Autophagy-Related Beclin 1 and Head and Neck Cancers

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Abstract: Beclin 1, a positive regulator of autophagy, behaves as a double-edged sword in tumorigenesis. Beclin 1 contributes to tumor suppression by removing defective or damaged organelles and other cellular components; however, its activity can also stimulate cancer initiation and progression. In head and neck cancer, Beclin 1 overexpression promotes autophagy, which limits DNA damage and chromosomal instability and increases necrosis and inflammation by impacting apoptotic and autophagic pathways. This paper reviews the relationship between Beclin 1, carcinogenesis and head and neck cancer prognosis.

Keywords: head and neck cancer, autophagy, Beclin 1, carcinogenesis

Introduction

Head and neck cancers are common malignant tumors, with most tumors being squamous cell carcinomas (SCCs); however, their pathogenesis, occurrence, development, and optimal treatment remain unclear. Tumor development is a multi-step, multi-gene mutation process. Multiple factors are also involved in the chemoradiotherapy resistance of malignant tumors.

Autophagy, a new focus of tumor research, plays an important role in tumorigenesis, development, and sensitivity to chemoradiotherapy. 1–3 Autophagy has a dual role; it is a barrier to prevent cancer invasion and inhibit cancer growth, and it is an adaptive response to the relatively harsh environment of cancer in promoting cancer progression. 4 Basic autophagy can maintain the survival of cancer cells, but excessive and continuously activated autophagy can activate self-degradation of cancer cells to lead to death 4 (Figure 1).

Beclin 1 is an essential autophagy protein, which was shown using a yeast two-hybrid screen to interact directly with the Bcl-2 protein. Beclin 1 was named in 1998 and is encoded by the Beclin 1 autophagy-related gene (BECN1). 5 Beclin 1 plays an important role in tumorigenesis. 1 However, Beclin 1 may be either a suppressor or supporter under different conditions. This article reviews the significance of Beclin 1 in head and neck cancers.

The Autophagy BECN1 Gene and Beclin 1 Protein

The human BECN1 gene is located on chromosome 17q21 and is highly homologous with the yeast autophagy gene, Atg-6. It was the first mammalian autophagy gene to be discovered and it was identified as a haploid-insufficient tumor suppressor. 6 BECN1 is important for the activity of the Class III phosphatidylinositol 3-kinase (P13KIII)/Beclin 1/JNL/VPS34 signaling pathway, and the encoded
Beclin 1 protein functions as the central adapter module within the PI3K-III complex. 6

Beclin 1 is a 60-kDa protein of 450 amino acids. It has three different functional domains. 7 The B cell CLL/lymphoma-2 (Bcl-2) homology 3 (BH3, amino acids 105–130) domain is located in a disordered N-terminal region and is sufficient for interaction with the anti-apoptotic family of proteins (Bcl-XL and Bcl-20). 8-16 The central coiled-coil domain (CCD) is a flexible helix domain containing amino acids 141–171 which binds to the protein encoded by the UV irradiation resistance-associated gene (UVRAG) and to PI3-III. 8-16 The central CCD can produce an inactive Beclin 1 homodimer or promote complex formation with Autophagy-related genes (ATG) 14 or UVRAG, which forms an active PI3K-III complex. The Beclin 1 BH3 domain interacts with BCL2/BCL-XL, which may stabilize the CCD-mediated Beclin 1 dimers, resulting in effective suppression of autophagosome biogenesis. 17 The third domain, an evolutionarily conserved domain (ECD, amino acids 175–264), also termed a β-α repeated autophagic-specific domain (BARA; amino acids 265–450), binds to PI3K-III and takes part in the binding of lipid membranes of cell organelles. 8-17 Beclin 1 may suppress cancer by coupling with lipid kinase rather than associating with BCL2. In addition, the ECD domain is essential for recruitment of VPS34, which not only induces autophagy but also leads to inhibition of tumors. 18 Bax-interacting factor-1 is also a positive regulator of Beclin 1 through interaction with UVRAG 18 (Figure 2).

