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Role of Autophagy in Cancer Metabolism

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Abstract

Cancer cells undergo a wide range of metabolic reprogramming to take advantage for supporting rapid growth and survival. Autophagy plays a critical role in directly regulating cellular metabolism as a main catabolic process mediated by lysosomal degradation in response to the metabolic stress. During cancer development, autophagy plays opposite functions in suppressing or promoting tumors dependent of distinct stage. Autophagy maintains cellular homeostasis by degrading unnecessary cellular molecules and oncogenic products, thereby suppressing tumorigenesis. By contrast, autophagy enables to promote cancer growth in advanced tumor by supplying nutrients and relieving metabolic stress.

In this book chapter, recent progress indicates how autophagy is integrated with cellular metabolic alteration during cancer development, particularly focusing on distinct metabolic substrates including glucose or glutamine. Multiple mechanisms would be suggested to explain the functions of autophagy at distinct stage of tumor progression. Cancer metabolic alterations associated with autophagy can be determined by certain oncogenic activators and/or tumor suppressors. Understanding the molecular mechanism of autophagy and metabolic alteration during cancer development may suggest potential targets for therapeutic intervention.

Keywords: autophagy, glucose metabolism, glutamine metabolism, macropinocytosis

1. Introduction

Autophagy is a lysosome-mediated self-degradation process in which cytosolic components and organelles are sequestered into membrane-bound vesicles called autophagosomes and are delivered to lysosomes. Autophagic cargo contents are ultimately degraded and recycled back to the cytoplasm for supporting cell metabolic processes. Most Atg genes have been identified

and characterized by genetic screening in yeast. The mutants harboring autophagy genes showed severe growth defect upon nitrogen starvation, although some genes were identified by the other-distinct nutrient starvation conditions. Autophagy process is evolutionally conserved from yeast to mammals, and more than 30 Atg proteins have also been found in mammals. Accordingly, autophagy process is utilized to maintain constant nutrient balance, which is important for certain stages of cellular development and physiology. Autophagy process can be categorized at distinct steps from phagophore induction, vesicle nucleation, expansion, fusion to the lysosome, and degradation of autophagic cargoes [1–3].

A key nutrient-signaling molecule, mammalian target of rapamycin (mTOR), has been identified as a major regulator of autophagy activity. mTOR activated by nutrients and growth factors usually suppresses autophagy through the direct phosphorylation of ULK1/2 and Atg13 [4, 5]. A well-known energy-sensing factor, adenosine monophosphate-activated protein kinase (AMPK), also positively regulates autophagy depending on the ratio of intracellular AMP/ATP levels. Activated AMPK by low-energy levels phosphorylates a series of autophagy proteins including ULK1 and Beclin1/VPS34complex distinctly, thereby enhancing autophagy activity. AMPK-mediated autophagy regulation occurs either through mTOR inactivation or through direct phosphorylation of ULK1 [6–8].

Cancer cells generally enhanced metabolic demands for supporting rapid proliferation, thereby ultimately altering cell metabolism toward anabolic-addicted condition. Metabolic stress often occurs in fast-growing tumor cells and the tumor microenvironment, which is caused by the lack of sufficient nutrient and oxygen. To overcome this metabolic hurdle, tumor cells engage in metabolic reprogramming and autophagy to increase intracellular nutrient supplies to support cell growth and survival [9, 10]. A typical catabolic process, autophagy, might support anabolic pathways such as macromolecule synthesis by supplying intracellular metabolites to the cell through degradation of cellular constitutes in lysosome-mediated manner. Substantial evidence for the integration of autophagy and metabolic alterations is reported, even though it is still not completely understood how these two processes are mechanistically balanced to promote cancer development.

2. Tumor-suppressing role of autophagy

Autophagy is considered to have both tumor-suppressing and tumor-promoting roles during cancer progression. This functional duality can be determined by the oncogenic feature of the primary tumor including oncogene types or the levels of tumor suppressors. In addition, the cellular context of the tumor such as tumor type and tumor stage might also be critical determinants for explaining the complex interactions of autophagy and tumor development.

