrane, in addition to the Golgi apparatus and the ER.[45]

Because of its neuronal expression, most of the studies defining the activity of TMBIM2/LFG in apoptosis have been performed in neurons. *Tmbim2* transcript and protein are strongly upregulated during postnatal brain development, and its expression is essential for the survival of neurons during development, in addition to fine-tuning the susceptibility of cells to FasL.[44] The mechanism proposed for the antiapoptotic activity of TMBIM2/LFG suggests that it interferes with caspase-8 activation (Figure 1), but not with its recruitment to the death-inducing signaling complex (DISC) or the binging of FADD.[42] Knocking down TMBIM2/LFG in the central nervous system results in a mouse with reduced cerebellar size. This phenotype correlates with the spontaneous activation of caspase-8 and caspase-3 in Purkinge cells, in addition to triggering an increment in the susceptibility of the cerebellum to Fas-mediated cell death.[44]

Apparently, both TMBIM1/RECS1 and TMBIM2/LFG inhibit Fas signaling, but at different levels of the pathway (Figure 1). The possible synergism between both proteins in the control of extrinsic apoptosis remains to be determined. We predict that a robust phenotype may be observed in the control of apoptosis in *tmbim1/tmbim2* double KO mice, similar to the results obtained when BAX and BAK were targeted simultaneously.[46] More studies are needed to assess the impact of the TMBIM family in the control of extrinsic apoptosis in settings where this pathway has demonstrated function, including the immune system, autoimmunity and certain forms of cancer.

TMBIM FAMILY OF PROTEINS AND THE REGULATION OF INTRINSIC APOPTOSIS

As mentioned above, TMBIM6/BI-1 was discovered in a genetic screening to identify genes that are able to inhibit cell death induced by BAX in yeast.[24] In that study, the overexpression of TMBIM6/BI-1 in human embryonic kidney cells reduced cell death induced by many stimuli, including BAX overexpression, treatment of cells with etoposide or staurosporine and growth factor withdrawal. Nevertheless, the antiapoptotic effects of TMBIM6/BI-1 overexpression were less pronounced when compared with BCL-2 overexpression.[24] Although BAX activation is inhibited by the overexpression of TMBIM6/BI-1 in mammalian cells, the mechanism of action still remains obscure. Interestingly, TMBIM6/BI-1 forms a complex with BCL-2 and BCL-X_L, but not with BAX or BAK.[24,47] On the basis of this observation, it may be feasible that the antiapoptotic activity of TMBIM6/BI-1 is mediated by an interaction with BCL-2. Besides, TMBIM6/BI-1-deficient cells (fibroblasts, hepatocytes and neurons) display selective hypersensitivity to apoptosis induced by agents that trigger ER stress, but not to other intrinsic or extrinsic apoptosis stimuli.[28] The effects of TMBIM6/BI-1 on ER stress-mediated apoptosis have been linked to the modulation of ER calcium homeostasis (Figures 2 and 3) and the inhibition of the unfolded protein response (UPR) (see next sections). Although *tmbim6/bi-1*-deficient mice are viable and develop normally, they are more susceptible to tissue damage induced by stroke, ischemia–reperfusion and experimental ER stress in the brain,[48] liver[49–51] and kidney,[49] respectively.

TMBIM4/GAAP was identified in vaccinia virus as a highly hydrophobic protein that regulates apoptosis.[52] TMBIM4/GAAP is also highly conserved in mammals, insects, fish, plants and protozoa. In humans, the TMBIM4/GAAP homolog has an extraordinarily high degree of conservation with camelpox virus TMBIM4/GAAP (73% of identity).[52] This degree of conservation contrasts with other viral proteins that modulate innate immunity and have orthologs of human proteins.[53,54] TMBIM4/GAAP is expressed in most tissues, and it is distributed to the Golgi apparatus and the ER.[52] At the functional level, TMBIM4/GAAP activity appears to be conserved and is essential for cell survival. Inhibition of human GAAP expression by short interfering RNA resulted in spontaneous apoptosis,[52] similar to the effects of knocking down TMBIM6/BI-1,[24] or TMBIM2/LFG[45]. This contrasts with the impact of targeting most BCL-2 antiapoptotic family member where no spontaneous cell death is observed. Human or viral TMBIM4/GAAP has broad antiapoptotic activity, protecting cells from Bax overexpression, or treatments with staurosporine, doxorubicin and C_2-ceramide.[52] In contrast to TMBIM2/LFG and TMBIM1/RECS1, overexpression of TMBIM4/GAAP also inhibits cell death induced by tumor necrosis factor (TNF)-α and FasL.[52] It remains to be determined if the control of both intrinsic and extrinsic apoptosis by TMBIM4/GAAP is commanded by the same molecular mechanism.

In contrast to other members of the TMBIM family of proteins, TMBIM5/GHITM is the only component localized to the mitochondrial inner membrane,[55] similar to BCL-X_L in certain experimental systems.[56] The name GHITM was assigned because its mRNA was found deregulated in a gene expression analysis of transgenic mice expressing a growth hormone antagonist.[57] TMBIM5/GHITM is ubiquitously expressed in mouse embryo and adult tissues[58] and has been suggested that the cleavage of the N-terminal portion regulates its own expression.[58,59] However, *Oka et al.*[55] redefined TMBIM5/GHITM as MICS1 (*mi*tochondrial morphology and *c*ristae *s*tructure) based in their own characterization of its function. In this report, knocking down TMBIM5/GHITM induced mitochondrial fragmentation and cristae disorganization. Targeting TMBIM5/GHITM enhanced the release of cytochrome *c* and Smac/Diablo from the mitochondria triggered by actinomycin D treatment.[55] So far, up to date TMBIM5/GHITM is the only TMBIM family member shown to be located in the mitochondria in mammals and that directly regulates MOMP (Figure 2).

