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GABARAPL1 (GEC1)
Original or copycat?

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Abbreviations: AMPA receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; AP-1, activator protein-1; Atg, autophagy-related; ER, estrogen receptor; ERE, estrogen response element; FoxO, forkhead box proteins; $GABA_A$, gamma-aminobutyric acid type A; $GABA_AR$, gamma-aminobutyric acid type A receptor GABARAP; $GABA_A$ receptor-associated protein GABARAPL1, GABARAPL2, GABARAPL3, GABARAP-like 1, 2 and 3; GATE-16, Golgi-associated ATPase enhancer of 16 kDa; GEC1, glandular epithelial cell protein 1; GFP, green fluorescent protein; GnRH, gonadotropin releasing hormone; KOR, kappa opioid receptor; MAP1LC3, microtubule-associated protein 1 light chain 3; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NBR1, neighbor of BRCA1; NSF, N-ethylmaleimide sensitive factor; *Per1*, period 1 gene; SN, substantia nigra; SNpc, substantia nigra pars compacta; SP-1, specificity protein-1; SQSTM1, sequestosome-1

The *GABARAPL1* (*GABARAP-LIKE 1)* gene was first described as an early estrogen-regulated gene that shares a high sequence homology with *GABARAP* and is thus a part of the *GABARAP* family. GABARAPL1, like GABARAP, interacts with the $GABA_A$ receptor and tubulin and promotes tubulin polymerization. The GABARAP family members (GABARAP, GABARAPL1 and GABARAPL2) and their close homologs (LC3 and Atg8) are not only involved in the transport of proteins or vesicles but are also implicated in various mechanisms such as autophagy, cell death, cell proliferation and tumor progression. However, despite these similarities, GABARAPL1 displays a complex regulation that is different from that of other GABARAP family members. Moreover, it presents a regulated tissue expression and is the most highly expressed gene among the family in the central nervous system. In this review article, we will outline the specific functions of this protein and also hypothesize about the roles that GABARAPL1 might have in several important biological processes such as cancer or neurodegenerative diseases.

Introduction

The *GEC1* gene (also known as *GABARAPL1* or *ATG8L*) was first identified in 1993 as an early estrogen-induced gene in quiescent guinea-pig endometrial glandular epithelial cells (GEC).[1,2] In 1999, a new protein named GABARAP ($GABA_A$-receptor-associated protein) was described to bind both $GABA_AR$ (γ-aminobutyric acid receptor) and tubulin, and to be involved in intracellular $GABA_A$ receptor trafficking.[3] The discovery of this new protein, which shares 87% identity with GABARAPL1, led to the classification of GEC1 as a member of the GABARAP family.[4] We therefore propose to unify the somewhat confusing nomenclature and spelling of this protein and to refer to GEC1 (or GABARAP-L1) as GABARAPL1 ($GABA_A$-receptor-associated protein-like 1).

In this review, we will show that although GABARAPL1 shares a high sequence homology with other GABARAP family members, its expression pattern and regulation differ. Together, these findings suggest that GABARAPL1 might have essential and specific functions.

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The GABARAP Family

GABARAPL1 belongs to the GABARAP family, which is one of 2 subfamilies of the yeast Atg8 (autophagy-related 8) ortholog, the other being the MAP-LC3s (consisting of the light-chain of microtubule-associated proteins MAP1A, MAP1B and MAP1C, also known as LC3A, LC3B and LC3C). The GABARAP family comprises GABARAP, GABARAPL1 and GABARAPL2/GATE-16 (GABARAP-like 2/Golgi-associated ATPase enhancer of 16 kDa) (**Fig. 1**). GABARAPL1 shows 87% identity with GABARAP and 61% identity with GATE-16 [5,6] and also shares a distant homology with LC3A (30.8% identity), LC3B (29% identity) and LC3C (35.8% identity).[7-10] Members of the GABARAP and LC3 families are composed of 117 to 145 amino acids and all possess a conserved C-terminal glycine, essential for their role in autophagy (**Fig. 1**).[11,12] In addition to their sequence similarity, the crystal structures of GABARAP,[13-17] GATE-16,[6] LC3,[18] and

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GABARAPL1     MKFQYKEDHPFEYRKKEGEKIRKKYPDRVPVIVEK-APKARVPDLDKRKYLVPSDLTVGQF-60
GABARAP       MKFVYKEEHPFEKRRSEGEKIRKKYPDRVPVIVEK-APKARIGDLDKKKYLVPSDLTVGQF-60
GABARAPL2     MKWMFKEDHSLEHRCVESAKIRAKYPDRVPVIVEK-VSGSQIVDIDKRKYLVPSDITVAQF-60
LC3A    MKMRFFSSPCGKAAVDPADRCKEVQQIRDQHPSKIPVIIERYKGEKQLPVLDKTKFLVPDHVNMSEL-66
LC3B        MPSEKTFKQRRTFEQRVEDVRLIREQHPTKIPVIIERYKGEKQLPVLDKTKFLVPDHVNMSEL-62
LC3C  MPPPQKIPSVRPFKQRKSLAIRQEEVAGIRAKFPNKIPVVVERYPRETFLPPLDKTKFLVPQELTMTQF-70
Atg8          MKSTFKSEYPFEKRKAESERIADRFKNRIPVICEK-AEKSDIPEIDKRKYLVPADLTVGQF-60

