Review

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G protein-coupled receptors and the regulation of autophagy

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Autophagy is an important catabolic cellular process that eliminates damaged and unnecessary cytoplasmic proteins and organelles. Basal autophagy occurs during normal physiological conditions, but the activity of this process can be significantly altered in human diseases. Thus, defining the regulatory inputs and signals that control autophagy is essential. Nutrients are key modulators of autophagy. Although autophagy is generally accepted to be regulated in a cell-autonomous fashion, recent studies suggest that nutrients can modulate autophagy in a systemic manner by inducing the secretion of hormones and neurotransmitters that regulate G protein-coupled receptors (GPCRs). Emerging studies show that GPCRs also regulate autophagy by directly detecting extracellular nutrients. We review the role of GPCRs in autophagy regulation, highlighting their potential as therapeutic drug targets.

Nutrients regulate autophagy

Autophagy is a catabolic process in which proteins, organelles, and other cytoplasmic contents are engulfed by autophagosomal double-membrane vesicles and delivered to lysosomes where they are degraded (Box 1). A basal level of autophagy in cells helps to remove damaged proteins and organelles. However, autophagy can be dramatically increased under different physiological conditions, such as hypoxia or nutrient deprivation, an adaptive response crucial for cell and organism survival [1]. For example, during starvation autophagy is induced through the inhibition of the mechanistic target of rapamycin (mTOR), an evolutionarily conserved protein kinase and a central regulator of cell growth [2]. Dysregulation of autophagy is associated with numerous diseases including cancer, metabolic syndromes, and cardiovascular diseases [1] (Boxes 2 and 3). For example, mouse models of obesity and insulin resistance show decreased autophagy in the liver, and restoration of autophagy in these mice significantly improves insulin action in the liver, suggesting that the reduction in autophagy is causally related to obesity-induced insulin resistance [3]. The role of autophagy in the development of human disease and how this process may be pharmacologically manipulated will be reviewed throughout this article.

Numerous mechanisms are used by cells in the surveillance of their environment to balance properly their metabolic decisions. Extracellular nutrients present in the serum are transported into cells by multiple specialized transporter systems. When they detect sufficient nutrient levels, intracellular nutrient sensors such as AMP-activated kinase (AMPK) and mTOR initiate anabolic processes including protein and lipid synthesis, and reduce catabolic processes such as autophagy [4]. Conversely, these same sensors initiate catabolic processes when there is a depletion of nutrients.

GPCRs are direct nutrient sensors that regulate autophagy

Cell surface nutrient receptors, including GPCRs in single cell organisms such as fungi, are well characterized [5]. Traditionally, mammalian non-sensory GPCRs were thought to be receptors for endocrine, paracrine, and autocrine signaling hormones and neurotransmitters produced and secreted in response to physiological cues. However, recent studies have expanded this view by indicating that GPCRs function in the surveillance of the fed state by directly detecting nutrients. Examples of these receptors include the amino acid responsive receptors GPRC6A, taste receptors type 1 members 1 and 3 (T1R1/T1R3), and the calcium sensing receptor (CaSR); long chain fatty acid receptors GPR120 and GPR40; and short chain fatty acid receptors GPR41 and GPR43 [6,7]. There are also many orphan GPCRs that may serve as nutrient sensors. The nutrient receptors are known to be linked to different G protein α subunits including Gs, Gq/G11, Gi, Go, and gustducin. Agonist stimulation causes activation and dissociation of the Gα subunits from the Gβγ subunits, both of which initiate downstream signaling. Gq/G11 activation leads to an increase in phospholipase C (PLC) activity, causing an elevation of intracellular inositol triphosphate (IP_3) and diacylglycerol (DAG) concentrations. IP_3 binds to the IP_3 receptor (IP_3R) on the endoplasmic reticulum (ER), which causes the ER to release stored Ca^{2+} (Figure 1). Gi and gustducin activation lead to decreases in intracellular cAMP concentrations, whereas Gs activation increases intracellular cAMP levels. Changes in cAMP, IP_3, and Ca^{2+} concentrations regulate a multitude of signaling molecules such as mTOR, AMPK, and the mitogen-activated kinases (MAPKs) ERK1/2 that modulate autophagy by a variety of mechanisms (Figure 1).