Tmbim1b, also called *lfg5*, was proposed as another putative member of the TMBIM protein family.[32,33] *Tmbim1b* is found

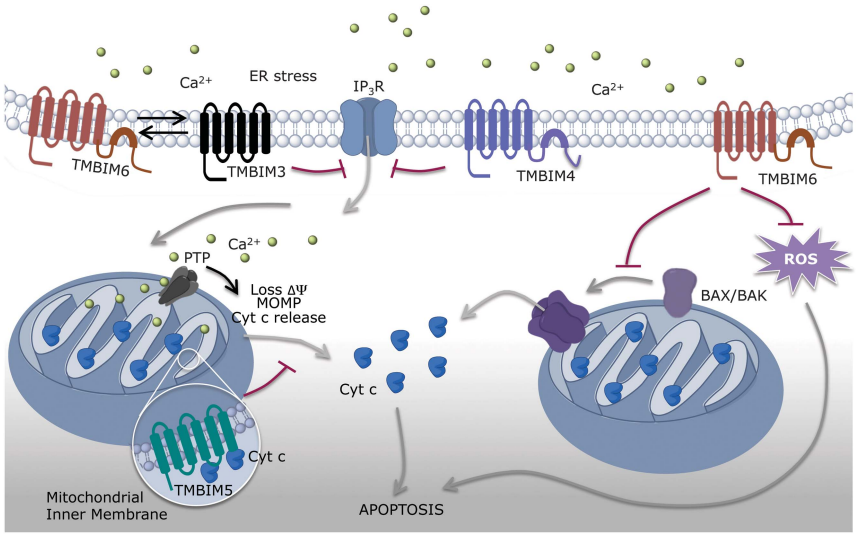

Figure 2. TMBIM family of protein and the control of intrinsic apoptosis. At the ER membrane, both TMBIM3/GRINA, TMBIM6/BI-1, and TMBIM4/GAAP inhibit Ca^{2+} release from the ER through IP_3Rs. Mitochondrial Ca^{2+} overload induces the PTP, which leads to the loss of $\Delta\Psi_m$, ionic imbalances, matrix swelling and MOMP. PTP has been associated with cytochrome *c* release and caspase activation. It has also been proposed that TMBIM5/GHITM directly inhibits cytochrome *c* release at the mitochondrial inner membrane. TMBIM6/BI-1 could also block ROS generation, BAX and BAK oligomerization at the mitochondria and cytochrome *c* release by unknown mechanisms.

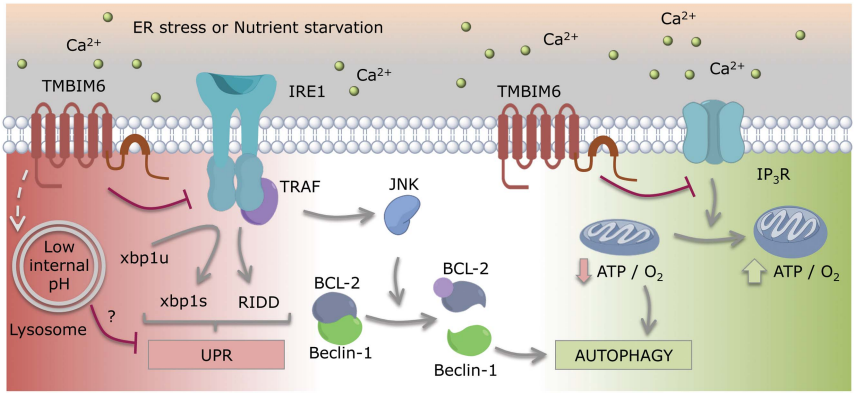

Figure 3. Regulation of stress pathways by the TMBIM protein family. TMBIM6/BI-1 interacts with the ER stress sensor IRE1α and inhibits its RNAse activity, diminishing XBP-1 mRNA splicing and regulated IRE1-dependent decay of mRNA (RIDD). TMBIM6/BI-1 could also displace TRAF2 from IRE1α, repressing c-Jun N-terminal kinase activation downstream of IRE1α. In this context, TMBIM6/BI-1 inhibits the c-Jun N-terminal kinase/IRE1α pathway, which could promote autophagy through the phosphorylation of BCL-2/BCL-X_L, releasing Beclin-1 to promote autophagosome formation. On the other hand, TMBIM6/BI-1 could impact mitochondrial bioenergetics though a pathway mediated by ER calcium release, reducing both oxygen consumption and ATP levels, and stimulating autophagy. Alternatively, TMBIM6/BI-1 expression reduces pH in lysosomes, which may indirectly affect ER stress levels and/or global UPR signaling through an unknown mechanism.

exclusively in eutherian mammals, and in mouse its expression is restricted to adult testis.[33] *Tmbim1b* expression in human cells has not been described in detail, and a premature termination codon was identified in two genomic sequences and two ESTs (reviewed in Hu *et al.*[33]). Thus, the possible contribution of *tmbim1b* to apoptosis remains to be tested.

INTERACTIONS BETWEEN TMBIM FAMILY MEMBERS ON THE CONTROL OF APOPTOSIS

An initial report identified TMBIM3/GRINA in *Rattus* sp. neurons (NMDARA1) as a glutamate-binding protein.[60] However, further studies led to doubts about its identity as a glutamate receptor-related protein.[61] TMBIM3/GRINA is highly conserved in mammals, insects, fish and plants, and was also reported as an oligodendrocyte transmembrane protein, expressed in oligodendrocytes acquiring a myelinogenic phenotype.[62] TMBIM3/GRINA is strongly expressed in the central nervous system,[63] similar TMBIM2/LFG. At the subcellular level, TMBIM3/GRINA is primarily located at the ER[34] and the Golgi compartment.[63] A viable *tmbim3/grina*-deficient mouse was generated and confirmed the neuronal expression of TMBIM3/GRINA.[63] However, no functional analysis of this animal model was performed. Manipulation of TMBIM3/GRINA levels in *Drosophila melanogaster* and zebrafish revealed a relevant antiapoptotic activity *in vivo* at basal levels and under experimental ER stress conditions.[34]