GABARAPL1  YFLIRKRIHLRPEDALFFFVN-NTIPPTSATMGQLYEDNHEEDYFLYVAYSDESVYGK-117
GABARAP    YFLIRKRIHLRAEDALFFFVN-NVIPPTSATMGQLYQEHHEEDFFLYIAYSDESVYGL-117
GABARAPL2  MWIIRKRIQLPSEKAIFLFVD-KTVPQSSLTMGQLYEKEKDEDGFLYVAYSGENTFGF-117
LC3A       VKIIRRRLQLNPTQAFFLLVNQHSMVSVSTPIADIYEQEKDEDGFLYMVYASQETFGF-125
LC3B       IKIIRRRLQLNANQAFFLLVNGHSMVSVSTPISEVYESEKDEDGFLYMVYASQETFGMKLSV-125
LC3C       LSIIRSRMVLRATEAFYLLVNNKSLVSMSATMAEIYRDYKDEDGFVYMTYASQETFGCLESAAPRDGSSLEDRPCNPL-147
Atg8       VYVIRKRIMLPPEKAIFIFVN-DTLPPTAALMSAIYQEHKDKDGFLYVTYSGENTFGR-117
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Figure 1. Alignment of the GABARAP and LC3 family members. Amino acid sequences (obtained from NCBI or GeneBank databases) of GABARAPL1 (NP_113600), GABARAP (CAG47031), GABARAPL2 (NP_009216), LC3A (NP_852610), LC3B (NP_073729), LC3C (NP_001004343) and Atg8 (NP_009475) proteins were aligned using the NCBI Protein BLAST tool. The amino acids that differ from those present in GABARAPL1 are indicated in red. The terminal glycine (position 116, 120 or 124), which is essential for congugation to phospholipids, is displayed in blue.

GABARAPL1 (Structural Genomic Consortium, code: 2R2Q) are also highly similar. It is also worth mentioning that the GABARAPL1 protein sequence is highly conserved throughout evolution from plants to mammals (100% identity) suggesting that this protein plays an essential role in these organisms.[4,7]

Regulation and Expression of the *GABARAPL1* Gene

GABARAPL1 mRNA is expressed in a variety of tissues within the mouse, rat and human. The highest expression levels were initially observed in the brain, heart, liver, skeletal muscle, kidney, spleen, ovary, small intestine, placenta and peripheral blood leukocytes.[4,7,19] In contrast to its differential expression in adult human tissues, *GABARAPL1* is present at comparable mRNA levels in all fetal tissues.[20]

Regulation by estrogen. The human and guinea pig *GABARAPL1* cDNA consist of 1,959 and 1,921 nucleotides, respectively. Their coding sequences are composed of 351 nucleotides, which share 93% identity and translate into the exact same protein. The guinea-pig *Gabarapl1* gene has a full-length ERE sequence (estrogen responsive element, reviewed in ref. 21) that is located in the first exon and described as the primary response element necessary for gene activation by Estradiol-17β (E_2) via ERα (estrogen receptor α).[22]

Sequence analysis of the human *GABARAPL1* gene (on chromosome 12) by the Matinspector program from Genomatix[23] or the Cister software[24] reveals the presence of several possible regulatory sequences such as ERE, SP-1 (Specificity Protein-1) and AP-1 (Activator Protein-1), to which estrogen receptors could bind (**Fig. 2**).[25,26] However, the precise functions of these sequences have not yet been determined.

In contrast, analysis of the DNA sequences of *GABARAP* (on chromosome 17), *GABARAPL2* (on chromosome 16) and *LC3A, B* and *C* (on chromosomes 20, 16 and 1, respectively) did not indicate the presence of any ERE in the 1,200 bp region upstream of the initial start codon. Indeed, no other *GABARAP* or *LC3* family member has been shown to be upregulated by estrogen as is the case for *GABARAPL1*. This specific regulation of the *GABARAPL1* gene by estrogen might indicate that it exerts particular functions in tissues or pathologies in which estrogens play a major role such as breast cancer or Parkinson disease as discussed further below.

Expression in the central nervous system. Real-time RT-PCR experiments performed on mRNA from male rat cerebral extractions showed that *Gabarapl1* displays the highest expression levels followed by *Lc3*, *Gabarap* and *Gabarapl2*.[27] Transcript expression levels of each member, however, differ depending on the specific region of the brain. *Gabarapl1* mRNA is the most highly expressed in all regions of the brain examined and its expression is the highest in the pons and diencephalon (thalamus and hypothalamus).[27] These results were confirmed in the adult rat using in situ hybridization experiments, which showed that *Gabarapl1* is indeed widely expressed in the brain, most likely exclusively within neurons, ranging from the olfactory bulb to the brain stem and spinal cord.[28] More specifically, *Gabarapl1* appears to be expressed more predominantly in neurons that are involved in somatomotor and neuroendocrine functions and to a lesser extent in sensory and reticular structures.[28] *Gabarapl1* is also highly expressed in gonadotropin-releasing hormone (GnRH) neurons,[29,30] a neuronal population that is regulated by estrogen. Indeed, estrogen receptors are highly expressed in the hypothalamus[21,29] and play a major role in the regulation of the reproductive axis through their control on the release of luteinizing hormone and follicle-stimulating hormone at the anterior pituitary. Moreover, the primary neurotransmitter inputs to these neurons are $GABA_A$ and glutamate, which exert their effects in the neurons through $GABA_A$ receptors

