National Journal of Physiology, Pharmacy and Pharmacology

REVIEW ARTICLE

Hypoxia-induced autophagy in hepatocellular carcinoma and anticancer therapy

Nadia Kabir, Yang Yong

Department of Pharmacology, State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, Center for New Drug Safety Evaluation and Research, China Pharmaceutical University, Nanjing, China

Correspondence to: Yang Yong, E-mail: yy@cpu.edu.cn

Received: March 03, 2017; **Accepted:** April 02, 2017

ABSTRACT

Autophagy is a genetically programmed dynamic process for the lysosomal degradation and recycling of bulk cytoplasmic contents, abnormal proteins, and damaged organelles. Along with cellular homeostasis maintenance, autophagy plays a fundamental role in cancer as tumor cells activate autophagy under stressful conditions such as hypoxia, nutrient derivation, and anticancer therapy. Increasing reports suggest the protective role of autophagy in hepatocellular carcinoma (HCC). Despite recent developments in HCC anticancer treatment, the overall patient survival remains poor. Tumor hypoxia, a common characteristic of most solid tumors like HCC is correlated with poor prognosis for patients partly because hypoxia promotes resistance to cancer therapy. Hypoxia preferentially selects apoptosis-resistant cells and activates autophagy as a survival mechanism leading to malignant and aggressive phenotype. In this review, we summarized the molecules involved in hypoxia-induced autophagy for HCC progression and anticancer therapy resistance. In addition, the molecular pathways involved in hypoxia-induced autophagy were provided. We also focused on some recent researches between hypoxia-induced autophagy and microRNAs in HCC. We believe understanding the novel function of hypoxia-regulated autophagy may coin new targets for the betterment of HCC treatment and improved clinical outcomes.

KEY WORDS: Hepatocellular Carcinoma; Autophagy; Hypoxia

INTRODUCTION

Hepatocellular carcinoma (HCC), the most common primary malignancy of liver is categorized as the second and sixth common cause of cancer-related deaths among men and women, respectively. Approximately, more than 748,000 new cases of HCC are diagnosed annually worldwide. The major risk factors of HCC development includes viral hepatitis, alcohol-related cirrhosis, and metabolic syndrome with non-alcoholic liver diseases.[1] Surgery, radiation and chemotherapy are the most common treatment for HCC. Even though with great advancement in HCC management the overall 5-year survival rate remains 47-53% even in patients with early, small HCC (<3 cm) who underwent surgical resection.[2] Recent reports suggest that autophagy can play an important role in tumor cell survival or death under metabolic stress conditions including hypoxia and anticancer treatment.[3] Tumor hypoxia underlies treatment failure and yields more aggressive and metastatic cancer phenotypes. Hypoxia is present in 90% solid tumors (e.g., HCC) as high proliferation rate and abnormal vascular system of solid tumor cannot supply adequate oxygen.[4] To adapt such

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0305702042017	

hypoxic stress tumor microenvironment would activate autophagy as a protective mechanism that impedes drug or radiation access too. Reports suggest that in liver autophagy was increased in tumor interior rather than in cancer margins, contributing tumor cell survival under hypoxia.[5] Therefore, in this review, we felt the necessity to explore the role of autophagy activation in HCC under hypoxia and anticancer therapy.

AUTOPHAGY AND HCC

Autophagy Introduction

Autophagy is a genetically programmed dynamic process for the lysosomal degradation and recycling of bulk cytoplasmic contents, abnormal proteins, and old or damaged organelles.[6] A stressed condition such as hypoxia, starvation, protein aggregation, and oxidative stress induce autophagy to maintain the cellular metabolic homeostasis. Recently, researchers pay attention on autophagy due to its close association with both tumorigenesis and therapy resistance. Autophagy involves sequential stages: Initiation, elongation, autophagosome formation, and autophagosome fusion with lysosomes and degradation.[7,8] These steps are regulated by autophagy-related genes (Atgs). In 2016, a Japanese researcher Yoshinori-Ohsumi has been awarded Nobel Prize in Physiology or Medicine because of his extraordinary contribution to identify Atgs in Yeast back in 1990.[9] Till date, more than 30 Atgs functions have been evaluated extensively. There have been three kinds of autophagy: Macroautophagy, chaperone-mediated autophagy, and microautophagy.[5,8] Macroautophagy is generally referred as "autophagy" due to the limited data for the other forms.[8] Phagophore, a double membrane structure forms within the cell, elongates and starts to encompass the cytoplasmic components (cargo). The membrane seals around the cargo forming a structure called the autophagosome which is a major indicator of autophagy induction. Later, the autophagosome fuses with the lysosome, which provides hydrolases and the sequestered contents undergo degradation and recycling.[7,8] Defective autophagy is correlated with diverse pathologies, including neurodegeneration, heart and muscle diseases, aging, and cancer.[7-10]

Autophagy is controversial for being involved in both tumor cell survival and death. Hypothesis reflects that during initial stages of tumorigenesis autophagy acts as a tumor suppressor by recycling damaged organelles to maintain genomic stability. However, once a tumor is established, autophagy acts as a cell survival mechanism in response to various stresses and in turn promotes tumor growth.[11] Increased autophagic activity is associated with 50%-60% of tumor cell survival under hypoxic conditions.[12] For example, hypoxia-induced autophagy promotes HCC cell survival[13] with malignant progression and poor prognosis.[14] Other types of solid tumors like colorectal, breast cancers[15,16] also experienced survival and aggressive phenotypes due to hypoxia-induced autophagy referring this phenomenon as a prosurvival factor for cancer.

However, autophagy is also associated with a kind of programmed cell death (PCD), termed as autophagic cell death. In comparison to apoptosis and necrosis, the other two kinds of PCD, autophagy plays a dual role in cell viability.[3,17] As previously discussed, besides of playing a cytoprotective role, autophagic cell death participates in tumor suppression under certain circumstances. For instance, treatment with ursolic acid (UA) resulted in Atg5-dependent autophagic cell death in cervical cancer where Atg5 inhibition along with UA increased cell survival.[18] Tai et al. reported that sorafenib and Sc-59, a potent sorafenib derivative-induced autophagic cell death with tumor growth inhibition in HCC xenograft through Mcl-1-dependent activation of Beclin-1.[19] Adriamycin monotherapy for advanced HCC is shown to activate a sustained mitogen-activated protein kinase (MAPK)/ERK pathway leading to prolonged autophagy and subsequent autophagic cell death.[20] Thus, autophagic cell death is associated with tumor suppression. It seems like autophagic cell death is a failure attempt by cancer cells to survive stress stimuli by activating sustained autophagy, followed by an irreversible stage and ultimately cell death. Therefore, for cancer therapy improvement molecules involved in such dual role of autophagy under hypoxia is necessary.