We reported that overexpression of mouse TMBIM3/GRINA specifically reduced cell death induced by several ER stress agents, but not by other agents that activate both intrinsic and extrinsic apoptosis. Silencing of TMBIM3/GRINA expression does not induce spontaneous cell death in mammalian fibroblasts,[34] in contrast with the observed phenotype obtained after knocking down

TMBIM6/BI-1 or TMBIM4/GAAP. Remarkably reducing the expression levels of TMBIM3/GRINA in *tmbim6/bi-1*-null cells led to spontaneous apoptosis, suggesting complementary and redundant activities of both proteins in the control of cell death (Figure 2). Consistent with this concept, the formation of a protein complex between TMBIM3/GRINA and TMBIM6/BI-1 was also observed. This hypothesis is also supported by *in vivo* experimentation in *D. melanogaster* where knocking down TMBIM3/GRINA or TMBIM6/BI-1 alone is not lethal, but reduces the survival of flies to experimental ER stress. In sharp contrast, TMBIM3/GRINA and TMBIM6/BI-1 double deficiency generates lethality and these animals are extremely susceptible to pharmacological ER stress.[34] The impact of targeting these two TMBIM family members on cell death/animal survival in flies highly contrasts with several studies showing poor or almost null PCD-related phenotypes of BCL-2/buffy or BAX/debcl mutants in *D. melanogaster*.[64,65] In addition, targeting TMBIM3/GRINA expression in zebrafish using morpholino demonstrated enhanced apoptosis during development of the nervous system. Ectopic expression of mouse TMBIM3/GRINA in this vertebrate model also provided strong protective effects on an *in vivo* paradigm of ER stress,[34] as shown when mammalian BCL-2 family members are expressed in zebrafish.[66] These studies demonstrated for the first time an interconnection between different TMBIM family members in the control of apoptosis, contributing to define a functional family of cell death regulators across species.

CONTROL OF ER CALCIUM HOMEOSTASIS BY THE TMBIM FAMILY OF PROTEINS

Several components of the TMBIM protein family have been linked to the control of calcium (Ca^{2+}) homeostasis. Ca^{2+} is a known signaling molecule involved in apoptosis. For example, rises in cytosolic calcium could sensitize cells to apoptosis through the mitochondria and the release of cytochrome *c*. In this context, the opening of the mitochondrial permeability transition pore (PTP), a nonspecific pore in the inner mitochondrial membrane, is observed under conditions of mitochondrial Ca^{2+} overload, especially when accompanied by oxidative stress.[67,68] Opening of the PTP leads to dissipation of $\Delta\Psi_m$ and an influx of solutes, resulting in sufficient swelling to rupture the outer mitochondrial membrane and cytochrome *c* release. Alternatively, ER Ca^{2+} depletion can also trigger cell death by inducing ER stress.

Several TMBIM family members are able to modulate the content of ER Ca^{2+}, including TMBIM6/BI-1 (reviewed in Henke *et al.*[30]), TMBIM4/GAAP[69] and TMBIM3/GRINA,[34] in addition to affect the stimulation of Ca^{2+} release through the inositol trisphosphate receptor (IP_3R) (Figure 4). The expression of TMBIM family members reduces ER calcium release and sometimes also decreases the steady-state ER calcium content, which has been suggested to impact the susceptibility of cells to apoptosis. A similar activity has been extensively described for several antiapoptotic members of the BCL-2 family at the ER, such as BCL-X_L and BCL-2 (reviewed in Kiviluoto *et al.*[70]). Cell lines derived from TMBIM6/BI-1 KO mice release more ER Ca^{2+} after stimulation with IP_3R agonists or by the inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase pump to trigger the passive release of Ca^{2+}.[28,71,72] These observations are explained in part because TMBIM6/BI-1 induces a reduction of the absolute amount of Ca^{2+} stored at the ER. Three different mechanisms have been proposed to explain the control of Ca^{2+} homeostasis by TMBIM6/BI-1: (i) TMBIM6/BI-1 may have Ca^{2+} channel properties;[71,73] (ii) it could act as a Ca^{2+}/H^+ antiporter;[28,71,74] or (iii) it could function as an IP_3R-sensitizing protein.[75] The carboxyl-terminal region of TMBIM6/BI-1 can form Ca^{2+} channels *in vitro* in artificial lipid bilayers, which may be responsible for its Ca^{2+} leak properties. This activity involves negative residues in the TMBIM6/BI-1 sequence,[73] which are also modulated by changes in pH.[76] Unexpectedly, under low cytosolic pH, the Ca^{2+}/H^+ antiporter activity may turn TMBIM6/BI-1 into a proapoptotic protein