The tumor-suppressing role of autophagy was characterized that monoallelic deletion of *BECN1* was observed in various human cancers in breast, ovarian, and prostate [11]. Mice with monoallelic loss of BECN1 spontaneously develop lymphoma, hepatocellular carcinoma, and lung adenocarcinomas, suggesting that Beclin1 is a haploin-sufficient tumor-suppressor protein [12]. Moreover, Beclin1-interacting autophagic proteins such as UVRAG and Bif-1

exhibited tumor-suppressor roles in different mouse models [13, 14]. Similarly, mice harboring monoallelic deletion for the BECN1 interactor autophagy/beclin-1 regulator 1 (AMBRA1) also exhibit increased spontaneous tumorigenesis, through that preventing AMBRA1-mediated deregulation of c-Myc [15]. Moreover, mice bearing a systemic mosaic deletion of Atg5 or a liver-specific knockout of Atg7 spontaneously develop benign hepatic neoplasms more frequently than their wild-type counterparts [16].

To address the role of autophagy as tumor suppressor, a series of reports have suggested that multiple oncogenes can be degraded by autophagy processes. An autophagy cargo receptor p62/SQSM1, an autophagic cargo receptor which is expected to be degraded by autophagy, plays an oncogenic role in promoting cancer progression. Overexpression of p62/SQSM1 in KRas-induced tumor cells exhibits to increase pro-inflammatory responses through the Nrf2 and nuclear factor-kappa B (NF–κB) activation, thereby leading to tumor progression. These results indicate that p62 accumulation due to autophagy defect is strongly correlated with tumor development [17–19].

The mechanism of how p62/SQSTM1 regulates Nrf2 activity elucidates the tumor-suppressing role of autophagy. The transcription factor Nrf2 is known to activate the expression of oncogenes involved in angiogenesis and cell survival. The p62/SQSTM1 is competing with Nrf2 for binding Keap1 in the E3 ubiquitin ligase complex. Keap1 usually enables Nrf2 to be ubiquitinated, thereby inducing its degradation under normal conditions. Under autophagy-defective conditions, accumulated p62/SQSTM1 directly competes with Nrf2 to interact with Keap1, thereby preventing Keap1-mediated Nrf2 degradation. Thus, Keap1 sequestration by p62/SQSTM1 prevents Nrf2 degradation, which facilitates Nrf2-mediated tumor survival and aggressive angiogenesis [20].

Moreover, p62/SQSTM1 is phosphorylated by mTORC1 at S351 and increases its affinity for Keap1, which eventually enhances Nrf2-associated tumor progression [21]. ULK1 also plays a role in phosphorylation of p62/SQSTM1 in response to proteotoxic stress including defective proteasome or protein aggregate insult. In this condition, the phosphorylation directs p62/SQSTM1 to be ubiquitinated, thereby leading to efficient degradation of p62/SQSTM1 [22]. Although mTOR and autophagy protein ULK1 inversely regulate autophagy activity, p62/SQSTM1 is a substrate of both protein kinases and p62/SQSTM1 can be phosphorylated at distinct sites and regulate autophagy activity distinctly. In addition, p62/SQSTM1 also acts as an important role for activating mTORC1 through interaction with TNF receptor-associated factor 6 (TRAF6), showing that TRAF6-p62 complex recruits mTORC1 to the lysosomal membrane to be activated under the amino acids-abundant conditions [23]. Furthermore, significant activation of Nrf2 through p62 accumulation was observed in multiple cancer types including hepatocellular carcinoma cells (HCCs) [24]. Taken together, the levels of p62/SQSM1, an autophagic cargo receptor can be regulated by multiple mechanisms, thereby influencing tumor progression.

Interestingly, the function of p62/SQSTM1 in tumor microenvironment is also critical for tumor progression. Tumor-associated stromal cells contain reduced p62/SQSTM1 levels compared to cancer cells, which eventually enhances malignant tumorigenesis of epithelial prostate tumor. Low levels of p62 in stromal cells inactivate mTOR and c-Myc pathway resulting in downre-

gulation of glucose and glutamine metabolism. Associated metabolic defects in tumor microenvironment ultimately fail to maintain redox balance and increase interleukin-6 (IL-6) secretion, thereby leading to promote adjacent tumor progression [25]. Accordingly, the levels of p62/SQSTM1 enable tumor-associated stromal cells to work coordinately with adjacent tumor cells, which ultimately alters stromal metabolism and influences on tumor development

3. Tumor-promoting functions of autophagy

The importance of autophagy during tumor development can be elaborated as a feature of its survival mechanism. Autophagy supports cell survival and growth by supplying degraded and recycled nutrients, in response to various metabolic stresses, often facing rapidly proliferating or hypovascularizing well-developed tumors. Cancer cells can utilize autophagy to provide alternative bioenergetics and effective precursors for macromolecule biosynthesis, which is required for fulfilling metabolic alteration in malignant tumor.