Atg in HCC

Autophagy as a tumor suppressor involves some housekeeping genes which are vital for its own regulatory pathway. Among them Beclin-1, ATG4C, ATG5, ATG7, ATG8, ultraviolet irradiation resistance-associated gene (UVRAG), and Bif1 have already confirmed their tumor suppression role in mouse models.[21]

Beclin-1, the mammalian ortholog of yeast Atg6, promotes the initial stages of autophagy through binding with the prototypic apoptosis inhibitor Bcl-2.[8] and plays critical role in the regulation of autophagy. It binds to several proteins, such as Vps34, Atg14L, UVRAG, Bif-1, p150, and therefore regulate the maturation of the autophagosome.[3,8] Delaying cell cycle progression, suppressing tumor proliferation, angiogenesis particularly under hypoxia, and limiting chromosomal instability are some of the underlying tumor suppressive mechanism of Beclin-1. In fact, the initial studies of Beclin-1 helped researchers to connect impaired autophagy with cancer development which is monoallelically deleted in 40-75% of cases of prostate, breast, and ovarian cancer.[3] Mice with heterozygous Beclin-1 disruption have a high frequency of spontaneous HCC.[22] microarray analysis of HCC tissues revealed a significant decreased expression of Beclin-1 compared to adjacent nontumorous tissue.[23,24] Consistent with this report, increased Beclin-1 expression is also correlated with longer free survival and overall survival

(OS) in HCC.[24] indicating the role of defective autophagy in carcinogenesis development. Like Beclin-1, the expression and activity of Atg5 or Atg7 autophagy gene are also reduced in HCC cell line as compared to normal hepatocytes *in vitro* and their deletion resulted in increased HCC.[25]

Atg8 also known as light chain 3 (LC3) (microtubule-associated protein-LC3) converts from soluble LC3-I into lipidated LC3-II on autophagy activation. Later, LC3-II is recruited for autophagosome formation. Such LC3-I/LC3-II conversion is an indicator of autophagy activation.[8] Aberrant expression of LC3 is correlated with cancer prognosis for several tumor types.[26,27] In HCC, significant high expression of LC3 is found compared with noncancerous tissues.[28] and associated with longer OS in advanced stage of HCC.[29] In addition, LC3 is an independent predictor of HCC recurrence after surgery only in the context of large tumors.[28] These data support aberrant autophagy activates tumorigenesis. Autophagy defective tumor cell accumulate p62, an ubiquitinated protein which aggregates in response to stress contributing tumorigenesis. In normal cells, p62 interacts with LC3, merge to the autophagosome and thereafter degrades by autophagy. Specific deletion of p62 in ATG7-deficient mice strongly suppressed the growth of liver adenomas.[8] Again, p62 expression was found in the HCC samples but not in the surrounding nontumorous hepatocytes.[30] which implies the role of p62 in tumorigenesis. By studying the role of autophagy-regulating gene, it is evident that autophagy plays a pivotal role in tumor suppression, and defective autophagy or autophagy deficiency leads to tumorigenesis like HCC. However, mechanisms responsible for defective autophagy are yet to be elucidated.

HYPOXIA-INDUCED AUTOPHAGY IN HCC

Hypoxia, Hypoxia Inducible Factor (HIFs), and HCC

Tumor hypoxia, a common feature in 90% solid tumors like HCC is intricately associated with more malignancy, therapeutic resistance, and poor patient survival.[4] To survive under such reduced oxygen concentration, tumor cell adapts some transcriptional expression where HIF is a key transcriptional factor.[31]

HIF transcription factors (HIF-1 and HIF-2) act as heterodimers comprising an alpha and beta subunit. Under normoxic condition alpha subunit is unstable due to proteasomal degradation. However, under hypoxia, stable alpha subunit moves to nucleus to dimerize with beta subunit. In cooperation with transcriptional coactivators, this HIF heterodimer binds with hypoxia response element (HRE) and induces transcription of more than 60 HIF-targeted gene. Regulation of these genes are widely associated with metabolic processes, tumor cell proliferation, angiogenesis, survival, and therapeutic resistance.[4,31]

HIF-1α plays a major role in the development of characteristic tumor phenotypes including growth rate, angiogenesis, invasiveness, and metastasis.[4,31-33] Increased expression of angiogenesis marker, vascular endothelial growth factor (VEGF), and metastasis marker, matrix metalloproteinase 2 (MMP-2) are associated with increased HIF-1α in HCC patient with lymph node metastasis[34] where knockdown of HIF-1α repressed both VEGF and MMP-2 resulting decreased angiogenesis and metastasis, respectively.[35] HIF-1α is also involved in the regulation of gene expression during inflammation. Xia et al. demonstrated that by the induction of tumor necrosis factor-α (TNF-α), HIF-1α directly binds with Forkhead box M1 promoter which is a proliferation-specific transcription factor and subsequently promote HCC cell proliferation with apoptosis resistance.[33] HIF-1α stabilization also fuels hypoxic solid tumor with survival potential through glycolysis by upregulating many enzymes of glycolysis pathway. For instance, hypoxia stimulates HCC cellular growth through HIF-1α dependent induction of hexokinase 2 (HK2) enzyme. Inhibiting HK2 expression in a murine HCC model increased tumor cell apoptosis and limited tumor growth.[36]

HIF-2α another HIF isoform is also associated with cell proliferation, tumor angiogenesis, and metastasis by regulating their targeted genes such as transforming growth factor (TGF)-α, VEGF, cyclin D1, and SERPINB3.[37] Its expression has also been reported to reduce hepatic gluconeogenesis with increased HCC malignancy.[38] Moreover, increased HIF-2α also confers sorafenib resistance by TGF-α/epidermal growth factor receptor pathway activation. These reports support the positive correlation between HIF-2α and HCC pathogenesis.[39] Recently, HIF-2α targeted antagonist, PT2399 is shown to impart antitumor activity in human renal cell carcinoma cell lines, and xenografts with higher expression of HIF-2α by dissociating HIF-2α/HIF-1β heterodimer with decreased HIF-2α targeted genes.[37] Therefore, HIF-2α targeted therapeutic approach is essential for HCC which is yet to be experimented. However, decreased expression of HIF-1α was found to serve proapoptotic effect and negatively correlated with tumor size both in HCC patients and xenografts making HIF-2α controversial in liver cancer.[40]

Activating autophagy, metabolic reprogramming, drug efflux and inhibiting apoptosis, senescence, DNA damage, mitochondrial activity are some of the major hypoxia-mediated therapy resistance mechanisms well summarized in Rohwer and Cramer paper.[32] In addition, tumor hypoxic microenvironment is shown to evade chemotherapy through generating dormant, or slow cycling disseminated tumor cells (DTCs) in an irreversible manner in endoplasmic reticulum (ER)+ breast cancer and head and neck squamous cell carcinoma. Authors speculated that these DTCs may be the source of disease relapse and poor prognosis associated with hypoxia.[41]

Molecular Pathway of Hypoxia-induced Autophagy

Besides HIF-1α regulated pathway to induce autophagy as a cellular response to hypoxia several other pathways have been identified as follows: Unfolded protein response (UPR), inhibition of the mammalian target of rapamycin (mTOR) kinase signaling pathway, and activation of AMP-responsive protein kinase (AMPK). Therefore, signaling pathways of hypoxia-induced autophagy can be divided into two: HIF-1 dependent pathway and HIF-1 independent pathway (Figure 1).