Beclin 1 plays multifunctional roles in the cell (Figure 3). The main function of Beclin 1 is to regulate autophagy. It is involved in the initial stage of autophagolysosome formation, which means that Beclin 1 plays a crucial role in many biological cellular processes, including development, endocytosis, response to stress, aging, and cell death. Beclin 1 dysfunction may therefore cause many diseases. 7-9,19,20 There are two interesting paradigms regarding the function and mode of regulation of Beclin 1, involving the promotion of autophagy and suppression of autophagy. 21 Beclin 1 regulates autophagy through its phosphorylation. 9,19,20 Bcl-2 interacting mediator (Bim) is a member of the Bcl-2 family that only contains BH3, which interacts with Beclin 1 and binds Beclin 1 to dynein light chain 1 (DLC1; also called LC8), which is a component of microtubules. Once Bim is phosphorylated, Bim and Beclin 1 dissociate from DLC1 to induce autophagy. 22 Phosphorylation of Beclin 1 occurs at serine 15 in the N-terminal region through the action of kinases including unc-51-like kinase 1 (ULK1), phosphoglycerate kinase 1 (PGK1), death-associated protein kinase (DAPK3), mitogen-activated protein kinase-activated protein kinase 2/mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK2/MAPKAPK3), calcium/calmodulin-dependent protein kinase II (CaMKII), and adenosine 5’-monophosphate-activated protein kinase (AMPK); at

Figure 1 The dual roles of autophagy. Depending on the context and stage of cancer development, autophagy may play neutral, cancer-inhibiting, or cancer-promoting roles. In general, the function of autophagy is to suppress cancer at early stages of carcinogenesis by preventing cancer initiation, proliferation, growth, invasion, and metastasis. Impaired autophagy results in inadequate removal of intracellular components, damaged mitochondria, defective proteins and, ultimately, inflammation and microenvironment instability. Under these conditions, chemoradioresistance, invasion, metastasis, and a poor patient prognosis occur.
serine 30 via ULK1/2; in the BH3 domain via serine/threonine kinase-4 (STK4), DAPK1, DAPK2, and Rho associated coiled-coil containing protein kinase 1 (ROCK1); in the CCD domain via FAK; in the CCD, ECD-BARA domains via serine-threonine kinase (AKT) 1; in the CCD and BARA domains via epidermal growth factor receptor (EGFR) and BCR-ABL; and in the BARA domain via AMPK and Casein kinase 1 gamma 2 (CSNK1G2). Mst1 may inhibit autophagy by phosphorylating the BH3 domain of Beclin 1. Mst1 also induces apoptosis by enhancing its interaction with Bcl-2/Bcl-xl, which phosphorylates Beclin 1, leading to Bax activation. Akt phosphorylates Beclin 1, leading to Beclin 1 sequestration to the cytoskeleton and suppression of its function in autophagy. Some metabolites and metabolic stimuli also regulate autophagy, including nutrient starvation, glucose, amino acids, glutamine, lipids and free fatty acids, hypoxia, and reactive oxygen species.

**Beclin 1 and Tumorigenesis**

As previously mentioned, autophagy deficiency may be involved in a large number of diseases, including tumors. Dysfunctional Beclin 1 plays an important role in autophagy deficiency. Similar to the dual effects of autophagy, Beclin 1 has dual functions in tumorigenesis. It may impede tumor growth and progression by removing defective or damaged organelles and other cellular compounds.
or it may play a role in cancer initiation and progression by regulating autophagy.7

**Beclin 1 Affects Tumorigenesis and Tumor Development by Regulating Autophagy**

In 1999, Liang et al first found that Beclin 1 may inhibit the proliferation of breast cancer MCF7 cells to prevent tumorigenesis.27 Beclin 1 may be a haploid-insufficient tumor suppressor. Thus, defective Beclin 1 plays a role in tumorigenesis.27

**Beclin 1 as a Tumor Oncosuppressor**

Many studies have shown that increased Beclin 1 expression in a number of cancers may activate autophagy and inhibit tumorigenesis.5-31 Beclin 1 plays a role in the induction of autophagy. Monoallelic loss of BECN1 may cause the occurrence of some cancers, including breast, ovarian, and prostate cancer. In BECN1+/− mice, lymphoma, lung carcinoma, and liver carcinoma may occur spontaneously.5,28-31 Haploinsufficiency in Beclin 1 impairs autophagy and promotes tumorigenesis in mice, and Beclin 1 is monoallelically deleted in many breast, prostate, and ovarian tumors, indicating an effect on autophagy in tumor suppression.5,28,29,31 In a study of mammary tumorigenesis, allelic loss of BECN1 was involved in breast tumorigenesis by increasing DNA damage and genomic instability.33 In immortalized baby mouse kidney epithelial (iBMK) cells, autophagy in Beclin1+/− and Beclin1+/− iBMK cells was reduced, even under normal conditions.32 Autophagy induced in Beclin1+/− iBMK cells temporarily maintained homeostasis under stress in the background of a defect in apoptosis, and defective autophagy induced by Beclin 1+/− promoted tumorigenesis in iBMK cells. These results suggested that autophagy deficiency acts through a separate pathway to alleviate harsh stress in accordance with apoptosis to prevent death by necrosis.32