As a direct example, when hematopoietic cells dependent of IL-3 are exposed to IL-3-deprived conditions, glucose utilization is decreased, instead autophagy process is upregulated, which provides energy and nutrients to prolong cell survival [26]. The tumor-promoting role of autophagy has been largely investigated in multiple oncogene-driven cancers in vivo and in vitro system, including oncogenic Ras expression. Genetic deletion of ATG genes in both oncogenic HRas-transformed MEFs and human breast carcinoma cells, harboring oncogenic KRas, leads to reduced tumorigenic transformation and proliferation as well as decreased glycolysis [27]. Similarly, a breast cancer mouse model driven by the polyoma middle T (PyMT) oncogene, when FIP200, an essential protein for autophagy initiation, was deleted, exhibited defective glycolysis in vitro and significantly blocked mammary tumor progression in vivo [28].

Rapidly proliferating tumor cells primarily depend on glycolysis as main glucose metabolism, which is mediated by the activation of oncogenes or the inactivation of tumor suppressors. This metabolic alteration of glycolysis is proposed to provide a major portion of metabolic intermediates for newly activated biosynthetic pathways [29]. Established tumors exhibited Increasing anabolic reactions as the main cellular metabolism, which is supplied with metabolic precursors that are generated by autophagic degradation. Specific oncogenic transformation such as oncogenic Ras promotes autophagic catabolic pathways, although most oncogenic pathways are clearly associated with anabolic processes such as cell growth and proliferation.

Multiple in vivo studies using genetically engineered mouse models (GEMMs) of cancer have provided additional support for cancer-promoting functions of autophagy. Genetic deletion of Atg5 or Atg7 showing early tumorigenesis, however, revealed to reduce advanced tumor development driven from certain oncogene activation.

Using KRas mutant-driven PDAC or lung mouse model, autophagy is an important pathway that exacerbates tumor development. In a pancreatic cancer mouse model harboring a

pancreas-specific KRas mutant, when autophagy genes Atg5 or Atg7 were deleted, the progression of PDAC was significantly inhibited [30]. In a lung cancer model driven by oncogenic KRas or BRAF mutant, autophagy deficiency due to the deletion of Atg5 or Atg7 significantly decreased the tumor burden. These autophagy-deficient mice still harbored benign oncocytomas, which are different from adenocarcinoma generally induced by additional oncogenic insult [31, 32]. As a mechanism for generating oncocytomas, the importance of p53 was raised. The loss of p53 in the KRas-induced lung cancer model suppressed fatty acid oxidation and showed lipid-accumulated oncocytomas, which phenotype might be due to defective mitophagy caused from when autophagy genes were deleted [33].

Interestingly, the suppression of autophagy in oncogenic KRas-driven PDAC mouse models revealed conflicting results depending on the p53 status. Tumor-promoting effect of autophagy mostly was observed in p53-intact condition. In the background of p53 deletion, autophagy inhibition is not sufficient to block tumor progression in oncogenic Ras-mutant mice. Moreover, in the oncogenic KRas-mutant mice with p53 deletion, genetic or pharmacological inhibition of autophagy significantly increased PDAC development. As a survival mechanism, glycolysis especially pentose phosphate pathway(PPP) is activated in tumor cell lines derived from KRas G12D-mutant mice with both deletion of p53 and Atg7, which contribute to tumor progression in PDAC [30]. Since the pentose phosphate pathway can generate NADPH as reductive molecule to scavenge reactive oxygen species (ROS) and produce the metabolic intermediates supporting for biosynthesis efficiently, glycolysis and PPP activated in Ras-driven, p53-deficient tumors might play a role in supplying the metabolic precursors, which are reduced due to the lack of autophagy. Therefore, the p53 can determine cellular metabolic status in coordinating with autophagy and directs to undergo tumor progression.