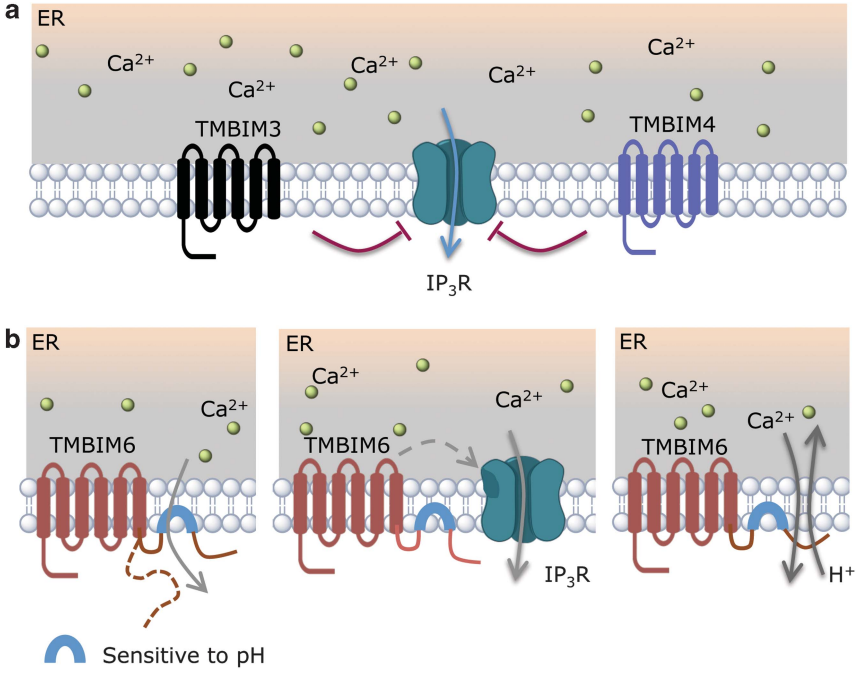

Figure 4. Control of ER calcium homeostasis by the TMBIM family of proteins. (**a**) At the ER membrane, TMBIM3/GRINA expression does not disturb the steady-state ER calcium content; however, it diminishes stimulated calcium release through IP_3R under ER stress conditions by IP_3 release. TMBIM4/GAAP expression reduces ER calcium content, and diminishes calcium release mediated by IP_3R. (**b**) Three different models have ben proposed to explain the effects of TMBIM6/BI-1 on calcium homeostasis: its C-terminal region may have calcium channel properties (left panel), it may function as an IP_3R-sensitizing protein (middle panel) or it could act as a Ca^{2+}/H^+ antiporter (right panel).

because ER Ca^{2+} stores are depleted.[71] This contrasting activity of TMBIM6/BI-1 in the control of cell death was corroborated in deficient cells for this protein because they were less sensitive to apoptosis triggered by lowering the pH.[74] These data suggest a dual and contrasting activity of TMBIM6/BI-1 in regulating cell survival depending on the context. Interestingly, although this function depends on the charged amino acids present on its carboxyl terminal, this sequence is not present in its yeast homolog.

As both BCL-2 and BCL-X_L physically interact with TMBIM6/BI-1,[24] the possible relationship between BCL-X_L and TMBIM6/BI-1 was investigated in the context of ER calcium homeostasis. The overexpression of BCL-X_L reduces ER Ca^{2+} content, and this effect is abolished in TMBIM6/BI-1-deficient cells.[77] These results suggested that TMBIM6/BI-1 acts downstream of BCL-X_L. Finally, mechanistic studies have shown that the BH4 domain sequence derived from BCL-2 or BCL-X_L, or phospholipids such as cardiolipin and phosphatidylserine, stimulate the putative Ca^{2+}/H^{+} antiporter function of TMBIM6/BI-1.[78]

The activity of TMBIM6/BI-1 in the handling of ER Ca^{2+} can impact additional cellular processes. For example, the increment in ER Ca^{2+} in hepatocytes derived from TMBIM6/BI-1 KO mice can promote dephosporylation of the transcription factor NFAT (nuclear factor of activated T cells) and its traslocation to the nucleus, enhancing the regeneration of hepatocytes.[79] Besides, the overexpression of TMBIM6/BI-1 indirectly affects actin polymerization, mediated by an increase of store-operated Ca^{2+} entry.[80] Accordingly, TMBIM6/BI-1 expression increases cell adhesion because it is able to induce actin polymerization, also involving an interaction with G-actin.[80] TMBIM6/BI-1 can also prevent the increment of ROS that arises as a consequence of ER stress, mainly by the upregulation of heme oxygenase 1,[81] but also by interfering with NADPH-dependent cytochrome *P*450 reductase.[82] Overexpression of TMBIM6/BI-1 may also suppress indirectly mitochondria-mediated ROS production in a manner dependent of ERK activation.[83]

Similar to TMBIM6/BI-1 both TMBIM4/GAAP and TMBIM3/GRINA are able to control ER Ca^{+2} homeostasis, where their overexpression reduces IP_3-mediated ER calcium release[34,69,75] (Figure 4). A physical association between TMBIM4/GAAP[69] or TMBIM3/GRINA[34] with IP_3Rs was reported using co-immunoprecipitation. Nevertheless, the global effect of these proteins in ER Ca^{2+} concentration at basal conditions is different between them. Although TMBIM4/GAAP overexpression reduces ER Ca^{2+} content,[69] the overexpression of TMBIM3/GRINA does not alter the steady-state ER Ca^{2+} levels.[34] However, TMBIM3/GRINA has a specific effect on controlling the activity of the IP_3R.[34] More importantly, TMBIM3/GRINA has synergistic activities with TMBIM6/BI-1 in the modulation of ER Ca^{2+} homeostasis, consistent with their synergistic effects on the regulation of apoptosis under ER stress[34] (Figure 4). TMBIM4/GAAP has the ability to oligomerize in a pH-regulated manner,[84] similar to TMBIM6/BI-1,[74] which may impact apoptosis.[84] However, although this characteristic is conserved between viral and human TMBIM4/GAAP, a mutant viral TMBIM4/GAAP that is unable to oligomerize retained both its antiapoptotic activity and its effects on Ca^{2+} homeostasis. Finally, a recent report indicated that the activity of TMBIM4/GAAP on Ca^{2+} signaling has an impact on focal adhesion dynamics, cell adhesion and migration.[85] These biological effects were explained in part by the stimulation of Ca^{2+} influx across the plasma membrane via store-operated Ca^{2+} entry, modulating calpain activation. Similarly to TMBIM6/BI-1, the C-terminal region of TMBIM4/GAAP mediated its impact on both store-operated Ca^{2+} entry and apoptosis.[84]

In summary, data available indicate that there is a direct correlation between both the antiapoptotic activity of several TMBIM family members and their impact on Ca^{2+} homeostasis associated with a subcellular distribution at the ER and Golgi compartments. However, the exact mechanism connecting both processes is not well defined, and the involvement of the PTP in the modulation of apoptosis by TMBIM family members remains to be determined.