Some interactors of Beclin 1 may induce dissociation of the Beclin 1-Bcl-2 complex to reduce the Beclin 1/P13K-III complex, leading to promotion of autophagy. The Autophagy and Beclin 1 Regulator 1 (AMBRA1) gene, which encodes a Beclin 1 interactor (Ambr1), enhances autophagy in a positive feedback mechanism by promoting autophagosome formation by activating the PI3K-III complex.34 In mice, spontaneous tumors occur in the liver and lung after a monoallelic deletion of AMBRA1, with the absence of a well-structured fibrous capsule, which suggests tumor aggressiveness. High mobility group box 1 (HMGB1) may inhibit formation of the Beclin 1/Bcl-2 complex to activate autophagy, which exerts multiple functions in various cancers including lung cancer, hematological malignancies, breast cancer, and osteosarcomas.35 Mitosis (NIMA)-related kinase 2 (NEK2) may bind and stabilize Beclin 1 to enhance autophagy, and NEK2 combined with chloroquine (CQ) and chemotherapeutic bortezomib may prevent NEK2-induced drug resistance in multiple myeloma.36 Some glycolytic enzymes also regulate autophagy. Phosphoglycerate kinase 1 (PGK1) may phosphorylate Beclin 1 to induce autophagy. Under hypoxia or glutamine deprivation, PGK1 may cause the interaction between PGK1 and Beclin 1 to result in phosphorylation of Beclin 1 at serine 30, which activates the ATG14-associated PIk3C3 vacuolar protein sorting 34 (VPS34)-BECN1-PIK3R4/VPS15 complex to generate the initiation stage of autophagy, and support cell proliferation and survival during stress.37 In nucleophosmin (NPM1)-mutated acute myeloid leukemia (AML), pyruvate kinase isoenzyme M2 may increase the phosphorylation of Beclin 1 to activate autophagy, leading to cell survival and poor survival of patients with NPM1-mutated AML.38 Chen et al found that knockdown of Beclin 1 inhibited cisplatin-induced autophagy and increased cisplatin-induced apoptotic cell death in A549 lung cancer cells.39 It is also suggested that Beclin 1 is involved in the degradation of oncogenic proteins such as P53. Wild-type P53 protein interferes with oncosuppressive functions. Cancer cells with Beclin 1 deletion tend to accumulate increased amounts of mutant P53, whereas overexpression of Beclin 1 results in mutant P53 depletion. Wild-type P53 may inhibit malignant transformation.40

Beclin 1 expression in human solid cancers is decreased, which is associated with progression, invasion, and metastasis. Enhanced efficacy of anticancer therapies has been demonstrated by upregulating Beclin 1 expression to trigger autophagosome formation and accumulation.41 In some malignant tumors, decreased expression of Beclin 1 may enhance the therapeutic effect, although the underlying mechanism remains unclear.42,43 In the SGC-7901 gastric carcinoma, Beclin 1 binds to the N- and C-termini of lysosome-associated 4β (LAPTM4B) transmembrane protein, which competes with EGFR for LAPTM4B. Using this process, Beclin 1 can inhibit gastric cancer cell growth.42 In MDA-MB-231 triple negative breast cancer cells, Beclin 1 promotes cell proliferation and colony formation, while knockdown of Beclin 1 inhibits the migration and invasion of MDA-MB-231 cells.43
Beclin 1 Is Also an Oncosupportive Protein

Some studies have shown the opposite result that Beclin 1 acts as an oncogenic component in tumorigenesis. The expression of Beclin 1 in some cancers, including colorectal cancers and gastric carcinomas, is higher while there is little or no expression in normal mucosal cells. The expression of Beclin 1 shows no significant correlation with clinicopathological characteristics.44 These results suggest that high Beclin 1 expression plays a role in the progression of tumors. In this regard, Jung et al suggested that Beclin 1 plays an oncogenic role by increasing cell proliferation under harsh conditions such as hypoxia and nutrient starvation.45 Because Beclin 1 plays a role in protein sorting, in an oncogenic role, Beclin 1 might mediate the evasion of specific receptors from lysosomal degradation, thus downregulating receptor degradation.46