HIF-1 Dependent Pathway

Mitochondria are one of the major sites of adenosine triphosphate (ATP) production and dysfunctional mitochondria are main source of reactive oxygen species (ROS) resulting DNA damage and cell death. Elimination of such damaged mitochondria is mediated by selective autophagy-mitophagy. Studies revealed that mitophagy is an HIF-1 dependent endogenous progress in response to hypoxia.[42] Bcl-2/E1B-19K-interacting protein 3 (BNIP3), a BH3-only protein, is induced under hypoxic condition through the BNIP3 promoter which has the HRE recognized by HIF-1. BNIP3L (BNIP3 Like, also called Nix) shares 55% sequence similarity to BNIP3 and also a member of BH3-only subfamily. Upregulation of BINP3 and BINP3L induced by HIF-1 is crucial for the initiation of hypoxia-induced autophagy both *in vivo* and *in vitro*.[43,44]. HIF-1-induced BNIP3 expression can mediate hypoxia-induced mitophagy to prevent increased ROS and cell death in hypoxic mouse embryonic fibroblasts (MEFs).[42] Usually, autophagy is inhibited by the Beclin-1/Bcl-2 complex but under hypoxic condition, BH3 domains of BNIP3 and BNIP3L interferes the Beclin-1-Bcl-XL/Bcl-2 interaction. This results in more release of Beclin-1 to promote autophagy.[45]

HIF-1 Independent Pathway

Besides HIF-1 dependent pathway under severe hypoxia (<0.01% oxygen) or even anoxia, autophagy is mediated by AMPK. On long-term hypoxic stress, there is an energy deprivation in tumor cell which results in elevated AMP/ATP ratio, leading to the activation of AMPK, a major regulator of cellular energy homeostasis. Activated AMPK activates tuberous sclerosis complex 1/2 which inhibits small GTPaseRheb resulting mTOR inactivation.[46] Autophagy

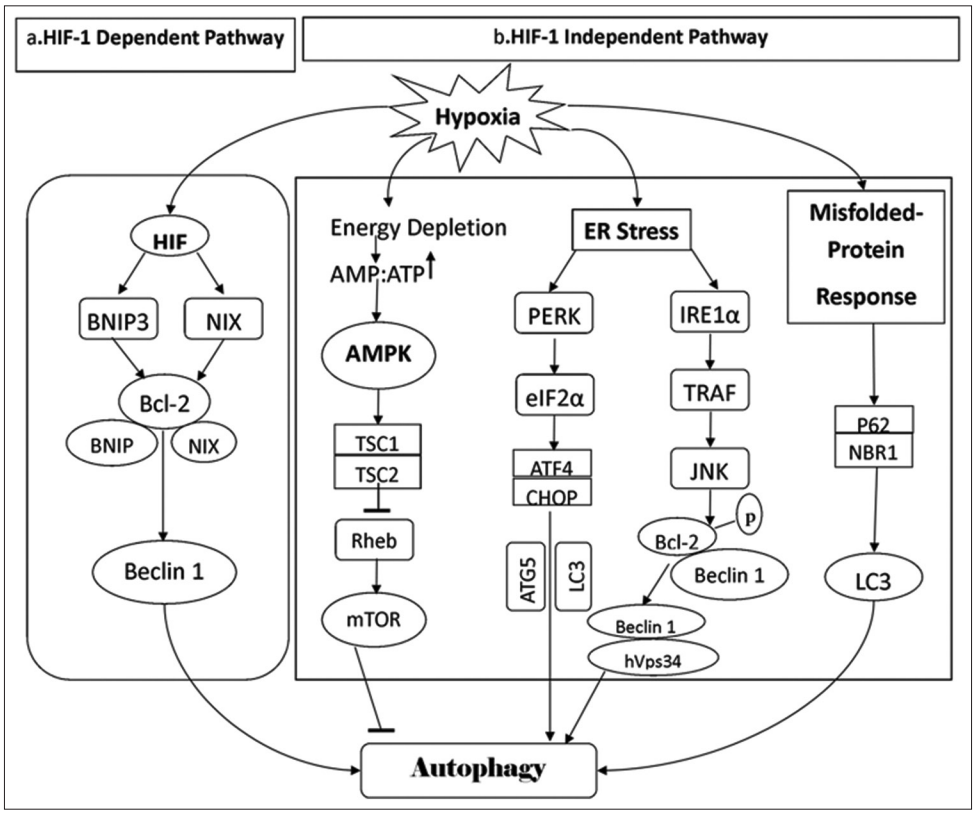

Figure 1: Molecular pathway of hypoxia-induced autophagy: (a) Hypoxia-inducible factor (HIF)-1 dependent pathway and, (b) HIF-1 independent pathway: (a) Under hypoxia, stable HIF-1 α moves to the nucleus and induces transcription of BNIP3/NIX (BNIP3L) which in turn disrupt Beclin-1-Bcl-XL/Bcl-2 interaction and releases Beclin-1 that triggers autophagy, (b) under severe hypoxia, energy depletion activates AMP-responsive protein kinase which inhibits the activity of mammalian target of rapamycin. Hopoxic stress-induced unfolded or misfolded proteins can be cleared by autophagy activation. Unfolded protein response pathway is mainly regulated by PERK-eIF2α and IRE1-tumor necrosis factor receptor-associated factor 2-Jun N-terminal kinase pathways. Misfolded protein binds to light chain 3 through p62 and neighbor of breast cancer 1 and activate autophagy

regulated by AMPK pathway contributes to cell death in HCC. Vara et al. found that cannabinoid reduced the growth of two different models of HCC subcutaneous xenografts with decreased mTORC1 activation, enhanced AMPK phosphorylation with increased autophagy and apoptosis in those tumors.[47] AZD8055 which is a potent inhibitor of mTORC1 and mTOR2 also activated AMPK pathway and resulted HCC cell death with autophagic features but not apoptosis.[48]