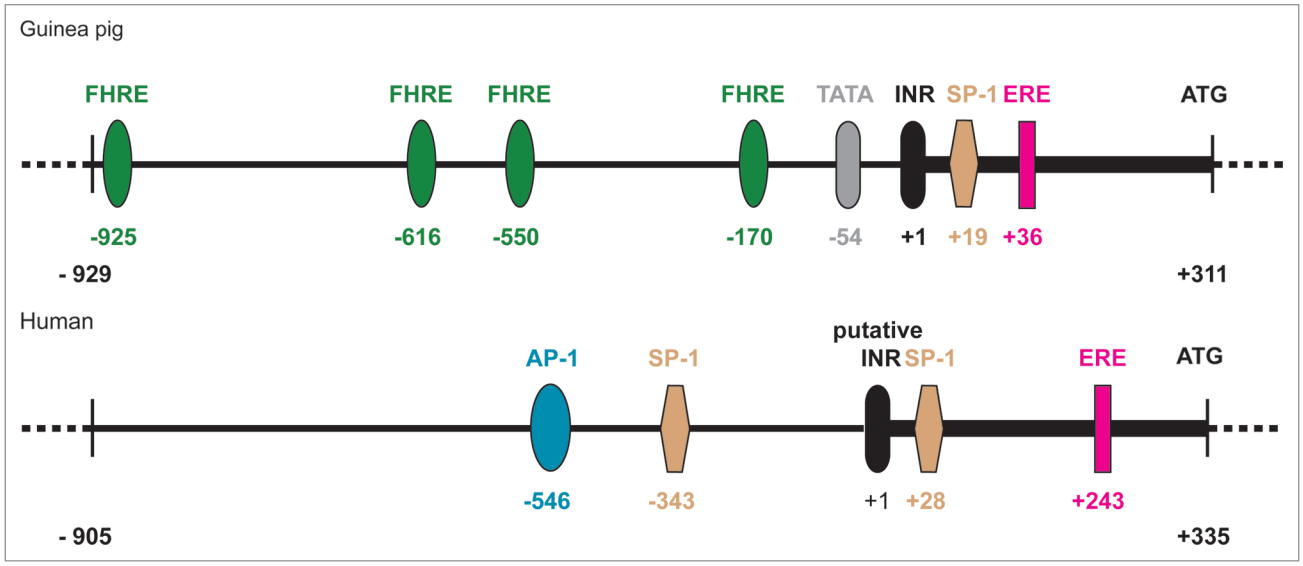

Figure 2. Comparison of the human and guinea pig *GABARAPL1* gene promoter regions. Predictive computer analysis was conducted on promoter sequences. In the guinea pig gene, the INR element is sufficient to direct transcription and the ERE located in the first exon permits induction of the gene by Estradiol-17β via ERα.[4] These sequences are conserved in the human gene but their roles have not yet been addressed. A similar analysis of the equivalent region for the *GABARAP* gene revealed no estrogen responsive cis-regulatory sequences. AP-1: binding site of the Activator Protein-1; SP-1: binding site of the transcription factor Specificity Protein-1; ERE: Estrogen Response Element; FHRE: ForkHead Responsive Element; INR: transcription initiator element; TATA: TATA box; Atg: translation initiation codon. Cister and Matinspector softwares were used to predict the presence of estrogen response cis-regulatory sequences.[23,24] Only sequences identified by both software programs are represented in the figure. Matinspector software was used to predict FHRE.

and glutamate receptors, respectively.[31,32] As an estrogen-regulated gene, *Gabarapl1* may play a role in the responsiveness of GnRH neurons to estrogen through its implication in $GABA_AR$ trafficking to the plasma membrane.

Regulation by circadian rhythms. In mammals, the central pacemaker in the hypothalamus, the suprachiasmatic nucleus, and the intracellular oscillation generators within several peripheral organs all work together to generate circadian oscillation.[33,34] Two separate studies have shown that *Gabarapl1* expression varies depending on the day/night rhythms.[30,35] The first study showed that *Gabarapl1* expression peaks halfway through the light phase of a 24-h cycle (12 h light/12 h dark) in mouse liver.[35] The second study demonstrated that *Gabarapl1* expression is temporally related to the oscillations of Circadian Locomotor Output Cycle Kaput (Clock) transcription factors and dependent on *Period 1* (*Per1*) expression in GnV-3 cells (conditionally immortalized gonadotropin-releasing hormone-expressing neurons from rat).[30] Among the *Gabarap* and *Lc3* family members, *Gabarapl1* is the only gene identified thus far to be regulated by circadian rhythms.

Several recent publications have hypothesized a relationship between autophagy and circadian rhythms, although the mechanism is not yet determined (reviewed in ref. 36). It has been shown that the autophagy-related genes *Vps4b* and *Bnip3*, like *Gabarapl1*, undergo rhythmic variations. *Gabarapl1* may, therefore, be regulated by *Per1* in order to participate or regulate autophagy during the diurnal cycle. Moreover, unlike the studies done on *Vps4b* and *Bnip3*,[37] *Gabarapl1's* rhythmic expression was demonstrated in vitro, suggesting that autophagic activity may vary intrinsically in the cell, independent of the circadian pattern of the whole animal. This finding may indicate that *Gabarapl1* is particularly important for the specific autophagic degradation of unwanted proteins in the cell and not just involved in nutrient recycling, a hypothesis that will require further examination.