In some systems, the role of Beclin 1 in tumorigenesis may be independent of autophagy.34 Beclin 1 may bind to UVRAG, which is monoallelically mutated in a high percentage of gastric and colonic tumors, while UVRAG overexpression and Beclin 1 interdependently activate autophagy, and UVRAG increases Beclin 1-P13K III interaction and P13KC3 enzymatic activity.47,48 In addition, UVRAG contributes to genomic stability in an autophagy-unrelated manner.49 Although Bcl-2 has an anti-apoptotic effect, Oh et al found that Bcl-2-binding to Beclin 1 was independent of its interaction with proapoptotic Bcl-2 protein in MCF7 breast cancer cells, and Bcl-2 mutants inhibited Beclin 1-dependent autophagy to induce tumorigenesis.50 In normal NCM-460 colon epithelial cells, 0.2 mM deoxycholic acid induced a significant decrease in the levels of Beclin 1 protein expression mediated through activated oxidative stress, which induced autophagy that activated a pro-survival pathway to play a role in colon carcinogenesis.51

Beclin 1 Affects Tumorigenesis and Tumor Development by Regulating Apoptosis

Beclin 1 also has non-autophagy functions. These include endocytosis, protein targeting/protein sorting, cytokinesis, and cell death.45 Beclin 1 may therefore regulate cell death in an autophagy-independent manner, such as by apoptosis. Apoptosis regulated by Beclin 1 may also play an important role during tumorigenesis and tumor development.

It has been suggested that mutual interaction between apoptosis and autophagy plays a novel role in tumorigenesis and progression.52 Beclin 1 plays an important role in this mutual process. Crosstalk between autophagy and apoptosis is complicated. Beclin 1 is a bridge linking autophagy, apoptosis, and differentiation. The BH-3 domain in Beclin 1 binds to both Bcl-2 and Bcl-xL.12 Once the BH-3 domain interacts with Bcl-2, the binding impedes Beclin 1-mediated autophagy, which might be considered as a convergence of apoptosis and autophagy. Simultaneous dysfunction of apoptosis and autophagy may cause accelerated DNA damage and genomic instability that promotes tumorigenesis.53 In gastric carcinomas, Zhou et al found that Beclin 1 showed low expression in four gastric carcinoma cell lines (MKN-45, MKN-28, BGC-823, and SGC-7901), while Bcl-xL showed high expression. They also found that Beclin 1 was moderately expressed in 153 gastric carcinoma tissues but was strongly expressed in the normal adjacent gastric mucosal tissues, as detected by immunohistochemistry (IHC) and Western blotting. However, the expression of Bcl-xL was high in these gastric carcinoma tissues compared to the normal adjacent gastric mucosal tissues.52 Low Beclin 1 expression was associated with poor differentiation and poor prognoses of gastric carcinomas. It has been shown that Beclin 1 overexpression in human gastric cancer (MKN28) enhances apoptosis triggered by cis-diaminedichloroplatinum (CDDP).54 Oxaliplatin and bortezomib significantly inhibit the growth of colorectal cancer by activating apoptosis by inducing the JNK-Bcl-Xl-Bax pathway and dissociating Beclin 1 from Bcl-xL.55 In gastric carcinomas, Wang et al used IHC to show that Beclin 1 expression in tissues of gastric carcinomas was significantly decreased when compared to para-carcinoma tissues.56 Beclin 1 expression was negatively associated with the TNM stage and invasion status. In MKN-45 gastric carcinoma cells, they also found that high expression of Beclin 1 might enhance apoptosis. They suggested a possible mechanism where Beclin 1 may inhibit the anti-apoptotic factor Bcl-xL to induce the apoptosis pathway, as well as the mutual effect of autophagy and apoptosis on gastric tumorigenesis.56 Shi et al detected Beclin 1 expression in seven hepatocellular carcinoma (HCC) cell lines (Hep G2, Hep3B, SMMC-7721, MHCC97-L, MHCC97-H, HCCLM3, and GCCLM6).57 They found that Beclin 1 expression in all HCC cell lines was low, and Beclin 1 expression was significantly lower in high-grade malignant HCCs. They also found that Beclin 1 expression was absent in 68.3% (205/300) of HCCs and patients with positive Beclin 1 expression had a good prognosis. Further study showed that the prognostic value of Beclin 1 expression was only significant
in the Bcl-X<sub>L</sub> positive expression background. Thus, they suggested that there was a relationship between the autophagy defect and poor prognosis and apoptosis. In A549 human lung cancer cells, Li et al reported that cadmium may lead to both autophagy and apoptosis. This process was facilitated by Atg 4B binding to Bcl-2 to cause Beclin 1 release from the Bcl-2/Beclin 1 complex and thereby complete the Cd-induced switch from apoptosis to autophagy.