UPR pathway is another important pathway in response to severe hypoxia. Under hypoxic stress, tumor cell's ER fails to fulfill the great demand for enormous protein folding and assembly, which leads to the accumulation of unfolded or misfolded protein.[49] Therefore, to maintain ER homeostasis tumor cell utilizes autophagy mediated by UPR pathway. This UPR pathway is mainly initiated by ER stress sensors: PERK, ATF6 and IRE1, and the PERK-eIF2α and IRE1-TNF receptor-associated factor 2 (TRAF2)-Jun N-terminal kinase (JNK) pathways are the leading regulators for autophagy induction.[50] Hypoxia promotes autophagy involving transcriptional induction of Atg5 and LC3 through the PERK-responsive transcription factors ATF4 and C/EBP Homology Protein (CHOP).[51] Under hypoxic environment activated IRE1α can recruit TRAF2 switch subsequently activates JNK. Severe ER stress leads to activation of JNK that down-regulates the anti-apoptotic protein Bcl-2 by phosphorylating Bcl-2 on the mitochondrial and ER membrane. JNK-mediated phosphorylation of Bcl-2 releases Beclin-1 from its inhibitory interaction with Bcl-2 at ER membrane. Freed Beclin-1 induces autophagy through the formation of hVPS34 complexes.[50] UPR pathway is involved in the treatment of HCC. Shi et al. reported that sorafenib significantly increased the micro RNA (mRNA) and protein expression levels of the UPR target genes CHOP and IRE-1 as well as eIF2α phosphorylation. Thus, it can trigger the ER stress-induced autophagy. Briefly, autophagy conferred a survival advantage for sorafenib treatment. Inhibition of autophagy using either pharmacological inhibitors or essential autophagy gene knockdown enhanced cell death in sorafenib-treated HCC cell.[52] Therefore, it is clear that ER stress and autophagy are closely related to prosurvival mechanisms. However, the specific mechanisms linking UPR to autophagy during ER stress remain poorly understood.

In addition, hypoxia and oxidative stress can generate misfolded protein which are degraded by selective autophagy. p62 and neighbor of breast cancer 1 directly binds with LC3 and becomes a selective autophagy receptor. Thereafter, it can concentrate ubiquitinated protein aggregates to the emerging autophagosome for clearance.[53] Certainly, better understanding of molecular pathway responsible for hypoxia-induced autophagy is beneficial for future cancer research and treatment.

Hypoxia-induced Autophagy Plays as Pro-survival or Pro-death Role in HCC?

For survival purpose, HCC like solid tumors faces stressful microenvironment including energy shortage, ROS production, and accumulation of misfolded proteins and damaged organelles. Autophagy as a housekeeper of cellular homeostasis protects tumor cells from such stressed conditions. However, recent studies found role of autophagy controversial either having pro-survival or pro-death role under various conditions at different stages of tumor cells.[12]

Autophagy as a Survival Pathway for HCC under Hypoxia

Under hypoxia, autophagy is essential for the survival of various cancer cell lines including esophageal, colon, and prostate cancer.[12] Having said as a solid tumor, HCC is more likely to experience starvation where autophagy counters this hypoxic stress in hepatocellular tumor spheroids. HIF-1α-induced BNIP3 expression led to enhanced HCC survival. Even though BNIP3 acts as pro-death protein.[54,55] reports also verify its prosurvival role in colon and prostate cancer cells by autophagy induction under hypoxia.[45] In MEF, BNIP3 mediates the mitochondrial autophagy to remove ROS, preventing cell death.[42] Consistently, Cosse et al. have reported that BNIP3 protects HepG2 cells against etoposide-induced cell death under hypoxia.[56] In general, BNIP3 is induced by HIF-dependent pathway.[45] However, other pathways also exist. Park et al. reported that under hypoxia, BNIP3 is induced and degraded by autophagy in various types of tumor cell including human adenocarcinoma cells specifically triggered by ULK1-mediated autophagy, which could be regulated by mTORC1 and AMPK. mTORC1 inhibition resulted loss of BNIP3 with the limited survival of hypoxic tumor cells.[57] These reports are sufficient enough to support the protective role of BNIP3 under hypoxic stress. Du et al. reported that p62, a protein aggregate which is commonly induced under hypoxia is cleared by autophagy in HCC indicating a possible survival mechanism.[13]

Clinical data also reveal that tumors can utilize autophagy for survival and proliferation during hypoxia. Beclin-1 exhibits a tumor suppressor function and has been found to be monoallelically deleted in several tumors.[3] High Beclin-1 expression in advanced human nasopharyngeal carcinoma samples is correlated with poor patient survival.[58] Similarly, there was a significant correlation with elevated Beclin-1 when assessed with HIF-1α, indicating that hypoxia-induced activation of Beclin-1 and autophagy may drive tumor cells to survive treatment. Hypoxia-induced autophagy also plays a pivotal role in cancer development and progression. An experimental study, using a primary mouse melanoma tumor model, demonstrated that Beclin-1 deficiency was significantly associated with the aggressive phenotype and increased angiogenesis in tumors under

a hypoxic condition compared to normoxic condition.[16] Similarly, immunohistochemistry results on 65 HCC tumor specimen exhibited a significant association between reduced Beclin-1 and elevated HIF-1α expression with large tumor size, multifocal tumors, and advanced stage of cancer. The authors speculated that autophagic Beclin-1-induced clinicopathological effect was driven by hypoxia in HCC and reduced Beclin-1 with high HIF-1α expression collectively promotes malignancy and aggressive HCC.[23] Association of poor clinicopathological features either with increased or decreased expression of Beclin-1 and elevated HIF-1α support the hypothesis that defective autophagy can contribute to carcinogenesis.

Cancer stem cells (CSCs) have high tumor-propagating potentials with enhanced metastasis and therapy-resistant property. Therefore, even after tumor reduction CSCs can result in tumor relapse. Autophagy is reported to impart CSCs with survival, self-renewal, and metastatic property to adapt stressful tumor microenvironment and therapy. Under hypoxia and nutrient-deprived condition, autophagy contributes survival of CD133+ liver CSCs.[59] Hif-1α mediated autophagy is reported to promote epithelial-to-mesenchymal transition and metastatic ability of CD133+ pancreatic CSCs under hypoxia where blocking of autophagy attenuated this property.[60] Even the dynamic equilibrium between CSCs and non-CSCs is maintained by autophagy. Zhu et al. reported that under hypoxia HIF-1α induced autophagy is the modulator for CSCs to non-CSCs conversion in pancreatic cells.[61] On these ground, the functional link between cytoprotective hypoxia-mediated autophagy and CSCs should be considered for new therapeutic approach.

Autophagy as a Negative Regulator of HCC Survival under Hypoxia

Hypoxia-induced autophagy imparts tumor cell survival by clearing dysfunctional mitochondria resulting decreased ROS production through HIF-1α/BNIP3 pathway.[42] However, it is also evident that prolonged hypoxia could induce autophagic cell death similarly through BNIP3.[62] These controversial results reflected the dual role of autophagy under different conditions. Hypoxia-induced autophagy has been demonstrated to reduce cell survival in breast cancer, glioma, and human embryonic kidney cells independent of apoptosis. Prolonged hypoxia to all of these cell types resulted autophagic cell death.[62] Autophagy inhibition abrogated this decrease in tumor cell survival suggesting that cell death associated with autophagy may have been responsible for the reduction in cellular viability. ADRB2 associated adrenaline signaling is reported to stabilize HIF-1α which increased glycolysis in HCC. ADRB2 signaling inhibition resulted autophagic degradation of HIF-1α with reduced glycolysis, which is essential for cell survival. Thus, autophagy-mediated alteration in metabolism contributes to reduced cellular vitality which can be a partial reason for autophagic cell death.[63]

It can be hypothesized that the level of autophagy plays the dominant role in the regulation of its either pro-survival and pro-death. Autophagy regulates tumor cell survival under moderate hypoxia with no nutrient limitation while tumor cells faces autophagic cell death under severe hypoxia associated with metabolic stress. This concept goes same for HCC too where moderate hypoxia induces prosurvival autophagy while severe hypoxia leads to autophagic cell death. More attention is required to solve the dual autophagic role when considering the complex hypoxic tumor microenvironment.