REGULATION OF STRESS PATHWAYS BY THE TMBIM PROTEIN FAMILY

The ER operates as a platform where several stress signaling pathways are initiated, modulating the efficiency of protein folding, synthesis, protein degradation, gene expression, apoptosis and bioenergetics. Under a variety of physiological and pathological conditions, unfolded proteins can accumulate at the ER lumen, a cellular condition known as ER stress.[86] A successful adaptation to ER stress depends on the engagement of the UPR, an integrated signal-transduction pathway that reduces unfolded protein load. The UPR is initiated by the activation of three major stress sensors including activating transcription factor 6 (ATF6) α and β, protein kinase RNA activated-like ER kinase (PERK) and inositol-requiring enzyme 1 (IRE1) α and β[87]. PERK, IRE1α and ATF6 signaling govern in concert the expression of a large spectrum of partially overlapping target genes that mediate the recovery of ER homeostasis. IRE1α is the most conserved UPR signaling branch and the only one expressed in yeast. Upon activation, IRE1α dimerizes and autophosphorylates favoring a conformational change that activates an endoribonuclease domain located at the cytosolic region. Active IRE1α catalyzes the splicing of mRNA coding for XBP-1 (X box-binding protein 1), excising a 26-nucleotide intron, and thus shifting the coding reading frame of this mRNA. This triggers the expression of a potent transcription factor termed XBP-1s for the spliced form. Many different components modulate the activation and inactivation of IRE1α signaling at the ER membrane through a protein complex we have referred to as the UPRosome.[88,89] In the control of apoptosis by ER stress, the UPR upregulates the expression of a subset of BH3-only proteins mediated by downstream signaling events of PERK. This involves the UPR transcription factors ATF4 (activating transcription factor 4) and C/EBP homologous protein (CHOP: also named growth arrest and DNA damage-inducible 153, GADD153). Many additional signaling pathways contribute to the induction of apoptosis by irreversible ER stress (reviewed in Tabas and Ron[90] and Urra *et al.*[91]).

Under ER stress, *tmbim3/grina* is upregulated at the transcriptional level on a PERK- and ATF4-dependent manner, whereas *tmbim6/bi-1* expression is not altered.[34] An initial study performed by John Reed's group in TMBIM6/BI-1-deficient animals revealed that TMBIM6/BI-1-deficient mice subjected to ischemia show hyperactivation of the IRE1α pathway and possibly ATF6 in the kidney and liver.[49] Interestingly, in this analysis proximal PERK signaling events were not altered in TMBIM6/BI-1 KO animals. On the basis of these interesting observations, we explored the possible mechanism underlying the overactivation of ER stress signaling in TMBIM6/BI-1-null cells, in addition to mice and flies exposed to ER stress. We showed that TMBIM6/BI-1 directly inhibits IRE1α activity by binding to its cytosolic domain.[47] This event was essential for the attenuation of IRE1α signaling after prolonged ER stress. The modulation of IRE1α by TMBIM6/BI-1 was reconstituted *in vitro* on a cell-free system, and was mediated by its C-terminal region[47] (Figure 3). Of note, the same region of TMBIM6/BI-I is responsible for the control of Ca^{2+} and apoptosis. A subgroup of the BCL-2 family members, including BAX and BAK[92] and the BH3-only proteins BIM and PUMA,[93] can stimulate IRE1α activity associated with a physical interaction. TMBIM6/BI-1 may compete with BAX/BAK for their binding to the UPRosome.[47] Interestingly, as TMBIM6/BI-1 negatively regulates the expression of XBP-1, a potent adaptive factor of the UPR, it can reduce the survival of cells under ER stress, as demonstrated when XBP-1

levels were manipulated in the context of TMBIM6/BI-1 deficiency.[47] Thus, TMBIM6/BI-1 may have a dual role in controlling cell survival under stress by distinct mechanisms. Other studies showed that BAR, a RING (Really Interesting New Gene)-type E3 ligase on the ER membrane, interacts with TMBIM6/BI-1, induces its ubiquitination and proteosomal-mediated degradation, enhancing IRE1α signaling.[94] Moreover, the interaction between TMBIM6/BI-1 with IRE1α affects glucose metabolism *in vivo*. TMBIM6/BI-1 is downregulated in the liver and muscle of mice undergoing diet-induced obesity, as well as in genetically obese mice.[50] Overexpression of TMBIM6/BI-1 by adenoviral gene transfer improved glucose metabolism in both obese mice and inhibited IRE1α signaling, associated with a physical interaction between both proteins.[50] Similar effects of TMBIM6/BI-1 expression were reported on ER stress signaling using neuronal-specific transgenic mice in the context of brain ischemia.[48]

Other studies in cells overexpressing TMBIM6/BI-1 indicated that it can also have a global effects on the physiology of the ER, affecting the signaling of the three main UPR signaling branches indirectly, possibly due to lysosomal alterations.[95] This pathway also modifies cytochrome P450 expression and ROS levels, which may influence protein folding and the susceptibility of cells to ER stress.[82] Taken together with the impact of TMBIM family members in Ca^{2+} homeostasis, it is feasible to speculate that, depending on the cellular context and stimuli, TMBIM6/BI-1 may control ER stress signaling through IRE1α and possibly ATF6, or in a more generic way by affecting ER function (that is calcium, protein degradation or redox metabolism).