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Box 1. Regulation of Autophagy

Autophagy can be initiated when cells undergo metabolic stresses including hypoxia and nutrient limitation. These stresses inhibit mTORC1, resulting in an increase in the activity of UNC51-like kinase 1/2 (ULK1/2). ULK1/2 form a complex with the regulatory proteins Atg13 and FIP200 [74], which is necessary to initiate autophagy. The type III phosphoinositide-3 kinase (PI3K) VPS34 facilitates the nucleation of the pre-autophagosome (phagophore) by catalyzing the formation of phosphatidylinositol-3-phosphate (PI3P) [75]. An interaction between VPS34 and Beclin1, a Bcl-2-homology (BH)-3 domain only protein, is required to activate VPS34 [76]. Negative regulation of this complex occurs when the anti-apoptotic protein Bcl2 sequesters Beclin1 by binding to its BH3 domain. The phosphorylation of Bcl2 by JNK or the phosphorylation of Beclin1 by death-associated protein kinase (DAPK) activates autophagy by preventing the Beclin1/Bcl2 interaction, thus allowing VPS34/Beclin1 association [77,78]. Recent work by Guan and colleagues showed that ULK1 increases VPS34 activity by phosphorylating Beclin1 [79]. Another major activator of autophagy is AMPK. A high AMP/ATP ratio activates AMPK, increasing energy levels by inhibiting anabolic and inducing catabolic processes. AMPK enhances autophagy by directly activating ULK1 [80,81]. AMPK also phosphorylates Beclin1 to induce autophagy [82].

Expansion of the phagophore into the mature autophagosome requires two ubiquitin-like conjugation systems. The ubiquitin-like protein Atg12 is covalently attached to Atg5 by Atg7 and Atg10. The Atg12/Atg5 conjugate then binds to Atg16, which is essential for autophagy. The other ubiquitin-like protein LC3 is cleaved by Atg4 and subsequently conjugated to phosphatidylethanolamine (PE) by Atg7 and Atg3 to form LC3-II. LC3-II becomes incorporated in both the inner and outer membranes of autophagosomes. LC3-II, the formation of which is used as a measure of autophagy, is thought to play a role in autophagic cargo recruitment and autophagosome biogenesis. Sequestosome-1 (SQSTM1 or p62) is an adaptor protein that binds polyubiquitinated proteins and LC3-II, recruiting them to the autophagosome for disposal. The final step in autophagy is the fusion of the autophagosome with the lysosome and the degradation of its cargo [1,83]

Amino acid sensing GPCRs

Most of the known amino acid-responsive GPCRs belong to the GPCR class C, which includes the sweet taste receptor (T1R2/T1R3), the umami (savory) taste receptor (T1R1/T1R3), metabotropic glutamate receptors, the $GABA_B$ receptor, the Ca^{2+}-sensing receptor (CaSR), GPRC6A, and a few orphan receptors. Most members of class C are hetero- or homodimers and contain a large extracellular segment called the Venus flytrap module that is involved in agonist binding [7,8]. T1R1 and T1R3 dimerize to form a receptor that binds L-amino acids, specifically L-glutamate, thus enabling the body to sense the umami (savory) flavor [9]. The amino acid-responsive GPCRs have been reported to be coupled to gustducin, Gi, Gs, or Gq depending on the tissue in which they are expressed [7].