However, Beclin 1 small interfering RNA (siRNA) decreases CDDP cytotoxicity. This mechanism is related to caspase activity. Caspases are cysteine aspartyl proteases, which play a key role in apoptosis. Caspases cleave Beclin 1 during apoptosis, resulting in destruction of its pro-autophagic activity. Beclin1 siRNA markedly decreased apoptosis in cervical HeLa cells, while overexpression increased apoptosis and autophagy. Beclin 1 also regulates caspase-9 and overexpression of Beclin 1 can result in tumor regression in vivo. It has been suggested that Beclin 1 may be the key molecular switch involved in mutual tuning of autophagy and apoptosis by modulation of caspase-9, which is an important mechanism in tumorigenesis of cervical carcinomas.

**Beclin 1 and Head and Neck Cancers**

Head and neck cancers are heterogenous tumors that are considered to be promoted by excessive alcohol and cigarette consumption. Although advances in therapeutic methods have been improved, the outcomes regarding novel approaches have been relatively limited. As previously mentioned, Beclin 1 plays an important role in tumorigenesis; however, studies of Beclin 1 involvement in head and neck cancers are relatively limited. From the results of these studies, the key roles of Beclin 1 in head and neck are summarized as follows.

**Beclin 1 Is Involved in Carcinogenesis of Head and Neck Cancers**

**Beclin 1 Overexpression Inhibits Carcinogenesis of Head and Neck Cancers**

In many cancers, overexpression of Beclin 1 plays an oncosuppressive role. In esophageal adenocarcinoma (EAC), Beclin 1 expression in normal HET-1A esophageal epithelium cells (derived from normal squamous epithelium) and nondysplastic Barratt’s esophagus (BE) cells detected by IHC, immunoblotting, and real-time polymerase chain reaction (RT-PCR), was high, whereas Beclin 1 expression was low in EACs. Acute bile acid upregulates Beclin 1 expression and chronic bile acid stimuli decrease Beclin 1 expression. These results suggested that Beclin 1-mediated autophagy was initially upregulated during premalignant stages of esophageal carcinogenesis and then decreased during the EAC transition. In thyroid cancer, another study showed that Beclin 1 had autophagy-independent anticancer effects on the exposure of thyroid cancer cells (FRO82-1, KTC1, KTC3, 8305C, and 8505C) to the proteasome inhibitor, MG132. Beclin 1 is also a marker of papillary thyroid carcinoma (PTC). Beclin 1 expression was positive in 98.9% of 97 PTC cases, whereas Beclin 1 expression was positive in 57.1% and 21.4% of follicular carcinomas and follicular adenomas, respectively. Cancer-associated fibroblasts (CAFs) play an important role in carcinogenesis, progression, and metastasis of cancers. CAFs induced resistance to cisplatin in SCC9 and CAL27 tongue squamous cell carcinoma (TSCC) cells. The mechanism involved the cisplatin-induced overexpression of Beclin 1 and microtubule-associated protein light chain 3-II (LC3-II) after SCC9 or CAL27 cells were co-cultured with CAFs. Inhibition of Beclin 1 by siRNA or CQ reduced the level of autophagy, resulting in the promotion of cytotoxicity of cisplatin on TSCC cells. Expression of Beclin 1 together with LC3-II and sequestosome 1 (p62) showed an inverse relationship with precancerous stages in the same section of 19 tissues of patients with laryngeal carcinomas (the same section included normal epithelium, hyperplastic epithelium, dysplastic epithelium, and invasive SCCs), whereas there was a parallel relationship in laryngeal carcinomas. The authors reviewed past reports and suggested that nucleocytoplasmic translocation of Beclin 1 was associated with its autophagic function and tumor suppressive roles.
of 4QQ-induced tongue carcinogenesis. The authors also found that Beclin 1 expression was associated with the number of regulatory T cells (Tregs) in mice peripheral blood and spleens, while LC3B and p62 were associated with myeloid-derived suppressor cells. These results suggested that autophagy was associated with tumor inflammation during tongue carcinogenesis.66 In esophageal cancer cells, autophagy was suggested to play a cytoprotective role in stressed EC109 cells. Inhibition of Beclin 1 by siRNA suppressed the autophagy level induced by tunicamycin, resulting in the promotion of apoptosis.67

Notably, Obatoclax, a pan-Bcl-2 inhibitor, induced autophagy, and had a cytoprotective role in adenoid cystic carcinoma cell line ACC-M.68 The induced autophagy by Obatoclax was dependent on activation of Beclin 1 and ATG5. Knockdown of Beclin 1 or ATG5 by siRNA significantly inhibited caspase-3 cleavage, resulting in decreased cell death induced by Obatoclax. When apoptosis was inhibited by benzylxoyacarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (ZVAD-fmk), Beclin 1 siRNA or ATG5 siRNA still decreased the toxicity of Obatoclax. The possible mechanism may involve Beclin 1 and ATG5 playing dual roles in autophagy and apoptosis.68