HYPOXIA INDUCED AUTOPHAGY AND ANTICANCER THERAPY IN HCC

Surgery along with chemotherapy and radiotherapy combination fails to improve the survival rate of patients with advanced HCC due to resistance.[15] Therefore, scientist looks for the underlying mechanism that facilitates tumor microenvironment to adapt anticancer drug-induced stresses.

Resistance to Chemotherapy

Autophagy also acts as a survival mechanism from therapy-induced stress. Chemotherapeutic drugs, including etoposide, temozolomide, cisplatin, methotrexate, and 5-fluorouracil, could induce autophagy with cytoprotective effect.[15,64] Hypoxia-induced autophagy also imparted chemoresistance in HCC cells.[65] with increased Beclin-1, LC3-II, and autophagosome formation. Oxaliplatin and bevacizumab-treated HCC xenografts also experienced chemoresistance where autophagy decreased intracellular ROS level which were actually mediated by the drugs. Autophagy inhibitor either with oxaliplatin or bevacizumab markedly inhibited their tumor growth with enhanced apoptosis demonstrating autophagy as a survival factor.[66,67]

Hypoxia-induced autophagic response elicits chemoresistance by disrupting drug-induced apoptosis either by downregulating pro-apoptotic proteins or upregulating anti-apoptotic proteins. Very recently, Zhou et al. reported that low glucose and hypoxia-induced autophagy down-regulated the expression of the pro-apoptotic proteins Bad and Bim in the presence of chemotherapeutic agents in the HCC cells, while chloroquine (CQ) or 3-MA or the RNAi of Atg5 or Atg7 could counteract this. Thus, Bad and Bim downregulation plays an important role in autophagy-induced chemoresistance, consequently proving a clear mechanism of autophagy as a pro-survival factor.[68] In HCC, autophagy regulated chemoresistance is also correlated with the induction of HIF-1α which subsequently regulates BNIP3. Studies indicate that BNIP3 induces autophagy by disrupting the interaction of Beclin-1 with Bcl-2 and Bcl-XL resulting decreased expression of these major antiapoptotic proteins.[69]

Other than HIF-1α, transcriptional factors that are often high expressed in HCC with poor survival also confer chemoresistance by autophagy induction. Peng et al. showed that early growth response gene-1 (Erg-1) transcriptionally regulates hypoxia-induced autophagy by binding to LC3 promoter in HCC cells and enhanced cisplatin and epirubicin resistance.[70] Similarly, ATF4 being transcriptional regulator of the cellular hypoxic response to the UPR also showed resistance to bortezomib which actually a UPR-apoptosis mediator through autophagy induction as a survival key in HCC.[71] Inhibition of these transcriptional regulator increased chemosensitivity representing an attractive therapeutic approach.

Resistance to Radiotherapy

Hypoxia remains to be one of the major challenges of radiotherapy. Hypoxic regions in solid tumors are approximately 3 times more resistant to ionizing radiation (IR) than normoxic areas. Clinically, relevant hypoxia ($1\%O_2$) (moderate hypoxia, 1% O_2) can induce autophagy and consequently promote resistance of human tumor cells to IR *in vitro*. Chaachouay et al. reported that preconditioning of different tumor cells to moderate hypoxia increased their radioresistance through the induction of autophagy while autophagy inhibitors sensitized the tumor cells.[72] Peng et al. reported that Atg4B is upregulated at both mRNA and protein level in HCC patients where Egr-1 which can modulate autophagy facilitates HCC cells resistance to radiotherapy by direct upregulation of Atg4B transcription. Suppression of Egr-1 activity abrogates IR-induced autophagy and sensitizes HCC cells to IR treatment. This serves as how IR-induced autophagy can impart a protective mechanism against radiotherapy.[73]

Autophagy as a Pro-survival or Pro-death Mechanism against Anticancer Therapy for HCC

Just like the autophagic potential either to promote cell survival or cell death under hypoxia, more and more reports justify a paradoxical role of autophagy following anticancer treatments. In HCC, cytoprotective autophagy conferred resistance when treated with oxaliplatin, bevacizumab, and etoposide. The combination of autophagy inhibitor and bevacizumab markedly inhibited the growth of HCC.[67] Moreover, sorafenib with CQ can generate more ER stress-induced cell death in HCC both *in vivo and in vitro*.[24] Therefore, autophagy inhibition can be considered a promising therapeutic strategy to enhance chemotherapeutic effect for HCC patients. The anti-malarial drugs, CQ and its derivative Hydroxy CQ are the only autophagy inhibitors which have been approved by the Food and Drug Administration.

On the contrary, autophagy may also play as a death executioner to induce autophagic cell death. Sorafenib and SC-59, a novel sorafenib derivative, induce autophagic cell death in HCC cells in a dose and time-dependent manner.[19] Recently, for glioma, pancreatic cancer, and HCC, cannabinoids have been shown to exert their anticancer activity via stimulation of autophagy-mediated cell death.[47,74,75] Basically, it appears that whether anticancer drug-induced autophagy will respond as pro-survival or pro-death depends on different types of cancer. Moreover, drug concentration can also play important role conferring either apoptosis or autophagy for the same kind of cancer. tetrandrine, a bisbenzylisoquinoline alkaloid has been found to induce apoptosis at a high concentration (30 μM) by repressing AKT activity in HCC cells.[76] whereas at a low concentration (5 μM) it can induce autophagy in a dose- and time-dependent manner *in vivo* and *in vitro*. Mechanistically, it is due to partial activation of the MAPK ERK, and up-regulation of ATG7.[77] Therefore, the role of autophagy can be taken under consideration as novel target for anticancer therapy.