Coupled to the regulation of the UPR, TMBIM6/BI-1 has been implicated to the repression of macroautophagy (here referred to as autophagy) through IRE1α, initiated by the binding of the adapter protein TRAF2 to active IRE1α and downstream signal of c-Jun N-terminal kinase[96] (Figure 3). These events control the phosphorylation of BCL-2 locally at the ER membrane, releasing Beclin-1, an essential activator of autophagy.[97] TMBIM6/BI-1-deficient cells, and fly larvae with manipulated levels of this protein, presented a faster and stronger induction of autophagy, promoting efficient adaptation to nutrient starvation.[96] Moreover, liver and kidney of *tmbim6/bi-1*-deficient mice showed increased expression of autophagy indicators at basal levels or after induction of experimental ER stress.[96] These results are in agreement with the known inhibitory activity of antiapoptotic BCL-2 family members on the engagement of autophagy, where they converge into the regulation of Beclin-1. In contrast, on a different setting, TMBIM6/BI-1 expression was shown to enhance autophagy by a parallel antagonizing pathway. TMBIM6/BI-1 decreases ATP levels and O_2 consumption and augmented autophagy on an IP_3R- and calcium-dependent manner, possibly due to the modulation of mitochondrial Ca^{2+} uptake that affected global bioenergetics[98] (Figure 3). Thus, two independent pathways control autophagy by TMBIM6/BI-1, the IRE1α/c-Jun N-terminal kinase and the IP_3R/Ca^{2+} modules, which may be selectively engaged on a stimuli and cell type-specific manner.

In summary, the evidence presented in this section illustrates a critical role of TMBIM6/BI-1 as a stress integrator that modulates UPR and autophagy levels, and other interconnected homeostatic processes. Nevertheless, it is still unknown if other members of the TMBIM family of proteins share the same capability to fine-tune the UPR and autophagy.

AN ANCESTRAL TMBIM-RELATED PROTEIN IN YEAST: THE INTERSECTION WITH THE BCL-2 FAMILY?

As mentioned, Ynl305cp (Bxip/Ybh3p) have been proposed as a putative homolog of mammalian TMBIM6/BI-1 protein in yeast,[99,100] as well as a putative BH3-only protein.[37] However, it is important to mention that phylogenetic comparison of all human TMBIM family members with the protein sequence of YnL305cp indicated that all of them are almost equally close to the yeast ortholog.[34,47] Ynl305cp colocalizes with Sec63, an ER protein,[99] but it has been also detected at the vacuole (equivalent to the lysosomes). Yeast mutants in Ynl305cp are more sensitive to different stress insults,[99] including ER and oxidative stress conditions.[100]

In contrast to studies with mammalian TMBIM6/BI-1, one report showed that yeasts lacking Ynl305cp had a decreased UPR response.[99] However, we did not detect any effects on UPR signaling in Ynl305cp mutants, which was consistent with the fact that the C-terminal region of mammalian TMBIM6/BI-1 is not present in Ynl305cp.[47] Interestingly, another study showed that ectopic expression of human BAX in yeast induced IRE1p overactivation, suggesting that the UPRosome machinery may be present in these species.[101] In addition besides, the effects of Ynl305cp on autophagy are not conserved in yeast.[96]

Ynl305cp was shown to have a functional BH3 domain and it is able to interact with mammalian BCL-X_L.[37] In yeast, Ynl305cp translocates to the mitochondria and triggers apoptosis that is dependent on the putative BH3 domain in response to acetic acid or H_2O_2[37] (Figure 5a). Interestingly, as some BH3-only proteins instigate IRE1α activity,[93] it is feasible to speculate that Ynl305cp may enhance the UPR through the putative BH3 domain. Of note, other member of the TMBIM family, TMBIM1/RECS1, could contain a putative BH3 domain[37] (Figure 5b); however, its function has not been characterized. Importantly, it was recently indicated that the putative BH3 sequence of Ynl305cp overlaps the last transmembrane segment, which is not a characteristic among classical BH3-containing proteins in mammals.[38] Therefore, it is still unclear if

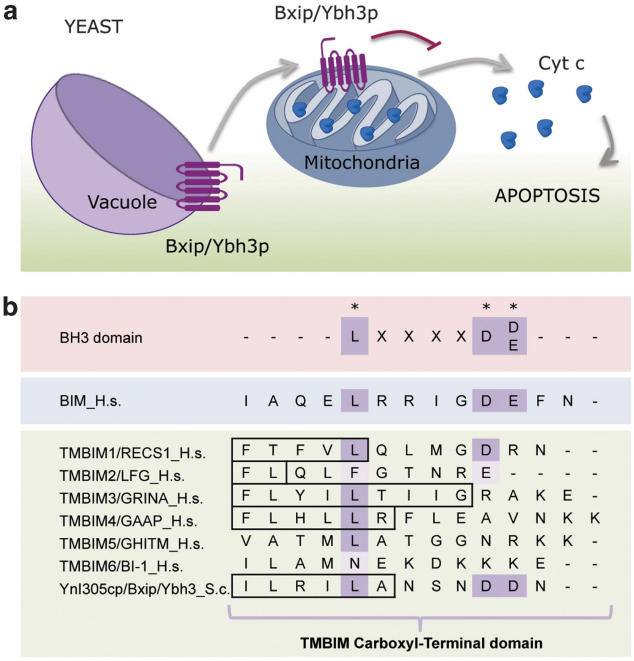

Figure 5. TMBIM protein in yeast and the putative BH3 domain. (**a**) In yeast, the gene *Ynl305cp* codifies for Bxip/Ybh3p, a protein that translocates from the vacuole to the mitochondria under stress conditions. At the mitochondria, Bxip/Ybh3p induces cytochrome *c* release through a putative BH3 domain, enhancing apoptosis. (**b**) Sequence comparison of the BH3 domain (L-4(X)-D-(D/E)) of the human BH3-only protein BIM (BCL-2 family member) with the putative sequence present in some TMBIM family members and yeast ortholog Bxip/Ybh3p. Asterisks indicate essential amino acids defining the BH3 domain. Amino acids with black outline correspond to amino acids contained in the final transmembrane domain.

Ynl305cp could represent an ancestral connector between both the TMBIM and BCL-2 family of proteins.