Regulation by the FoxO transcription factor family. Several groups have demonstrated that *Gabarapl1* is regulated by FoxO transcription factors. For instance, Sengupta and colleagues found that, in primary cultures of both cardiomyocytes from neonatal rats and mouse heart tissue, stress conditions, such as glucose deprivation or oxidative stress, cause the translocation of FoxO1 and FoxO3 to the nucleus, where they activate autophagy-related genes (*Gabarapl1, Atg12* and *Lc3*) and a *FoxO1/FoxO3* conditional double-knockout mouse displays reduced expression of *Gabarapl1* and *Lc3-II* following ischemia/reperfusion injury.[38,39] Another study indicated that the effects of insulin suppression on autophagy are mediated by a FoxO1-dependent transcription of genes implicated in autophagy (*Gabarapl1, Atg12* and *Vps34).*[40] Furthermore, *Gabarapl1* as well as several other genes implicated in either the process or the regulation of autophagy (*Bnip3*, *Bnip3l*, *Vps34*, *Lc3, Atg12, Beclin 1* and *Atg4B*) are upregulated in a model of muscle atrophy in vivo.[41,42] A recent study linked FoxO, AMPK and p38α in colorectal cancer cells (CRC) during stress-response. In this study, the authors used an inhibitor of p38α (SB20219) to study the expression of FoxO3A target genes and demonstrated that, of the genes involved in autophagy, *GABARAPL1* shows the highest transcriptional induction after treatment and accumulates in autolysosomes.[43]

Three FoxO target consensus sequences are located upstream of the promoter region in the mouse *Gabarapl1* gene[44] and

4 potential FoxO binding sites are present in the guinea pig gene (**Fig. 2**). While no FoxO binding sites are found in the human gene within the homologous region, they are present further upstream (2–3 kb respective to the start codon) in the human gene. Interestingly, even if *GABARAPL1* is not the only member of the *GABARAP* family found to be regulated by FoxO transcription factors, it is regulated by several FoxO transcription factors and in different physiological processes such as muscle atrophy or colorectal cancer, therefore demonstrating its wide range of action.

Expression and localization of the GABARAPL1 protein. As the protein sequences among the GABARAP family members, and in particular between GABARAP and GABARAPL1, share a high identity, a detailed analysis of their differential expression has been limited. To avoid the use of nonspecific antibodies, research efforts have focused on the analysis of *GABARAPL1* mRNA expression since mRNA probes, complementary to the 3'-untranslated region of the gene, are able to specifically identify *GABARAPL1.*

Despite the lack of a GABARAPL1-specific antibody,[27,28,45] some tissue expression analysis has been performed. High levels of GABARAPL1 have been found in the brain, in neurons but not in glial cells, and in the lungs.[46] In addition, GABARAPL1 protein levels decrease in the failing human heart after mechanical unloading, possibly due to a decreased demand in energy from the heart during this process.[47] Nevertheless, it is important to point out that it cannot be excluded that, in these studies, several members of the GABARAP family have been detected and not only GABARAPL1.

Cell lines that stably express GABARAPL1 linked to a fluorescent tag have also been utilized for protein expression experiments. These studies have shown that the cellular distribution of GABARAPL1 is highly variable, including the cytoplasm, Golgi complex, endoplasmic reticulum or the plasma membrane, but with a common feature, a staining linked to intracytoplasmic vesicles that can partially colocalize with autophagosomes or lysosomes.[45,46,48,49] Although these experiments make it possible to determine the expression pattern of exogenous GABARAPL1, the cellular distribution of endogenous GABARAPL1 remains to be confirmed.

Taken together these data show that *GABARAPL1* displays a specific regulation (estrogens and circadian rhythms) not shared by the other members of the *GABARAP* and *LC3* families. This regulation might allow the investigators to use specific inducers targeting only one member of these families. Nevertheless, several points will need to be addressed in the future: Do the differences observed at the mRNA level reflect differences at the protein level? Is this specific regulation linked to any cellular process, such as autophagy, or pathologies, such as cancer and neurodegenerative disease?

The Role of GABARAPL1 in Neuronal Signal Transmission

$GABA_A$ receptors are ligand-gated chloride ion channels found at neuronal synapses and are responsible for the majority of the fast inhibitory transmission in the brain.[50,51] They are involved in many different physiological processes such as anxiety, circadian rhythm, memory, learning, controlling excitability of the brain, synaptic plasticity and synaptogenesis.[52] It has been recently demonstrated that Gabarapl1 interacts with tubulin and the γ2 subunit of the $GABA_A$ receptor and is able to promote tubulin polymerization and bundling to form microtubules.[45,49,53] A similar role for the Gabarap protein has also been previously demonstrated, suggesting that the 2 proteins are redundant with regard to this function.[3,54]

This idea has been confirmed with the creation of the *Gabarap* knockout mouse.[55] These mice lack a phenotype, they do not display any change in localization or number of $GABA_A$ receptors, nor are Gabarapl1 or Gabarapl2 protein levels upregulated. siRNA knockdown of *Gabarap* in primary hippocampal neurons also gave similar results.[56] A double-knockout mouse of both *Gabarap* and *Gabarapl1* will therefore be essential in order to evaluate their collective role and to determine the role of the third member of the family, *Gabarapl2*, has in the transport of $GABA_A$ receptors in vivo.