EMERGING ROLE OF AUTOPHAGY REGULATION BY miRNAs UNDER HYPOXIA

Even though there are abundant evidence that autophagy plays a significant role in allowing tumor cells survival under stressful conditions such as hypoxia, the underlying molecular mechanism still remains to be clarified. One of the probable regulatory mechanisms is mRNAs which has been shown to mediate autophagic response to hypoxia very recently. mRNAs are noncoding single-stranded RNA molecules which participate in cell division, development, cell death, and cell migration by controlling post-transcriptional and translational regulations. Being able to modulate protein synthesis, mRNAs provides cells with advantages in response to starvation, genotoxic stress, and hypoxia.[78] A considerable number of mRNAs including miR-375, miR-31, miR-204, and miR-34 could mediate autophagic responses to hypoxia in different pathways.[79] Zhai et al. summarized that mRNAs can impact on cancer cell survival by modulating autophagy by regulating ATGs and their regulators at different autophagic stages including autophagic induction, vesicle nucleation, vesicle elongation, and completion.[79] For example, in human HCC, miR-375 is normally downregulated but when exogenously expressed it was able to inhibit autophagy in response to hypoxia by targeting ATG7 which is involved in the critical steps of conversion from LC3-I to LC3-II and for the elongation of the autophagosome. In mouse xenograft model, miR-375 expression could reduce tumor growth compared to control. This indicates that autophagy is activated as a protective mechanism against hypoxic stress, and miR-375 exerts tumor-suppressive activity via inhibition of autophagic vesicle elongation triggered by hypoxia.[80] Along with enhancing cell survival capability, such dysregulated mRNAs are reported to confer chemoresistance as well as multidrug resistance (MDR) by activating autophagy under hypoxia. Xu et al. reported that both HCC patients and HCC cell lines

represent down-regulated miR-199a-5p when treated with cisplatin where autophagy was activated as well by targeting ATG7 allowing increased cell proliferation particularly in Huh7 and HepG2 cells. Later autophagy inhibition attenuated such resistance demonstrating that autophagy activated by miR-199a-5p downregulation increased cisplatin resistance.[81] Yuan et al. showed that there is a HIF-2α-metastasis associated lung adenocarcinoma transcript (MALAT1)-miR-216b axis regulating MDR of HCC cells through modulating autophagy. MALAT1 is an oncogenic lncRNA which is usually upregulation in HCC cells and under hypoxia can be induced by HIF-2α. Bioinformatics analysis showed that MALAT-1 highly targets miR-216b which usually acts as a tumor suppressive mRNA in HCC.[82] Knockdown of MALAT-1 or overexpression of miR-216b reduced MDR resulting decreased autophagy.[83] These studies support the importance of autophagy regulation by mRNAs under hypoxia making the concept a viable target for cancer therapy in future research.

CONCLUSION

Recent studies agree with the idea that hypoxia-induced autophagy plays a significant role in cancer cell survival. In this review, we discussed the molecules involved in hypoxia-induced autophagy which nurture HCC survival, progression, and in turn, impart therapy resistance. Targeting these molecules along with conventional therapy may improve clinical outcome. However, with only few exception experiments have generally been performed in xenograft models, thereby eliminating the involvement of the immune system, which might ultimately be proven to play a central role in determining the effectiveness of autophagy inhibition in patients.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.
2. Altekruse SF, McGlynn KA, Dickie LA, Kleiner DE. Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992-2008. Hepatology. 2012;55(2):476-82.
3. Eskelinen EL. The dual role of autophagy in cancer. Curr Opin Pharmacol. 2011;11(4):294-300.
4. Kizaka-Kondoh S, Inoue M, Harada H, Hiraoka M. Tumor hypoxia: A target for selective cancer therapy. Cancer Sci. 2003;94(12):1021-8.
5. Fang H, Liu A, Dahmen U, Dirsch O. Dual role of chloroquine in liver ischemia reperfusion injury: Reduction of liver damage in early phase, but aggravation in late phase. Cell Death Dis. 2013;4:e694.
6. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell. 2006;10(1):51-64.
7. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. Mol Cell. 2010;40(2):280-93.
8. Rosenfeldt MT, Ryan KM. The multiple roles of autophagy in cancer. Carcinogenesis. 2011;32:955-63.
9. Scientific Background Discoveries of Mechanisms for Autophagy; 2016. Available from: https://www.nobelprize.org/nobel_prizes/medicine/laureates/2016/advanced-medicineprize2016.pdf. [Last accessed on 2016 Oct 03].
10. Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, et al. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev. 2010;90(4):1383-435.
11. Lee YJ, Jang BK. The role of autophagy in hepatocellular carcinoma. Int J Mol Sci. 2015;16(11):26629-43.
12. Yang X, Yu DD, Yan F, Jing YY, Han ZP, Sun K, et al. The role of autophagy induced by tumor microenvironment in different cells and stages of cancer. Cell Biosci. 2015;5:14.
13. Du H, Yang W, Chen L, Shen B, Peng C, Li H, et al. Emerging role of autophagy during ischemia-hypoxia and reperfusion in hepatocellular carcinoma. Int J Oncol. 2012;40(6):2049-57.
14. Wu DH, Jia CC, Chen J, Lin ZX, Ruan DY, Li X, et al. Autophagic LC3B overexpression correlates with malignant progression and predicts a poor prognosis in hepatocellular carcinoma. Tumour Biol. 2014;35(12):12225-33.
15. Chen S, Rehman SK, Zhang W, Wen A, Yao L, Zhang J. Autophagy is a therapeutic target in anticancer drug resistance. Biochim Biophys Acta. 2010;1806(2):220-9.
16. Lee SJ, Kim HP, Jin Y, Choi AM, Ryter SW. Beclin 1 deficiency is associated with increased hypoxia-induced angiogenesis. Autophagy. 2011;7(8):829-39.
17. White E, Di Paola RS. The double-edged sword of autophagy modulation in cancer. Clin Cancer Res. 2009;15(17):5308-16.
18. Leng S, Hao Y, Du D, Xie S, Hong L, Gu H, et al. Ursolic acid promotes cancer cell death by inducing Atg5-dependent autophagy. Int J Cancer. 2013;133(12):2781-90.
19. Tai WT, Shiau CW, Chen HL, Liu CY, Lin CS, Cheng AL, et al. Mcl-1-dependent activation of Beclin 1 mediates autophagic cell death induced by sorafenib and SC-59 in hepatocellular carcinoma cells. Cell Death Dis. 2013;4:e485.
20. Manov I, Pollak Y, Broneshter R, Iancu TC. Inhibition of doxorubicin-induced autophagy in hepatocellular carcinoma Hep3B cells by sorafenib – The role of extracellular signal-regulated kinase counteraction. FEBS J. 2011;278(18):3494-507.
21. Mah LY, Ryan KM. Autophagy and cancer. Cold Spring Harb Perspect Biol. 2012;4(1):a008821.
22. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest. 2003;112(12):1809-20.
23. Nisreen AA, Dalia M, El-Rehim IM. Defective Beclin-1 and elevated hypoxia-inducible factor (HIF) -1α expression are closely linked to tumorigenesis, differentiation, and progression of hepatocellular carcinoma. Tumor Biol. 2015;36:364293-9.
24. Ding ZB, Shi YH, Zhou J, Qiu SJ, Xu Y, Dai Z, et al. Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma. Cancer Res. 2008;68(22):9167-75.
25. Kotsafti A, Farinati F, Cardin R, Cillo U, Nitti D, Bortolami M. Autophagy and apoptosis-related genes in chronic liver disease and hepatocellular carcinoma. BMC Gastroenterol. 2012;12:118.