TMBIM FAMILY OF PROTEINS IN CANCER AND OTHER HUMAN DISEASES

As apoptosis impairment is a known contributor to cancer progression, several groups have started to address the possible impact of the TMBIM family of proteins to various aspects of cancer progression. In this line, it is also important to highlight that the UPR and autophagy are also becoming interesting targets for cancer therapy as they have implications not only to solid tumor survival but also to metastasis, cell transformation, angiogenesis, among other events.[102,103]

The most studied TMBIM family member in the context of pathological conditions is TMBIM6/BI-1, with a remarkable connection with the progression of cancer (Table 2). For example, TMBIM6/BI-1 is overexpressed in lung adenocarcinoma,[104] breast cancer,[105] prostate cancer,[106] tumor-like glioma,[107] anaplastic large-cell lymphoma,[108] nasopharyngeal carcinoma[109] and acute myeloid leukemia.[110] In contrast, TMBIM6/BI-1 is downregulated in some forms of stomach, colon, kidney, lung and rectal cancers.[105]

So far, the expression pattern of the TMBIM family as a whole has not been properly assessed. Here, we have analyzed the mRNA expression profiles of all TMBIM family members in databases obtained from diverse cancer tissues, including the Oncomine cancer microarray database[111] (Table 3). Interestingly, from this analysis, TMBIM1/RECS1, TMBIM2/LFG and TMBIM5/GHITM were found to be downregulated in many types of cancer, whereas TMBIM3/GRINA, TMBIM4/GAAP and TMBIM6/BI-1 are significantly upregulated in most of the studies reviewed (Table 3).

TMBIM4/GAAP is upregulated in patients with a significantly reduced survival score associated with two single-nucleotide polymorphisms located in regions over 500 kb from the 3′-untranslated region of *TMBIM4/GAAP* gene.[112] Besides, TMBIM6/BI-1-derived peptides are also proposed as possible antigens associated with the development of leukemia.[110].

At the mechanistic level, the contribution of TMBIM6/BI-1 to cancer involves many aspects of the biology of the disease. TMBIM6/BI-1 may control cellular metabolic stages, promoting cell proliferation and tumor growth.[113] Other studies have shown that overexpression of TMBIM/BI-1 in both human fibrosarcoma and mouse melanoma cell lines can drive metastasis *in vivo*.[114] Short interfering RNA targeting of TMBIM6/BI-1 also reduces the occurrence of metastasis.[114] This effect was linked to the capacity of TMBIM6/BI-1 to reduce extracellular pH and to alter glucose metabolism.[114,115] TMBIM6/BI-1 expression can promote cancer cell survival in several experimental systems,[105,116] in addition to enhancing abnormal cellular transformation.[117] Moreover, *in vitro* assays indicated that TMBIM6/BI-1 might represent a pharmacological target of the chemotherapeutic drugs doxorubicin and daunorubicin.[118] Both anthracyclines were able to inhibit the Ca^{2+}/H^{+} antiporter-like activity of TMBIM6/BI-1, which correlated with the loss of the antiapoptotic effects of TMBIM6/BI-1 in cells exposed to ER stress.[118] All these reports illustrate the possible impact of TMBIM6/BI-1 in the development and progression of cancer.

TMBIM4/GAAP has been also linked to cancer progression and metastasis. In this context, TMBIM4/GAAP influences cell adhesion and migration in both human osteosarcoma and cervical cancer cells through a mechanism that involves store-operated Ca^{2+} entry.[85] TMBIM2/LFG is also overexpressed in some forms of human breast cancer,[119–121] operating as a survival factor in the cancer cell lines MCF-7 and SW872.[121] Similarly, the expression of TMBIM5/GHITM is also altered in several cancer cell lines (Table 2).[59] However, the functional impact of TMBIM5/GHITM to cancer has not been addressed.

Table 2. The role of the TMBIM family of proteins in cancer

Gene	*Cancer model*	*Observation*	*References*
Tmbim2/lfg	Human breast cancer cells (MCF-7 and MDA-MB 231)	Upregulated expression	Buc*an et al.*[119–121]
Tmbim4/gaap	Human osteosarcoma (U2-OS) and human cervical cancer (HeLa) cells	Controls cell adhesion and migration	Saraiva *et al.*[84,85]
	Lung cancer	Upregulated expression	Wu *et al.*[112]
Tmbim5/ghitm	A549, ACTL, HeLa, HepG2, MCF-7, SW-620, SW-872, U2-OS	Expressed	Reimers *et al.*[59]
Tmbim6/bi-1	Lung adenocarcinoma	Frequently expressed	Tanaka *et al.*[104]
	Human breast cancer cells (MCF-7)	Its expression is hormone independent	Grzmil *et al.*[105]
	Tumor-like glioma cells	Overexpressed compared with normal brain	Schmits *et al.*[131]
	Anaplastic large-cell lymphoma	Upregulated expression	Villalva *et al.*[108]
	Human nasopharyngeal carcinoma cells	Its downregulation induces apoptosis	Zhang *et al.*[109]
	Acute myeloid leukemia	Cancer antigen	Schmidt *et al.*[110]
	Tissues form stomach, colon, kidney, lung and rectal cancers	Downregulated in ≈50% of cases	Grzmil *et al.*[105]
	Human malignant nasopharyngeal carcinoma cells	Its downregulation reduces proliferation, cell viability and tumorigenicity	Li *et al.*[113]
	Human fibrosarcoma cell line (HT1080) and mouse melanoma cell line (B16F10)	Enhances metastasis and tumor growth	Lee *et al.*,[74,115]
	Human breast cancer cells (MCF-7)	Promotes cell survival	Grzmil *et al.*,[105] Hsu *et al.*,[116] Lima *et al.*[132]
	NIH3T3 cells	Induces cell transformation *in vitro* and promotes tumorigenesis *in vivo*	Xiang-yong *et al.*[117]
	Human hepatocellular carcinoma cells (HepG2)	Pharmacological target of the chemotherapeutic drugs doxorubicin and daunorubicin	Yun *et al.*[118]

Abbreviations: GAAP, Golgi antiapoptotic-associated protein; GHITM, growth hormone-inducible transmembrane protein; GRINA, glutamate receptor ionotropic NMDA protein 1; LFG, life guard; RECS1, responsive to centrifugal force and shear stress 1; TMBIM, Transmembrane BAX Inhibitor-1 Motif-containing. The table summarizes most reports indicating differential expression or functional effects of distinct TMBIM family members in models of cancer or in human-derived samples.