Thus, there are multiple mechanisms by which Beclin 1 is involved in carcinogenesis of head and neck cancers: (1) Beclin 1-mediated autophagy can limit DNA damage and chromosomal instability;69 (2) impairment of both autophagy and apoptosis increases necrosis and inflammation, and inflammatory cytokines are correlated with cancer development;70 and (3) chronic bile acid stimuli decrease Beclin 1 expression resulting in promotion of genomic instability and cancer progression.61

Abnormal Expression of Beclin 1 Affects Proliferation, Growth, Invasion, Metastasis, and Prognosis of Head and Neck Cancer Cells

Many Studies Have Demonstrated That Low or Absent Expression of Beclin 1 Is Associated with Cancer Progression and a Poor Prognosis

Laryngeal carcinoma: Using IHC, we found that Beclin 1 expression was lower in 44 head and neck cancer tissues when compared with paracarcinomaous tissues. Beclin 1 expression was negatively correlated with hypoxia-inducible factor-1α (HIF-1α) and Ki67. However, Beclin 1 expression was not significantly associated with clinicopathological features.71 Using IHC, Beclin 1 expression in 82 laryngeal carcinoma tissues (53.66%) was shown to be significantly lower than in paracarcinoma non-tumor tissues (72.5%). Absence of Beclin 1 expression was associated with increased lymph node metastasis, and low expression of Beclin 1 correlated with poor prognoses of laryngeal carcinomas.72

Hypopharyngeal carcinoma: Beclin 1 expression in hypopharyngeal squamous cell carcinomas (HSCCs) was significantly lower (42.7%) in 82 samples from patients with HSCC as detected by IHC when compared with that in adjacent non-cancerous tissues (79.6%, p < 0.001). RT-PCR analysis also showed similar results. Absence of Beclin 1 expression was also associated with poorer overall survival in patients with HSCC, and Beclin 1 was an independent prognostic factor for overall survival.73

Oral carcinoma: In 195 cases of oral cancer, Beclin 1 and Beclin 2 expression was localized in the cytoplasm ranging from a total absence to diffuse strong expression and was occasionally localized in the nuclei. There was a weak correlation between nuclear and cytoplasmic expression levels of Beclin 1.74 Beclin 1 expression was not associated with clinicopathological features. High expression of cytoplasmic Beclin 1 was associated with low disease survival, and absence of nuclear expression of Beclin 1 was associated with recurrence-free survival.74 In vitro, Beclin 1 and Beclin 2 were mainly localized in the cytoplasm of HEK293 oral cancer cells. Overexpression of Beclin 1 or Beclin 2 activated autophagy and promoted cancer cell growth, but the effects were independent of each other.5,74 In 90 primary oral squamous cell carcinomas (OSCCs), Beclin 1 expression was significantly associated with tumor grade and lymph node metastasis, and high expression of Beclin 1 was associated with a lower survival rate.75 Beclin 1 mRNA levels in 10 oral cancers were approximately eight times lower than in normal tissues.76 Beclin 1 expression was increased or decreased in TSCC SCC-9 and SCC-15 cells by transfection of lentivirus Beclin 1 and Beclin 1 short hairpin RNA (shRNA), respectively. Overexpression of Beclin 1 inhibited the proliferation, migration, and invasion of SCC-9 and SCC-15 cells, while decreased Beclin 1 promoted the proliferation, migration, and invasion of SCC-9 and SCC-15 cells. This mechanism may have involved Beclin 1 regulation of the production of VEGF, MMP-2, and MMP-9 in SCC-9 and SCC-15 cells.77 In TSCC cells, Beclin 1 expression in 14 TSCC tissues and five TSCC cell lines detected by RT-PCR and Western blotting was significantly decreased.78 Beclin 1 expression detected by
IHC was also decreased in 133 TSCC specimens. The decreased expression of Beclin 1 was associated with poor differentiation, lymph node metastasis, advanced clinical TNM, and a poor five-year overall survival. In 74 OSCCs, the positive expression of Beclin 1 was 27.4%, which was significantly associated with lymphatic invasion and differentiation. In OSCC KB cells, the expression of Beclin 1 was high. 3-methyladenine (3-MA) significantly inhibited Beclin 1 expression and increased cell death and apoptosis of KB cells under nutrient depletion conditions. The authors suggested that autophagy was dependent on Beclin 1 (canonical autophagy) apoptosis and that Beclin 1-mediated autophagy involved cross-talk in KB cells, which constituted a negative correlation. The mechanism might be that Beclin 1 suppressed the transformation of TSCC cells, colony formation, and growth by acting as a tumor suppressor. Beclin 1-independent noncanonical activity was found in TSCC TCA-8113 and CAL-27 cells. Erlotinib, a small-molecule EGFR tyrosine kinase inhibitor used clinically for many cancers, induced autophagy (increasing the LC3-II/I ratio, which did not affect Beclin 1 expression) in tongue cancer cells. Induced autophagy by erlotinib resulted in erlotinib resistance in TCA-8113 and CAL-27 cells. HCQ (an inhibitor of the fusion of autophagosomes with lysosomes) combined with erlotinib treatment significantly decreased cell viability. However, 3-MA (an inhibitor of PI3K, which inhibits by blocking the activity of the Beclin 1-Vps34/class-III-PI3K complex) combined with erlotinib treatment showed less effect when compared with the effect of erlotinib on TCA-8113 and CAL-27 cells. These data suggested that erlotinib resistance was independent of the Beclin 1 noncanonical autophagy pathway, but dependent on the Atg5 autophagy pathway. InTSCC, Beclin 1 and LC3-II showed lower expression levels using IHC in 50 TSCC samples when compared with adjacent noncancerous epithelial cells. Low Beclin 1 expression was associated with higher tumor stage, higher clinical stage, and greater differentiation. In vitro inhibition of Beclin 1 by 3-MA decreased autophagy levels, resulting in increasing proliferation, migration, and invasion in OTSCC Tca8113 cells.