Moreover, the loss of Atg5 with oncogenic KRas-driven p53-deficient lung tumors markedly increases tumor progression, due to the recruitment of regulatory T cells (T reg) on the tumors. These accumulated Treg cells in tumor lesion might prevent immune surveillance system against tumors and further promote lung cancer progression [34]. The distinct role of p53 in particularly autophagy-defective conditions might be associated with various aspects of tumor-favorable mechanisms including metabolic rewiring including increasing glycolysis, regulating redox balance in addition to controlling immune cell populations adjacent to tumor.

According to a recent report, dormant populations of tumor cells can be survived even after oncogene ablation which are derived from inducible KRas mutant in a heterozygous p53 mouse model. These surviving tumor cell exhibited substantial dependency of oxidative phosphorylation (OXPHOS) for generating energy and utilized autophagy for the survival of these cell populations. This result suggests that metabolic rewiring including autophagic catabolism widely occurs even in heterozygous p53 mouse model. Autophagy and its related mitochondria function are particularly crucial for the survival of tumor cells harboring features of cancer stem cells or tumor relapse [35].

Accordingly, autophagy defect in oncogenic Ras-driven tumor confers accumulated cellular stress including metabolic and redox imbalance leading to cell death, when a tumor suppressor, p53, might have limitation of massive metabolic reprogramming. Thus, loss of p53 in

oncogenic Ras driven cells enables autophagy-defective cancer to avoid cell death through substantial metabolic rewiring to support cell proliferation.

Similar to the function of autophagy in normal cells, autophagy basically plays a role in the effective clearance of unnecessary intracellular products, thereby maintaining cell viability in malignant-transformed cancer cells. However, since cancer cells are frequently exposed to metabolic stress condition as well as high anabolic demand for proliferation, the requirement of autophagy might be more crucial for satisfying metabolic demand of malignant cancer cells. Additionally, autophagy can be activated by multiple anticancer therapies to sustain cancer survival against the treatment, implying the ability of autophagy for drug resistance.

4. Autophagy in glucose metabolism

Autophagy regulates aerobic glycolysis, which supports rapid growth and proliferation in cancer cells. In HRas- or KRas-mutant cells, the deletion of Atg5 or Atg7 leads to reduced glycolysis significantly and then suppresses anchorage-independent colony formation, indicating inhibitory effect on tumor progression [27]. However, additional deletion of p53 in tumor driven from oncogenic KRas mutant enables autophagy-defective mice to increase the levels of glycolysis and markedly facilitates pentose phosphate pathway, thereby promoting PDAC progression [30].

Accordingly, the molecular regulatory mechanism between autophagy and glucose metabolism during cancer development should be studied. Particularly, the function of p53 as a metabolic determinant in autophagy-defective conditions should be investigated more.

Recent study identified specific glycolytic enzymes including hexokinase II (HK II) and phosphofructokinase (PFK) that regulate autophagy [36–38]. Inhibition of hexokinase II (HK II), the enzyme involved in the first step of glycolysis, markedly decreases autophagy and facilitates cell death under the glucose-starvation conditions. Autophagy is induced by HK II upon glucose deprivation through HK II-mediated mTOR inactivation [37]. Moreover, hexokinase II (HK II) is phosphorylated by Akt, leading to increased mitochondrial binding and mitochondrial protection against ROS, where phenotype is abrogated by the addition of glucose-6-phosphate [39].

Another key glycolytic enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFBs), is also involved in regulating autophagy, which converts fructose-6-phosphate to and from fructose-2,6-bisphosphate. An isoform of PFKFBs, PFKFB3 acts as a positive regulator of autophagy in T-effector cells [36], but PFKFB3 in human cancers shows inverse phenotype that the inhibition of PFKFB3 significantly increases autophagy activity due to suppression of glucose uptake and utilizes this pathway as cancer-survival mechanism. Therefore, the concomitant inhibition of autophagy and PFKFB3, using chloroquine (CQ) and PFKFB3 inhibitor, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), might provide a reasonable combinatorial strategy as an anticancer therapeutics, which can be more effective to the cancers harboring highly expressed PFKFB [38]. PFKFB4 is also suggested as a putative autophagy

regulator resulting from the shRNA screening, which activates pentose phosphate pathway. Inhibition of PFKFB4 increases autophagy activity due to lowering NADPH and enhancing cellular ROS levels, which was as a result of defective pentose phosphate pathway, but paradoxically this PFKFB4 knockdown revealed the accumulation of p62 [40].