Table 3. Global expression of the human TMBIM family in cancer

	TMBIM1/RECS1		*TMBIM2/LFG*		*TMBIM3/GRINA*		*TMBIM4/GAAP*		*TMBIM5/GHITM*		*TMBIM6/BI-1*	
	Down	*Up*	*Down*	*Up*	*Down*	*Up*	*Down*	*Up*	*Down*	*Up*	*Down*	*Up*
Bladder c			2			1			1			2
Brain and CNS cancer	1	2	15	1	4		1		10			6
Breast cancer	13					7	2	3	1		1	2
Cervical cancer	1		1						1			
Colorectal cancer	1		4	5		5		8	11		2	
Esophagealcancer	2				2		1		1			
Gastric cancer						8			1		1	
Head and neck cancer	1	2	2		1	2	1		1	3		3
Kidney cancer	3		3	1		1	2		5	2	1	2
Leukemia	7	2				2	1	8	2		6	
Liver cancer		1							1		2	
Lung cancer	1				1	1		2			1	2
Lymphoma				2	1	4	4	1	1	1		5
Melanoma	1					2						1
Myeloma							2			3		1
Other cancer		4	2	1		1		8	5	1	2	4
Ovarian cancer						1		1	1			1
Pancreatic		1								1		1
Prostate cancer					1	3	3		1			3
Sarcoma	9		3			1	1				5	
Significant unique analyses	39	12	30	10	10	39	31	18	43	11	21	33
Total unique analysis	348		414		415		383		394		395	

1 5 10 % 10 5 1

Abbreviations: TMBIM, Transmembrane BAX Inhibitor-1 Motif-containing. The Oncomine cancer microarray database (http: //www.oncomine.com/) was used to analyze the expression pattern of TMBIM family members in several cancer tissues. The table shows the number of studies where the fold change of expression was at least 1.5 for their mRNA in the cancer tissue relative to healthy subjects. A maximum *P*-value of 0.05 was used. The color code reflects the percentage range among the top 1, 5 or 10% group of the most altered genes (best gene ranking percentile). The number inside each cell indicates the amount of analyses or studies where changes in *tmbim* genes' expression were reported.

In other disease contexts, TMBIM6/BI-1 has been is implicated in experimental ischemia–reperfusion[49], diabetes and obesity[50] as mentioned before, in addition to liver regeneration[79] and splenomegaly.[83] Overexpression of TMBIM6/BI-1 reduces ER stress and provides protection from acute brain injury[48] and confers affective resiliency in an animal model of depression and anhedonia.[122] Interestingly, the human *TMBIM3/GRINA* gene maps are close to a locus that is genetically linked to epilepsy.[123] *Tmbim3/grina* mRNA is significantly upregulated in human samples of the prefrontal cortex of subjects with a major depressive disorder.[124] Besides, TMBIM2/LFG is involved in neuroprotection under brain ischemia.[125] Finally, some TMBIM family members can modulate pathogen handling, as reported after bacterial and viral infections as demonstrated for TMBIM3/GRINA,[35] TMBIM4/GAAP[52,126] and TMBIM6/BI-1.[127,128] In summary, increasing reports are highlighting the possible impact of the TMBIM family of proteins in diverse pathological conditions, where most of the literature indicates important activities in the development of cancer.

CONCLUDING REMARKS

In the past 15 years, a considerable progress has been made in understanding the biological function of the TMBIM family of proteins in the context of PCD and the regulation of apoptosis in different species. Mechanistic studies suggest the concept that the TMBIM family may operate as a 'stress integrator' pathway. This idea is based on the fact that this group of proteins impact several essential adaptive responses to environmental changes, where we highlight their contribution to calcium homeostasis, the UPR, autophagy, mitochondrial bioenergetics and lysosomal function. In addition, we also view the TMBIM family as 'stress sentinels', since depending on the context, they may even have opposite effects on apoptosis and cell survival in response to the same biological perturbation, possibly reflecting their pleiotropic impact in multiple interconnected signaling pathways. One of the major challenges in the field is to perform a systematic analysis and define how the TMBIM family is organized at the biochemical level and then construct the hierarchical map of the pathway as nicely shown for the BCL-2 family. In addition, mechanistic definitions are needed to further understand how the TMBIM family cross-talk with the canonical apoptosis pathway governed by BCL-2-related proteins in mammals. It is becoming evident that in terms of the evolution of PCD, the TMBIM family may represent a more fundamental and highly conserved pathway to control cell death in lower organisms that then probably diversified through the emerging of the BCL-2 family and other apoptosis pathways. From our view, one of the key points that is still open for investigation in this field is the definition of 'who is the killer' in the TMBIM family. Most theories of cell death evolution have depicted a clear balance between the expression of toxins and antidotes that operates through a fine-tuned rheostat to determine cell fate decision as described for the BCL-2 family and many primitive antibiotic and bacterial toxin systems.[129,130] To conclude, here we have discussed accumulating evidence that places the TMBIM family as a relevant regulator of cell death, highlighting its importance as a possible target for therapeutic interventions in many pathological conditions including cancer. We emphasize the need for systematic studies focused on assessing the function of this conserved group of proteins in physiology and disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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