Interestingly, N-ethylmaleimide-sensitive factor (NSF) was also identified as a Gabarapl1 binding partner in rat brain extracts and Chinese hamster ovary cells.[45] Although the relevance of the Gabarapl1/NSF interaction has not yet been elucidated, it is likely that this complex will be involved in the trafficking of neuronal receptors as it has been described for the Gabarap/NSF and Gabarapl2/NSF complexes.[5,57]

Another protein partner of Gabarapl1 is the kappa opioid receptor (KOR), which is a member of the G-protein coupled receptor family.[45] Immunocytochemical studies show that Gabarapl1 colocalizes with the KOR in the endoplasmic reticulum and Golgi where it appears to increase the number of cell-surface receptors.[45] KOR/Gabarapl1 binding involves hydrophobic interactions[53] and the Gabarapl1 residues that are responsible for these interactions are highly conserved in Gabarap and Gabarapl2. However, compared with Gabarapl1, Gabarap and Gabarapl2 affect the transport of the KOR to a lesser extent. Moreover, Gabarapl1 displays a higher affinity for KOR and, unlike Gabarap, can associate with and enhance the expression of this receptor without being processed at its G116 residue and associated to membranes.[58] These data suggest a different role of these proteins in the transport of the kappa opioid receptor. The residues within the opioid receptor that are necessary for Gabarapl1 interaction are located within a FPXXM motif at its C terminus. This motif is also present in the GluR1 (glutamate receptor type 1) subunit of the AMPA receptor and in the prostaglandin receptor EP3.f. The expression of these receptors is increased as a result of Gabarapl1 overexpression, suggesting that Gabarapl1 has a role that is more complex than merely in receptor transport.[53] Indeed, the authors of the previous study suggest that Gabarapl1 might act as a chaperone for the KOR and possibly other proteins.

The Role of GABARAPL1 in Autophagy

Autophagy is a highly catabolic process leading to the degradation of cytoplasmic components and sometimes also the degradation