In addition to the function of glycolytic enzymes on autophagy activity, conversely autophagy regulates specific steps of the glycolytic pathway. HK II is known as an oncogenic kinase to promote metabolic pathways that are important to overcome metabolic stress. HK II overexpression defends cell death after growth factor withdrawal through increasing glucose metabolism [41]. The mutation of a receptor tyrosine kinase FLT3 in nonacute myeloid leukemia cells activates autophagy to overcome metabolic stress. However, when both autophagy and FLT3 are blocked, chaperone-mediated autophagy (CMA) markedly promotes to degrade hexokinase II. Cellular degradation of HK II by CMA primarily inhibits glycolysis and increases metabolic stress in cancers, thereby facilitating cancer cell death [42].

CMA also directly degrades another glycolytic enzyme, pyruvate kinase, M isoform (PKM2) in lysosome-mediated manner, which supports tumor-promoting role during tumor progression [43]. Distinct from HK II, the degradation of PKM2 by CMA may enhance the accumulation of diverse glycolytic intermediates, which could be converted to biosynthetic precursors. Consequently, this process is beneficial to proliferation of cells during tumor progression [44].

Dimeric form of PKM2 harboring low activity is known to be abundant in cancer, which mainly converts from pyruvate to lactate [45].

Recently, acetyl-CoA is reported as an essential metabolite for regulating autophagy activity. The metabolic enzymes involved in the multiple nodes for generating acetyl-CoA have a role in the inhibition of autophagy activity. By contrast, the enzymes participate in reducing the levels of acetyl-CoA, which generally induces autophagy in vitro and in vivo system. Suppression of either glucose, branched chain amino acids (BCAAs) or fatty acid catabolism generates acetyl-CoA, which markedly induces autophagy regardless of intracellular ATP levels. Upregulated autophagy is eventually restored back to normal levels by exogenous treatment with acetyl-CoA [46].

5. Autophagy in glutamine metabolism

Glutamine exists in mammalian plasma with the highest levels among 20 amino acids, which and is utilized for diverse purpose depending on the cellular environment conditions. Glutamine has important functions as a nitrogen source for contributing biosynthesis of nucleotide, other nonessential amino acids (NEAAs), and hexosamine, and is also utilized as a key component of an antioxidant to maintain redox homeostasis.

In various cancers, glycolytic intermediates from the enhanced glycolysis largely support anabolic process, which is essential for rapid cell proliferation. Similarly, the tricarboxylic acid (TCA) cycle can provide metabolic intermediate for supporting biosynthetic pathways in addition to generating energy. Eventually, TCA cycle intermediates themselves can convert to

nonessential amino acids and fatty acids, which are used as primary precursors for anabolic processes. As a crucial carbon source, glutamine is converted to glutamate and then turns to α-ketoglutarate (α-KG), which replenishes intermediates for the TCA cycle and preserves mitochondrial function [9, 47, 48].

The levels of glutamine are elevated in multiple cancers, which are indispensable for cancer growth and survival [49]. Particularly, in certain cancer cells, glutamine tends to replace glucose for playing a role in carbon source through glutaminolysis. Distinct from glutaminolysis to replenish TCA cycle, glutamine can also be utilized to generate oxaloacetate by nonconventional metabolic pathways, which ultimately increase NADPH to maintain redox homeostasis and support cancer cell growth in pancreatic ductal carcinoma (PDAC) [50].

In addition to metabolic reprogramming of glutamine, autophagy is markedly upregulated in response to glutamine deprivation although glutamine is one of NEAAs and is not absolutely essential for regulating autophagy and growth in normal cell conditions. However, autophagy is required for tumor transformation and growth in PDACs. As a similar concept, glutamine might be supplied from the autophagic degradation of cellular macromolecules, which can compensate or restore metabolic stress often shown in progressed malignant tumor.