T1R1/T1R3 is expressed in most tissues [10,11] where it is responsive to most of the 20 amino acids with varying affinities, thus acting as a direct sensor of the fed state and amino acid availability [7]. It was recently shown that amino acids signal through T1R1/T1R3 to activate the mTOR protein complex 1 (mTORC1), which consists of mTOR, mLST8 (mammalian lethal with SEC13 protein 8), and Raptor (regulatory associated protein of mTOR), and functions as a master regulator of metabolism controlling protein synthesis and other processes. Amino acid deprivation inhibits mTORC1 activity in most cells, and silencing T1R3 causes a significantly greater decrease in mTORC1 activity, upon starvation, in several cell lines. These data suggest that amino acid signaling through T1R1/T1R3 is necessary for optimal mTORC1 activation

Box 2. Cancer as a metabolic syndrome and the interplay with GPCRs

The concept that cancer is a metabolic disorder has been long-standing, with early studies by Warburg suggesting a unique metabolic landscape for tumor cells compared to untransformed cells [84]. Warburg observed that tumor cells preferentially undergo glycolysis instead of oxidative phosphorylation, and his observations on the distinct metabolic phenotype of tumors were further supported by studies revealing their glutamine-dependency [85]. The metabolic changes observed in tumors are likely to be driven by one or more mechanisms operating within the tumor including: (i) microenvironmental conditions which may lead to stabilization and activation of the hypoxia-inducible transcription factor (HIF) pathway due to an anaerobic, improperly-vascularized tumor microenvironment [86], (ii) activation of oncogenes such as Ras, Myc, Akt, and others which can all adversely regulate the metabolic profile of transformed cells, (iii) loss of tumor suppressors such as p53, whose targets include SCO2 which is required for mitochondrial respiratory chain, and TP53-induced glycolysis and apoptosis regulator (TIGAR) which regulates glycolysis and gluconeogenesis [84,87], or even mutagenesis of tumor suppressors encoding mitochondrial proteins such as succinate dehydrogenase (SDH) and fumarate dehydrogenase (FH), subsequently result in mitochondrial dysfunction and disrupted metabolism [88]. Furthermore, studies have shown that, in a model of stepwise malignant transformation, increasing tumorigenicity correlated with increased sensitivity to glycolytic inhibition [89], and other studies showed inhibition of tumor growth as a result of activation of oxidative phosphorylation and blocking the Warburg effect [90,91].

Rapidly-proliferating tumors require nutrients and substrates for the biosynthesis of nucleotides, proteins, lipids, and other macromolecules. These are provided for, at least in part, by the altered metabolic state of tumor cells. For example, there is an increased dependence of particular tumors on glutaminolysis, which can be used as a source of amino acids but also to produce NADPH for lipid biosynthesis and oxaloacetate for Krebs cycle intermediates [92]. Another mechanism for tumors to obtain nutrients for biosynthetic programs is via autophagy. Autophagy has a dual role in tumors whereby it can play tumor-suppressive or tumor-promoting roles in a context-dependent manner [93]. The role of autophagy in providing biosynthetic intermediates is essential for tumor growth, with lipophagy, the degradation of lipids by autophagy [17], degradation of proteins by autophagy to generate amino acids, as well as autophagy-recycled sugars, all contributing essential products to sustain tumor cell growth, in particular under conditions of stress and starvation [93]. Autophagy may therefore play a major role in sustaining the unique metabolic status of tumors, providing valuable byproducts as well as relieving cellular stress.

The varying role of autophagy in different tumors makes it difficult to assess the benefit of targeting autophagy, but also provides impetus for studies to define more clearly its regulators as potential targets for anti-autophagic cancer therapeutics. With novel studies showing uncharacterized links between autophagy, GPCRs, and nutrient sensing, as well as a large body of literature detailing the significance of GPCRs and downstream signaling from GPCRs in driving tumorigenesis [94], it will be interesting to study the role of autophagy in GPCR-driven tumors, as well as the roles of GPCRs in tumor-promoting or tumor-suppressing functions of autophagy in cancer.