26. Giatromanolaki A, Koukourakis MI, Harris AL, Polychronidis A, Gatter KC, Sivridis E. Prognostic relevance of light chain 3 (LC3A) autophagy patterns in colorectal adenocarcinomas. J Clin Pathol. 2010;63(10):867-72.
27. Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, et al. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. Cancer Sci. 2008;99(9):1813-9.
28. Yang JD, Seol SY, Leem SH, Kim YH, Sun Z, Lee JS, et al. Genes associated with recurrence of hepatocellular carcinoma: Integrated analysis by gene expression and methylation profiling. J Korean Med Sci. 2011;26(11):1428-38.
29. Lee YJ, Hah YJ, Kang YN, Kang KJ, Hwang JS, Chung WJ, et al. The autophagy-related marker LC3 can predict prognosis in human hepatocellular carcinoma. PLoS One. 2013;8(11):e81540.
30. Bao L, Chandra PK, Moroz K, Zhang X, Thung SN, Wu T, et al. Impaired autophagy response in human hepatocellular carcinoma. Exp Mol Pathol. 2014;96(2):149-54.
31. Adams JM, Difazio LT, Rolandelli RH, Luján JJ, Haskó G, Csóka B, et al. HIF-1: A key mediator in hypoxia. Acta Physiol Hung. 2009;96(1):19-28.
32. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: Novel insights on the functional interaction of HIFs and cell death pathways. Drug Resist Updat. 2011;14(3):191-201.
33. Xia L, Mo P, Huang W, Zhang L, Wang Y, Zhu H, et al. The TNF-a/ROS/HIF-1-induced upregulation of FoxMI expression promotes HCC proliferation and resistance to apoptosis. Carcinogenesis. 2012;33(11):2250-9.
34. Xiang ZL, Zeng ZC, Fan J, Tang ZY, Zeng HY, Gao DM. Gene expression profiling of fixed tissues identified hypoxia-inducible factor-1a, VEGF, and matrix metalloproteinase-2 as biomarkers of lymph node metastasis in hepatocellular carcinoma. Clin Cancer Res. 2011;17(16):5463-72.
35. Choi SH, Shin HW, Park JY, Yoo JY, Kim DY, Ro WS, et al. Effects of the knockdown of hypoxia inducible factor-1α expression by adenovirus-mediated shRNA on angiogenesis and tumor growth in hepatocellular carcinoma cell lines. Korean J Hepatol. 2010;16(3):280-7.
36. Gwak GY, Yoon JH, Kim KM, Lee HS, Chung JW, Gores GJ. Hypoxia stimulates proliferation of human hepatoma cells through the induction of hexokinase II expression. J Hepatol. 2005;42(3):358-64.
37. Chen W, Hill H, Christie A, Kim MS, Holloman E, Pavia-Jimenez A, et al. Targeting renal cell carcinoma with a HIF-2 antagonist. Nature. 2016;539(7627):112-7.
38. Wei K, Piecewicz SM, McGinnis LM, Taniguchi CM, Wiegand SJ, Anderson K, et al. A liver Hif-2a-Irs2 pathway sensitizes hepatic insulin signaling and is modulated by VEGF inhibition. Nat Med. 2013;19(10):1331-7.
39. Zhao D, Zhai B, He C, Tan G, Jiang X, Pan S, et al. Upregulation of HIF-2a induced by sorafenib contributes to the resistance by activating the TGF-a/EGFR pathway in hepatocellular carcinoma cells. Cell Signal. 2014;26(5):1030-9.
40. Yang SL, Liu LP, Niu L, Sun YF, Yang XR, Fan J, et al. Downregulation and pro-apoptotic effect of hypoxia-inducible factor 2 alpha in hepatocellular carcinoma. Oncotarget. 2016;7(23):34571-81.
41. Fluegen G, Avivar-Valderas A, Wang Y, Padgen MR, Williams JK, Nobre AR, et al. Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments. Nat Cell Biol. 2017;19:120-32.
42. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem. 2008;283(16):10892-903.
43. Azad MB, Gibson SB. Role of BNIP3 in proliferation and hypoxia-induced autophagy: Implications for personalized cancer therapies. Ann N Y Acad Sci. 2010;1210(1):8-16.
44. Band M, Joel A, Hernandez A, Avivi A. Hypoxia-induced BNIP3 expression and mitophagy: *In vivo* comparison of the rat and the hypoxia-tolerant mole rat, Spalax ehrenbergi. FASEB J. 2009;23(7):2327-35.
45. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouysségur J, et al. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. Mol Cell Biol. 2009;29(10):2570-81.
46. Fang Y, Tan J, Zhang Q. Signaling pathways and mechanisms of hypoxia-induced autophagy in the animal cells. Cell Biol Int. 2015;39(8):891-8.
47. Vara D, Salazar M, Olea-Herrero N, Guzmán M, Velasco G, Díaz-Laviada I. Anti-tumoral action of cannabinoids on hepatocellular carcinoma: Role of AMPK-dependent activation of autophagy. Cell Death Differ. 2011;18(7):1099-111.
48. Hu M, Huang H, Zhao R, Li P, Li M, Miao H, et al. AZD8055 induces cell death associated with autophagy and activation of AMPK in hepatocellular carcinoma. Oncol Rep. 2014;31(2):649-56.
49. Healy SJ, Gorman AM, Mousavi-Shafaei P, Gupta S, Samali A. Targeting the endoplasmic reticulum-stress response as an anticancer strategy. Eur J Pharmacol. 2009;625(1-3):234-46.
50. Suh DH, Kim MK, Kim HS, Chung HH, Song YS. Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. Ann N Y Acad Sci. 2012;1271:20-32.
51. Rouschop KM, Ramaekers CH, Schaaf MB, Keulers TG, Savelkouls KG, Lambin P, et al. Autophagy is required during cycling hypoxia to lower production of reactive oxygen species. Radiother Oncol. 2009;92(3):411-6.
52. Shi YH, Ding ZB, Zhou J, Hui B, Shi GM, Ke AW, et al. Targeting autophagy enhances sorafenib lethality for hepatocellular carcinoma via ER stress-related apoptosis. Autophagy. 2011;7(2):1159-72.
53. Shaid S, Brandts CH, Serve H, Dikic I. Ubiquitination and selective autophagy. Cell Death Differ. 2013;20(1):21-30.
54. Gustafsson AB. Bnip3 as a dual regulator of mitochondrial turnover and cell death in the myocardium. Pediatr Cardiol. 2011;32:267-74.
55. Kothari S, Cizeau J, McMillan-Ward E, Israels SJ, Bailes M, Ens K, et al. BNIP3 plays a role in hypoxic cell death in human epithelial cells that is inhibited by growth factors EGF and IGF. Oncogene. 2003;22(30):4734-44.
56. Cosse JP, Rommelaere G, Ninane N, Arnould T, Michiels C. BNIP3 protects HepG2 cells against etoposide-induced cell death under hypoxia by an autophagy-independent pathway. Biochem Pharmacol. 2010;80(8):1160-9.
57. Park CW, Hong SM, Kim ES, Kwon JH, Kim KT, Nam HG, et al. BNIP3 is degraded by ULK1-dependent autophagy via MTORC1 and AMPK. Autophagy. 2013;9(3):345-60.
58. Wan XB, Fan XJ, Chen MY, Xiang J, Huang PY, Guo L, et al. Elevated Beclin 1 expression is correlated with HIF-1alpha in predicting poor prognosis of nasopharyngeal carcinoma.