Recently, multiple reports have suggested the mechanism of how glutamine controls autophagy activity, which is that glutamine and leucine act together to regulate mTORC1 activity and thereby regulating autophagy. Import of glutamine tends to be enhanced in cancer cells, which elevated levels of intracellular glutamine contribute to import leucine into cells with a bidirectional alpha-ketoglutarate transporting system. These bidirectional transport mechanisms of two amino acids control mTORC1 activity, thereby inversely regulating autophagy [51]. In addition, glutamine and leucine work coordinately to activate glutaminolysis and α-ketoglutarate (α-KG), production, which increases the activity and lysosomal localization of mTORC1, resulting in the inhibition of autophagy [52]. A key enzyme in the glutaminolysis, glutamate dehydrogenase (GLUD1) involved in converting glutamine to glutamate, shows a critical role in autophagy regulation, which works as a leucine sensor. Intracellular leucine levels and ROS levels activate mTORC1, respectively, to influence autophagy activity [53]. As a consequence of rewired glutamine metabolism, ammonia at physiological concentration is produced from the amino acid catabolism or glutaminolysis and eventually increases autophagy. This ammonia-mediated autophagy occurs independent of mTORC1 and ULK1/2. These results can suggest direct evidence for autophagy induction mediated by metabolite by-products [54, 55].

As aforementioned in tumor-promoting role of autophagy, Braf-driven lung cancer model harboring the Atg7 deletion showed significant reduction of tumor progression [31]. Dysfunctional mitochondria accumulation in Atg7-deficient cell lines generated from these tumors might be due to defective mitophagy. The addition of glutamine to the cells rescued from the mitochondrial functional defects and slow growth of autophagy-defective tumors. By contrast, the treatment with antioxidant reagents, N-acetyl-cysteine (NAC), is not completely restored starvation-mediated cellular growth defect. These results suggested that glutamine is one of metabolic intermediates derived from autophagy, which are critical for regulating metabolic

homeostasis and sustaining mitochondrial intactness, rather than the function for redox homeostasis [31].

Moreover, the functional connection between glutamine-dependent metabolism and autophagy during metabolic stress conditions is recently reported. Using WT and $atg5^{-/-}$ MEFs, metabolomic profiling, oxygen consumption, and quantitative real-time polymerase chain reaction (RT-PCR) analyses about the metabolic enzymes are altered, upon glutamine deprivation, suggesting that novel regulatory pathways between autophagy and glutamine utilization. Autophagy deficiency shows significantly decreased levels of intracellular glutamine, indicating that glutamine can be supplied from activated autophagy, especially under the nutrient starvation conditions. Interestingly, autophagy-deficient cells increase the uptake of essential amino acids (EAAs) and branched chain amino acids catabolism upon glutamine deprivations, implying that the defect of glutamine generation caused from autophagy deficiency in ATG5 null cells might lead to activation of an alternative mechanism to compensate glutamine limitation.

Furthermore, mRNA levels encoding enzymes used for glutamine-dependent anaplerosis to the TCA cycle and encoding glutamine/EAA transporters are upregulated in $atg5^{-/-}$ MEFs, indicating that autophagy can function at transcriptional regulation to compensate glutamine-deprived conditions, although the exact mechanism is still not understood yet [56]. Taken together, glutamine supplied from autophagy plays critical roles in fueling mitochondrial function and regulating gene expression. Glutamine generated from autophagy is also important for cell growth and survival under the specific conditions including nutrient starvation.

Moreover, general amino acid control (GAAC) pathway, which usually maintains intracellular amino acid levels, also regulates autophagy activity in addition to the uptake of amino acids. Upon glutamine deprivation, the uptake of amino acids is enhanced by activated GAAC pathway, which eventually restores mTORC1 activity and suppresses autophagy. This feedback mechanism can explain how GAAC controls the degree of autophagy by the regulation of amino acid uptake and amino acid synthesis [57].

Accordingly, glutamine metabolism and autophagy are not only reciprocally regulated to compensate each other, along with metabolic and transcriptional alteration, but also more complex and diverse molecular networks act to regulate for cell growth and survival.