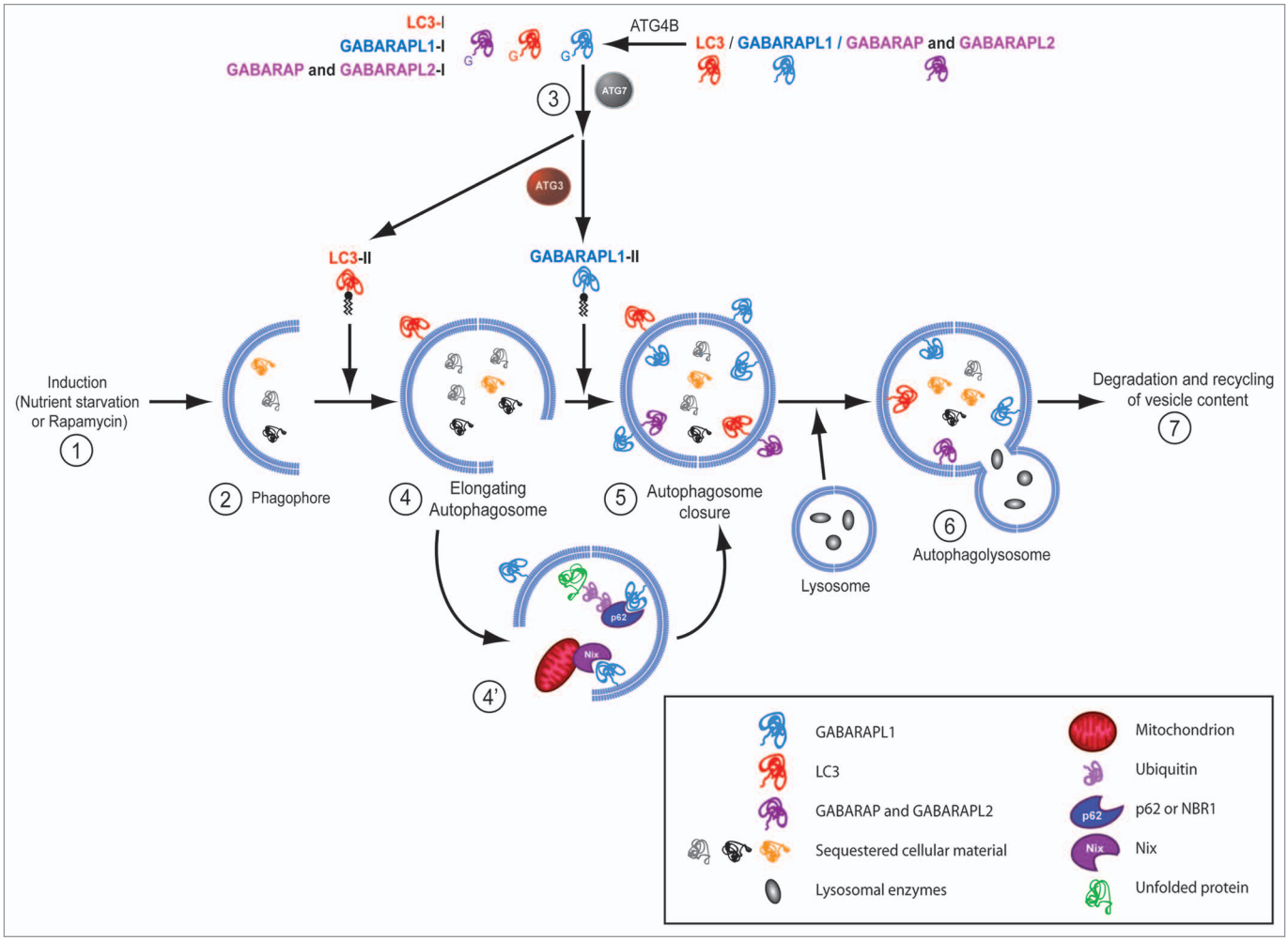

Figure 3. Role of GABARAPL1 in autophagy. GABARAP, GABARAPL2 and LC3 are cleaved in the cell by Atg4 enzymes to produce their mature forms; GABARAPL1-I, GABARAP-I, GABARAPL21-I and LC3-I. Under autophagy-inducing conditions (e.g., stimulation with rapamycin or nutrient starvation) (1), nucleation of phagophores is initiated (2) and followed by elongation of the pre-autophagosomal membrane, which leads to the creation of autophagosomes. During this process, GABARAPL1-I, GABARAP-I, GABARAPL2-I and LC3-I are conjugated to phospholipids by Atg7 and Atg3 (3) to produce the phospholipid-linked forms GABARAPL1-II, GABARAP-II, GABARAPL2-II and LC3-II. LC3-II is essential for elongation of autophagosomes (4), whereas GABARAP-II, GABARAPL1-II and GABARAPL2-II are required for the closure of autophagosomes (5). Autophagosomes then undergo maturation into autophagolysosomes (6), resulting in the degradation of their content and the recycling of the breakdown products (7). GABARAPL1 is also involved in selective autophagy through its interaction with cargo adaptor proteins, such as Nix, p62 or NBR1, to activate the clearance of specific unwanted proteins or organelles (4').

of the cell as a whole. It has been implicated in many biological processes from development to disease states (reviewed in ref. 59 and 60). Numerous recent studies have focused on elucidating the molecular mechanism of this process and it is now known that the elongation of the phagophore is regulated by 2 conjugation systems.[61] The first system involves the conjugation of Atg12 to Atg5 by the Atg7 and Atg10 enzymes. The second involves the conjugation of the LC3 protein onto phosphatidylethanolamine by the Atg7 and Atg3 enzymes (reviewed in ref. 62). Before conjugation, the precursor form of LC3 is cleaved by the cysteine protease Atg4B, a member of the Atg4 family of endopeptidases, to expose a glycine residue at its C terminus (**Fig. 1**). The mature LC3 is then attached to its target phospolipid, typically a phosphatidylethanolamine on the forming autophagosomal structure in vitro and in vivo (**Fig. 3**), or even a phosphatidylserine in vitro. This difference is thought to be governed by yet unidentified 'selective factors' that are only present in vivo.[63]

Recently, Gabarapl1 has been described as a new marker of autophagosomes[48] and can be cleaved by Atg4B in vitro, a maturation step described to be inhibited in *Atg4B*$^{-/-}$ mice.[48,64,65] The loss of the maturation of the Gabarap family members by Atg4B in this in vivo model leads to balance disorders owing to a loss of autophagic function within the inner ear. Another recent study shows that the proteins of the Atg8 family are involved in the formation of the autophagosomes but display different and specific function during this process: the LC3 family is necessary for the elongation of the phagophore, whereas the GABARAP family is implicated in later stages of the formation of the autophagosome.[66]

One main question remains: What is the specificity of function of the GABARAP and LC3 family members in autophagy?

Deletion of *Gabarap* does not result in an increased expression of *Gabarapl1* or *Gabarapl2*.[55] Similarily, *Lc3*β knockout mice do not display a compensation of *Lc3*α or *Gabarap* expression.[67] As such, one could assume that the members of each subfamily are simply redundant. Nevertheless, given their specific expression patterns, their role in autophagy might be tissue or cell specific or linked to specific types of autophagy such as selective autophagy as described below.

The Role of GABARAPL1 in Selective Autophagy

The autophagic pathway has evolved to include more specific processes in which certain autophagic targets are selected via autophagic receptors such as sequestosome1 (SQSTM1, also known as p62) or neighbor of Brca1 (NBR1).[68-70] These proteins act as cargo adapters and connect the ubiquitinated target proteins, protein aggregates or damaged organelles to the autophagosome-linked GABARAP and LC3 family members. They do so by binding both the cargo (i.e., the material to be sequestered) via their C-terminal ubiquitin-associated domain (UBA)[71] and the LC3 or GABARAP family members via their LC3-interacting domain (LIR).[69] Indeed, GABARAPL1 interacts with both p62 and NBR1,[69,72,73] and the interaction between GABARAPL1 and p62 facilitates the autophagy of ubiquitinated protein aggregates.[69] GABARAPL1 is also thought to be involved in mitophagy through its interaction with the mitochondria-associated protein NIX1 and its recruitment to damaged mitochondria in vitro.[74] It is therefore likely that GABARAPL1 constitutes a protein target for cargo adapters and thus is necessary for the degradation of unwanted organelles or protein aggregates, a function that could prove to be useful for the therapy of various diseases, such as cancer or neurodegeneration. Therefore, it will be of great interest in the future to determine whether the GABARAP and LC3 family members play redundant roles in this process or are involved in the degradation of different targets that are associated with specific pathologies, such as α-synuclein in Parkinson disease[75] or p62 in cancer.[76]