Thyroid carcinoma: Beclin 1 and ARHI, which is encoded by an anti-oncogene involved in tumorigenesis and modulation of autophagy activity, were detected by Western blotting in 80 tissues of patients with thyroid cancer. The expression levels of Beclin 1 and ARHI were significantly lower in thyroid cancer than in adjacent tissues. Lower Beclin 1 expression was associated with pathological stage, high pathological differentiation, and lymph node metastasis. Lower expression levels of Beclin 1 and ARHI were associated with poor prognoses. Beclin 1 expression was positively correlated with ARHI expression. The mechanism was studied in thyroid cancers. Knockdown of Beclin 1 expression by shRNA in FRO and KTC3 thyroid cancer cells promoted the invasive and migratory activities of cancer cells by upregulating N-cadherin and downregulating E-cadherin to induce the EMT. These onco-suppressor functions of Beclin 1 were mediated by posttranscriptional modulation of ZEB1 through the AU-binding factor 1 (AUF1) in thyroid cancer. ZEB1 is a crucial regulator of the EMT and AUF1. In sporadic medullary thyroid carcinoma (MTC), Beclin 1 expression was associated with the development of residual MTC; however, it was not associated with tumor size, extrathyroidal extension, or nodal metastasis. The expression levels of Beclin 1 and LC3-II detected in 50 papillary thyroid carcinomas (PTCs) by IHC and Western blotting were significantly decreased when compared with normal tissues, and Beclin 1 and LC3-II were associated with lymph node metastasis of PTC. In 86 PTCs, the expression levels of Beclin 1 in PTC and metastasis were higher than in normal tissues adjacent to tumors, distant normal tissues, and normal lymph nodes. Beclin 1 was significantly correlated with tumorigenesis and lymph node metastasis in human PTCs. Beclin 1 and HMGB1 expression levels were high in five thyroid cancer cell lines (K1, KTC-1, TPC-1, FTC-133, and FRO) and thyroid cancer tissues, but not in tissues derived from thyroid benign lesions. HMGB1 knockdown by shRNA downregulated the levels of LC3-II and Beclin 1 and increased p62 levels, which supported a key regulatory role for HMGB1 in autophagy.

Salivary adenoid cystic carcinoma (ACC): In ACC, Beclin 1 expression was also a prognostic factor. In 89 ACCs, Beclin 1 expression detected by IHC and RT-PCR was lower in ACC tissues than in normal salivary gland tissue samples. Low Beclin 1 expression was associated with distant metastasis and poor prognosis. In 79 ACCs, Beclin 1, along with low expression of LC3, was significantly associated with a progressive histological growth pattern, high histological grade, and poor survival.