In glioblastoma (GBM), glutamine metabolic alteration provides drug resistance to the mTOR inhibition because targeting mTOR increases the expression levels of glutaminase (GLS), a key enzyme for glutaminolysis. Inhibition of GLS can be expected to be more effective in addition to the treatment with mTOR inhibitor [58]. Accordingly, it is speculated that autophagy can associate with glutamine metabolism and glutamine derived from autophagy might ameliorate metabolic stress due to glutamine deprivation, especially in mTOR-activated tumors. Therefore, concomitant-targeting glutamine metabolism and autophagy in diverse cancer including mTOR active cells would be considered as a promising anticancer strategy.

6. Autophagy in macromolecule catabolism

In addition to massive metabolic alteration in various cancers, oncogenic Ras-driven tumors rely on macromolecule degradation including autophagy. Multiple reports have suggested that oncogenic Ras-driven tumors stimulate a unique endocytosis process called macropinocytosis, which engulf and break down extracellular macromolecules. Ras-driven tumors utilize this process as an effective nutrient-supply strategy. Moreover, associated with macropinocytosis driven by oncogenic Ras expression, core autophagy machinery is largely required for macropinocytosis process.

Lysosomal degradation of the extracellular cargoes commonly occurs dependent on nutrient availability and growth signaling. However, under the oncogenic Ras-activating conditions, autophagy is significantly activated despite the fact that this traditional degradation pathway is suppressed by increasing anabolic-signaling pathway.

Clearance of specific cargoes through activated lysosomal degradation, tends to maintain intracellular homeostasis, and recycling of nonspecific cargoes more likely ameliorates cellular metabolic stress of rapidly growing cells by supplying nutrients effectively.

As a typical scavenging pathway, autophagy is upregulated to support cancer cell proliferation and survival in oncogenic Ras-driven tumors including PDAC. In addition, oncogenic Ras-driven tumorigenic cells show increased uptake of extracellular materials for utilizing them as metabolic fuels after lysosomal degradation.

Macropinocytosis is a unique type of endocytosis that engulfs random portion of extracellular fluid without the need for specific vesicle-coat proteins. Multiple growth factor signals positively regulate macropinocytosis, which facilitates the plasma membrane ruffles and engulfs extracellular fluid to be internalized into the cell forming as vesicles, called macropinosome. Ultimately, these vesicles can fuse either with the lysosome, thereby degrading its cargo contents, or might follow regular secretory pathway to release its cargo contents out of the cells [59, 60].

Although oncogenic Ras is known to induce macropinocytosis, the exact function of macropinocytosis on cancer development is largely unknown. Recently, oncogenic Ras-mediated macropinocytosis has revealed to contribute to cancer cell growth and survival through the supply of the essential nutrients to overcome metabolic stress conditions of the cancer cells [59, 60].

In a recent report, macropinocytosis promotes the uptake of extracellular albumin as a cargo molecule, which can be degraded by the lysosome. Thus, overall nutrients including amino acids were generated from this macromolecule degradation. A couple of specific amino acids including glutamine are essential nutrients for supporting high metabolic demand of cancers. ^{13}C-labeled whole protein treated in the media is utilized as a macropinocytosis cargo molecule and degraded to generate amino acids through the detection of ^{13}C-labeled amino acid form. Amino acids labeled with ^{13}C can be thought as degraded products from extracellular macromolecule in oncogenic KRas mutant, indicating that these amino acids were derived from the

macropinocytosis-mediated degradation and mostly utilized for replenishing the TCA cycle [61].

Recent reports have demonstrated a novel regulatory mechanism for macropinocytosis, which is correlated with a representative anabolic signaling molecule, mTORC1 activity. mTORC1 acts as a key regulator to determine metabolic pathways depending on nutrient status. In nutrient-rich condition, active mTORC1 suppresses lysosomal catabolism including the degradation of extracellular proteins, whereas mTORC1 inhibition increases lysosomal degradation of proteins to supply nutrients and support cell growth under nutrient-deprivation conditions. Therefore, mTORC1 activity depending on environmental-nutrient conditions determines metabolic status in tumor either to addict to the anabolism or to rely on the degradation of extracellular macromolecules.

mTORC1 also shows its activity by the intracellular localization of this protein. mTORC1 is redistributed from cytoplasmic localization to the lysosomal membrane by nutrient abundance. Lysosomal localization of active mTORC1 is also exhibited by adding exogenous albumin to the nutrient-starved condition, similar to adding amino acids. This albumin-mediated mTORC1 re-localization is not restored by the blockade of macropinocytosis and lysosomal degradation [62, 63].