The Role of GABARAPL1 in Cancer

The first evidence for a potential role of GABARAPL1 and GABARAP in cancer was a study that described reduced *GABARAPL1* expression in different cancerous cell lines compared with normal tissues.[19] More recently, we investigated *GABARAPL1* expression in a large cohort of breast adenocarcinoma (265 samples) [77] and demonstrated that those patients who presented with high *GABARAPL1* expression levels had a lower risk of metastasis, specifically for lymph node-positive patients. Moreover, decreased *GABARAPL1* expression correlates with clinic-pathological features such as the histological grade of a given tumor. Reduced levels of *GABARAPL1* mRNA are observed in tumors of high histological grade, with lymph node-positive and estrogen- and/or progesterone receptor-negative status. These results suggest a role for *GABARAPL1* as a prognostic marker in breast cancer, specifically in lymph node-positive patients. Like *GABARAPL1*, *GABARAP* expression is also diminished in breast cancer cell lines, both at the mRNA and protein levels.[78] *GABARAP* also has a role in other cancers as *GABARAP* transcript expression correlates with a better survival rate for patients affected by neuroblastoma,[79] and GABARAP protein expression is significantly upregulated in colorectal cancer.[80]

Autophagy has been described to play a paradoxical role in tumor apparition and progression. Early in the process of tumorigenesis, autophagy prevents tumor progression by degrading damaged organelles such as mitochondria, which would otherwise be stressors in the cell.[81,82] Under metabolic stress, and at later stages of tumorigenesis, however, some tumors exploit their autophagic capabilities in order to provide themselves with the necessary nutrients to survive (reviewed in ref. 83–85). Later on, autophagy is also responsible for the development of drug resistance in many cancers.[86] Since GABARAPL1 is regulated by estrogens, and its gene expression is a good prognostic indicator for breast cancer patients,[77] it has a tremendous potential as a therapeutic target against cancer. In fact, anti-estrogen treatments (tamoxifen) are currently being combined with an inhibitor of autophagy (chloroquine) in clinical trials to treat breast cancer.[86]

The Role of GABARAPL1 in Neurodegeneration

In the last decade, there has been a growing body of evidence that supports a role for autophagy in the protection against unwanted protein aggregates in the brain. Anomalies in the autophagic process have been discovered in many neurodegenerative diseases including, but certainly not limited to, Alzheimer, Huntington and Parkinson diseases (reviewed in ref. 87). As discussed above, GABARAPL1 interacts with the autophagy cargo adaptors p62 and NBR1, which bind to ubiquitinated protein aggregates to identify them for degradation.[68-70]

However, GABARAPL1 not only binds to these autophagy cargo adaptors, but also confers an affinity for those mutated proteins that form aggregates in neurodegenerative diseases, such as α-synuclein oligomers in Parkinson disease.[88] Moreover, *Gabarapl1* mRNA is highly expressed in the *substantia nigra pars compacta* (*SNpc*), the region of the *SN* that consists of dopaminergic neurons, implicated in the progression of Parkinson disease, whereas its expression is lacking in the *pars reticula*.[28] Two recent microarray analysis showed that *GABARAPL1* expression, but not *GABARAP* or *LC3*, is significantly reduced in the prefrontal cortex of macaque monkeys in an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) experimental model of Parkinson disease,[89] and highly downregulated in laser microdissected dopaminergic neurons (DA) of the *SNpc* of Parkinson disease patients.[90] The latter observation might indicate a need for a decreased autophagic activity, by means of the degradation of *GABARAPL1* and/or other autophagy proteins, in order for these neurodegenerative diseases to progress, further suggesting the importance of GABARAPL1 in the prevention of neurodegenerative diseases.

It is worth noting that sex steroids, in particular estrogens, have a protective effect in various models of brain injury and, in particular, in a MPTP-murine model of Parkinson disease.[91] Since estrogens regulate *GABARAPL1* transcription, the GABARAPL1 protein may be one of the links between estrogen

Table 1. List of confirmed GABARAPL1-interacting partners

GABARAPL1-interacting partners	Potential significiance of the interaction	References
GABARAPL1-specific partners		
ARH (Autosomal recessive hypercholesterolemia)	Intracellular transport, outgrowth and elongation of axons	Mameza *et al.*, 2007
α-synuclein	Protein aggregates degradation in neurodegenerative diseases	Schnack *et al.*, 2008
Partners common with one or more GABARAP-family members		
$GABA_AR$ (Gamma-aminobutyric acid type A receptor)	Transport of the $GABA_A$ receptor to plasma membranes	Mansuy *et al.*, 2004
Tubulin	Tubulin assembly, microtubules bundling, transport of $GABA_A$ receptor *via* microtubules	Mansuy *et al.*, 2004
KOR (Kappa opioid receptor)	Transport of KOR from ER/Golgi to plasma membranes	Chen *et al.*, 2006
NSF (N-ethylmaleimide sensitive factor)	Intracellular transport, membrane fusion events	Chen *et al.*, 2006
PX-RICS	Transport of the N-cadherin/β-catenin complex from ER to Golgi	Nakamura *et al.*, 2008
Stbd1 (Starch binding domain containing protein 1/genethonin 1)	Vesicular transfer of glycogen to the lysosome	Jiang *et al.*, 2010
p62/SQSTM1 (sequestosome 1)	Degradation of ubiquitinated protein aggregrates by autophagy	Rual *et al.*, 2005; Pankiv *et al.*, 2007
Nbr1 (Neighbor of Brca1)	Degradation of ubiquitinated protein aggregrates by autophagy	Larsen *et al.*, 2010
Nix	Clearance of damaged mitochondria by mitophagy	Novak *et al.*, 2010

and neuroprotection. If this is indeed the case, it might constitute an attractive therapeutic target in the future.