Autophagy. 2010;6(3):395-404.
59. Song YJ, Zhang SS, Guo XL, Sun K, Han ZP, Li R, et al. Autophagy contributes to the survival of CD133 liver cancer stem cells in the hypoxic and nutrient-deprived tumor microenvironment. Cancer Lett. 2013;339(1):70-81.
60. Marcucci F, Ghezzi P, Rumio C. The role of autophagy in the cross-talk between epithelial-mesenchymal transitioned tumor cells and cancer stem-like cells. Mol Cancer. 2017;16(1):3.
61. Zhu H, Wang D, Liu Y, Su Z, Zhang L, Chen F, et al. Role of the hypoxia-inducible factor-1 alpha induced autophagy in the conversion of non-stem pancreatic cancer cells into CD133+ pancreatic cancer stem-like cells. Cancer Cell Int. 2013;13(1):119.
62. Azad MB, Chen Y, Henson ES, Cizeau J, Ward EM, Sara J, et al. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. Autophagy. 2008;4(2):195-204.
63. Wu FQ, Fang T, Yu LX, Lv GS, Lv HW, Liang D, et al. ADRB2 signaling promotes HCC progression and sorafenib resistance by inhibiting autophagic degradation of HIF1a. J Hepatol. 2016;65:314-24.
64. Carew JS, Kelly KR, Nawrocki ST. Autophagy as a target for cancer therapy: New developments. Cancer Manag Res. 2012;4:357-65.
65. Song J, Qu Z, Guo X, Zhao Q, Zhao X, Gao L, et al. Hypoxia-induced autophagy contributes to the chemoresistance of hepatocellular carcinoma cells. Autophagy. 2009;5(8):1131-44.
66. Ding ZB, Hui B, Shi YH, Zhou J, Peng YF, Gu CY, et al. Autophagy activation in hepatocellular carcinoma contributes to the tolerance of oxaliplatin via reactive oxygen species modulation. Clin Cancer Res. 2011;17(19):6229-38.
67. Guo XL, Li D, Sun K, Wang J, Liu Y, Song JR, et al. Inhibition of autophagy enhances anticancer effects of bevacizumab in hepatocarcinoma. J Mol Med (Berl). 2013;91(4):473-83.
68. Zhou Y, Sun K, Ma Y, Yang H, Zhang Y, Kong X, et al. Autophagy inhibits chemotherapy-induced apoptosis through downregulating Bad and Bim in hepatocellular carcinoma cells. Sci Rep. 2014;4:5382.
69. Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, et al. BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). Autophagy. 2007;3(4):374-6.
70. Peng WX, Xiong EM, Ge L, Wan YY, Zhang CL, Du FY, et al. Egr-1 promotes hypoxia-induced autophagy to enhance chemo-resistance of hepatocellular carcinoma cells. Exp Cell Res. 2016;340(1):62-70.
71. Rzymski T, Milani M, Singleton DC, Harris AL. Role of ATF4 in regulation of autophagy and resistance to drugs and hypoxia. Cell Cycle. 2009;8(23):3838-47.
72. Chaachouay H, Fehrenbacher B, Toulany M, Schaller M, Multhoff G, Rodemann HP. AMPK-independent autophagy promotes radioresistance of human tumor cells under clinical relevant hypoxia *in vitro*. Radiother Oncol. 2015;116(3):409-16.
73. Peng WX, Wan YY, Gong AH, Ge L, Jin J, Xu M, et al. Egr-1 regulates irradiation-induced autophagy through Atg4B to promote radioresistance in hepatocellular carcinoma cells. Oncogenesis. 2017;6(1):e292.
74. Salazar M, Carracedo A, Salanueva IJ, Hernández-Tiedra S, Lorente M, Egia A, et al. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J Clin Invest. 2009;119(5):1359-72.
75. Dando I, Donadelli M, Costanzo C, Dalla Pozza E, D'Alessandro A, Zolla L, et al. Cannabinoids inhibit energetic metabolism and induce AMPK-dependent autophagy in pancreatic cancer cells. Cell Death Dis. 2013;4:e664.
76. Liu C, Gong K, Mao X, Li W. Tetrandrine induces apoptosis by activating reactive oxygen species and repressing Akt activity in human hepatocellular carcinoma. Int J Cancer. 2011;129(6):1519-31.
77. Gong K, Chen C, Zhan Y, Chen Y, Huang Z, Li W. Autophagy-related gene 7 (ATG7) and reactive oxygen species/extracellular signal-regulated kinase regulate tetrandrine-induced autophagy in human hepatocellular carcinoma. J Biol Chem. 2012;287(42):35576-88.
78. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016;1:15004.
79. Zhai H, Fesler A, Ju J. MicroRNA: A third dimension in autophagy. Cell Cycle. 2013;12(2):246-50.
80. Chang Y, Yan W, He X, Zhang L, Li C, Huang H, et al. miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. Gastroenterology. 2012;143(1):177-87.e8.
81. Xu N, Zhang J, Shen C, Luo Y, Xia L, Xue F, et al. Cisplatin-induced downregulation of miR-199a-5p increases drug resistance by activating autophagy in HCC cell. Biochem Biophys Res Commun. 2012;423(4):826-31.
82. Yuan P, Cao W, Zang Q, Li G, Guo X, Fan J. The HIF-2a-MALAT1-miR-216b axis regulates multi-drug resistance of hepatocellular carcinoma cells via modulating autophagy. Biochem Biophys Res Commun. 2016;478(3):1067-73.
83. Liu FY, Zhou SJ, Deng YL, Zhang ZY, Zhang EL, Wu ZB, et al. MiR-216b is involved in pathogenesis and progression of hepatocellular carcinoma through HBx-miR-216b-IGF2BP2 signaling pathway. Cell Death Dis. 2015;6:e1670.

How to cite this article: Kabir N, Yong Y. Hypoxia-induced autophagy in hepatocellular carcinoma and anticancer therapy. Natl J Physiol Pharm Pharmacol 2017;7(8):771-780.

Source of Support: Nil, **Conflict of Interest:** None declared.