However, oncogenic KRas-expressing MEFs deleting Atg5 gene or PDAC cell line harboring Atg7 shRNA show the accumulation of extracellular proteins internalized by macropinocytosis, compared to complete degradation of the protein shown in WT control. Moreover, the lysosomal degradation of extracellular proteins leads to restore the decreased mTORC1 activity, which phenotype is not observed in *atg5⁻/⁻* MEFs. These results suggest that the generation nutrients from environmental extracellular proteins are mostly directed by the major autophagy machineries [63]. When the effect of mTORC1 inhibition was examined using a mouse model with pancreas-specific Kras mutations or xenograft experiment with KRas-mutant PDAC cell lines, PDAC-bearing mice showed rapid tumor growth along with the treatment with mTORC1 inhibitor rapamycin, compared to the nontreated group of mice. As results of histology analysis after rapamycin treatment, well-vascularized outer regions of the tumor revealed low number of Ki-67-positive, proliferating cells, whereas tumor cells in interior and hypovascularized regions are markedly increasing the number of proliferating cells, suggesting that poorly vascularized tumor microenvironment of PDAC is easily exposed to the nutrients and oxygen deprivation. This tumor microenvironment tends to alter tumor metabolism, which requires lysosomal degradation of extracellular macromolecule to generate an alternative nutrient source to support tumor growth.

In addition to the effect of mTORC1 on tumor progression in KRas- and p53-mutant mouse model (KPC), concomitant inhibition of mTORC1 and upregulated macropinocytosis-driven autophagy leads to inhibit tumor growth significantly compared to single inhibition of either mTORC1 or macropinocytosis/autophagy in mouse xenograft experiment using KRas-mutant PDAC cell lines. These results implied that macropinocytosis associated with autophagy can fulfill cellular metabolic requirements for promoting cell growth under the mTOR-compromised conditions. In other words, anabolic perturbation by mTORC1 inhibition results in more active access of nutrients from the degradation of extracellular macromolecules, which

ultimately promotes cell proliferation and survival under nutrient-deprived conditions. Thus, mTORC1 plays an opposite regulating role in tumor growth depending on environmental nutrient availability, implying that the utilized catabolic pathways could be distinguished depending on the nutrient status of the tumor microenvironment.

Accordingly, it raises the possibility of potential novel anticancer strategies interrupting these metabolic balances during tumor progression can open promising avenues.

7. Concluding remarks

Cancer cells undergo metabolic change to support cell proliferation and survival during tumor development.

As a representative catabolic process, autophagy can be suggested a key regulator. Most cancers in advanced stage show "autophagy-addiction" phenotype and need autophagy as a type of metabolic reprogramming during cancer development. Despite the controversial role of autophagy in cancer development, metabolically dynamic cancer cells utilize autophagy to supply the bioenergetic fuels and biosynthetic precursors that support cancer cell growth and survival.

As a new functional mechanism, autophagy also contributes to the metabolism of tumor microenvironment including stroma and immune cells adjacent tumor, which integrated with cancer metabolic alterations. These functional interactions and metabolic re-modulation within heterogeneous tumor microenvironment allow to overcome metabolic stress often facing to the cancer and to sustain in the harsh tumor microenvironment.

Furthermore, autophagy is induced by oncogenic stress such as Ras activation, which is implicated in oncogene-mediated transformation and proliferation. Most cancers driven by oncogenic Ras require autophagy to recover from the metabolic stress. Understanding the molecular mechanism on how autophagy is integrated to major metabolic change including glucose or glutamine metabolic rewiring in cancer and how these pathways are mutually regulated to each other to support cancer development are important for the development of cancer therapeutics with novel strategy. Accordingly, accumulated knowledge of molecular interactions among growth-signaling pathways and the metabolic alteration including anabolic-addicted phenotypes and autophagy dependency during cancer development shed a light to identify the effective target combination for anticancer therapeutics.

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