Other Functions of GABARAPL1

GABARAPL1 mRNA expression is upregulated in peripheral blood mononuclear cells from chronic fatigue syndrome patients compared with normal blood donors.[92] GABARAPL1 is the only member of the GABARAP family that has been shown to interact with the autosomal recessive hypercholesterolemia protein[93] in the brain. Lastly, GABARAPL1 interacts with the starch binding domain-containing protein 1, a protein thought to play a role in glycogen metabolism (**Table 1**).[94,95]

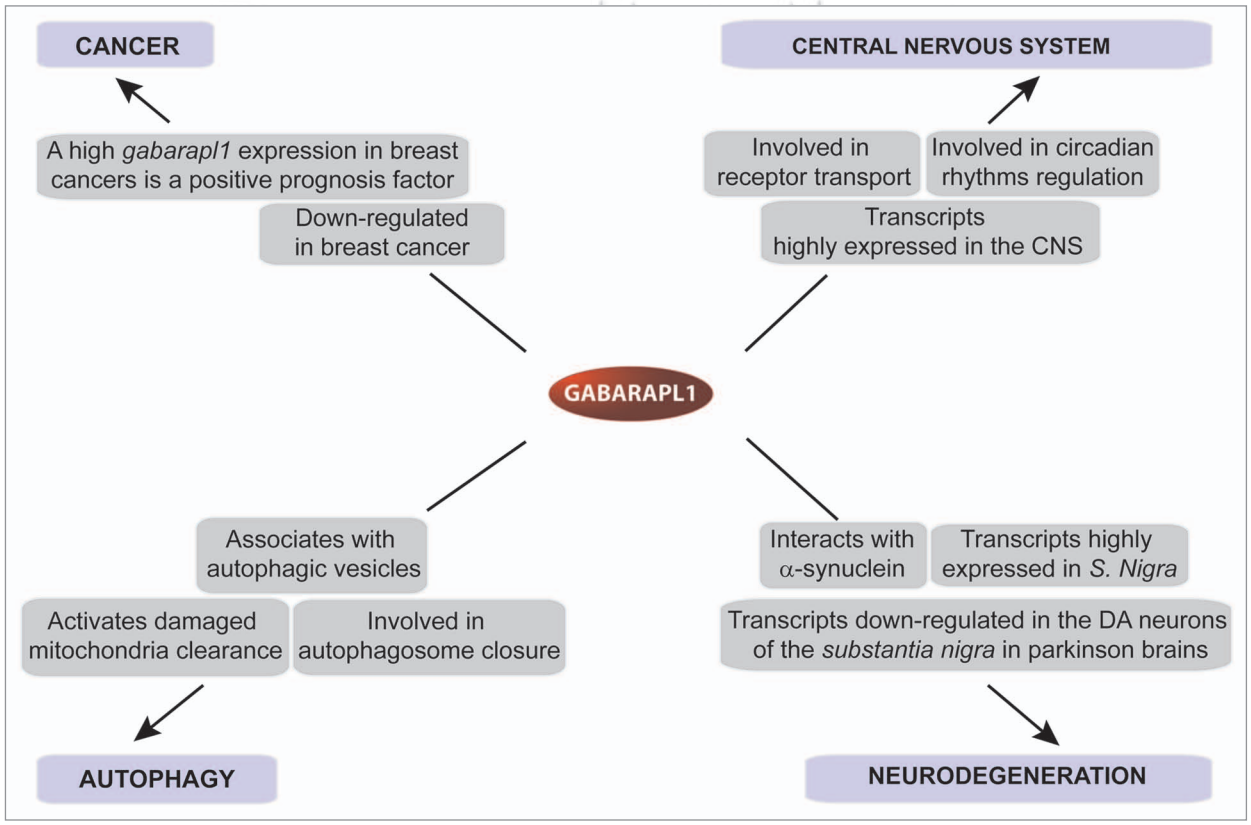

Figure 4. Confirmed and putative roles of GABARAPL1. Schematic illustration of described or putative roles for GABARAPL1 and regulation of *Gabarapl1* gene and GABARAPL1 protein in physiological processes or pathologies.

Conclusion

GABARAPL1, like GABARAP, is implicated in several different cellular processes and presents a specific regulation including the estrogen hormones, the FOXO family and the circadian rhythms. Moreover, it is differentially regulated in various pathologies, such as breast cancer, colorectal cancer, neurodegenerative models and chronic fatigue syndrome (**Fig. 4**).

Together, these data suggest an essential and specific role for this protein that is distinct from that of its closest homolog, GABARAP. At the molecular and cellular levels, the main question to address will be the specificity of their protein partners: are they identical for all the members of the family or are they specific to different pathologies? The necessary studies to answer these questions, however, will require a specific antibody to further characterize the interaction of GABARAPL1 with its protein partners. The therapeutic potential of GABARAPL1 looks promising since this protein displays a specific regulation that is not shared by the other members of the GABARAP family. In particular, GABARAPL1 might prove a useful therapeutic target for estrogen responsive cancers and in neurodegenerative diseases. The example of GABARAPL1 demonstrates the importance to differentiate between the different members of the GABARAP family when studying their role and no longer consider these proteins as being functionally redundant.

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