Nasopharyngeal carcinoma (NPC): ANXA1 is a calcium-dependent phospholipid binding protein that was initially characterized with phospholipase A2-inhibitory and anti-inflammatory activities, which may regulate BECN1 and...
LC3-II. Upregulated BECN1 and LC3-II along with autophagy-associated protein Sequestosome-1 (SQSTM1), which is an autophagic substrate that can be degraded by autophagy, was detected in ANXA1 knockdown 5-8F nasopharyngeal carcinoma cells. Conversely, overexpression of ANXA1 decreased BECN1 and LC3-II expression levels along with elevation of SQSTM1 levels. Inhibition of BECN1 by siRNA or 3-MA in ANXA1 knockdown NPC cells led to NPC cell migration, invasion, and metastasis by activating the PI3K/Akt pathway. Pretreatment with angiotensin-(1-7) [Ang-(1-7)] inhibited cell proliferation, migration, and invasion in NPC-TW10 cells. The mechanism involved upregulation of Beclin 1 expression and downregulation of Bcl-2 expression, which led to increased autophagy.

Esophageal carcinoma: Beclin 1 expression detected by IHC in 54 specimens of primary esophageal squamous cell carcinomas (ESCCs) was significantly lower than in normal epithelial cells of the esophagus. The decreased Beclin 1 expression was associated with decreased invasion, lymph node metastasis, and clinical stage. ESCC patients with overexpressed Beclin 1 had a good prognosis. In this study, Beclin 1 expression was associated with HIF-1α expression, which activated metabolic and pathogenic pathways leading to tumorigenesis, growth, invasion, and metastasis. Wei et al also reported decreased Beclin 1 expression with progression from normal tissues to BE with dysplasia, and in 115 EAC biopsies from 51 patients. They found that Beclin 1 loss in EACs was associated with advanced cancer stage and grade. They suggested that Beclin 1 might be a prognostic indicator. Further mechanistic studies showed that Beclin 1-dependent and Beclin 1-independent autophagy (noncanonical pathway) involved in suppressing progression of EACs. C-PAC, a cranberry proanthocyanidin extract, reduced Beclin 1 expression and increased LC3-II expression, resulting in a noncanonical autophagy-activated pathway. C-PAC reduced Beclin 1 through dephosphorylation at serine 15, serine 93, and serine 234, resulting in the death of EACCJHAD1 and OE19 cells. Rapamycin increased Beclin 1 levels by phosphorylation of Beclin 1, resulting in long-term cell survival. These results showed that Beclin 1 functioned as an onco-suppressor.

High Expression of Beclin 1 Was Associated with Cancer Progression and Poor Prognosis

Another study showed that high expression levels of Beclin 1 and LC3 detected by IHC and Western blotting in 150 samples from patients with II-IVa ESCC were associated with poor survival. Elevated Beclin 1 expression detected by IHC and Western blotting in 128 advanced NPC was associated with poor overall survival and progression-free survival. Moreover, Beclin 1 was also closely correlated with HIF-1α expression, and elevated Beclin 1 expression predicted poorer prognoses in a subset of NPC patients with positive HIF-1α expression. The authors explained the mechanism of elevated Beclin 1 expression and predicted that opposite prognosis results in different solid cancers might result from different intrinsic properties and the nature of the therapeutic regimen in different cancers. In addition, nuclear Beclin 1 had nonautophagic functions in DNA repair according to the results of Havaki et al, who reported that Beclin 1 expression in the nucleus in successive stages of laryngeal carcinogenesis was gradually decreased, which might indicate an impaired DNA repair process that increases genomic instability.

Chemoresistance: In NPC, Beclin 1 and LC3-II/lc3-I were highly expressed in tissue samples of NPC and C666-1 NPC cells after cisplatin treatment, which led to upregulated autophagy. The authors suggested that increased autophagy induced resistance to cisplatin-mediated cell death. They also found that inhibition of Beclin 1 by CQ improved the sensitivity to cisplatin by reducing cell viability and promoting cell apoptosis. Cisplatin is the main chemotherapeutic treatment for hypopharyngeal carcinoma. CQ, an anti-malarial and anti-rheumatoid drug, is also known as an inhibitor of autophagy. CQ suppressed autophagy and promoted apoptosis in cisplatin-treated mice bearing human hypopharyngeal carcinoma FaDu cells. Inhibition of Beclin 1 by shRNA also had a similar effect to CQ on mice. In ESCC, O’Donovan et al found that knockdown of Beclin 1 by siRNA only reduced cell viability at the early stage of ESCC KYSE450 cells after 5-FU treatment. Combined knockdown of Beclin 1 with ATG7 by siRNA significantly enhanced the anticancer
effect of 5-FU. They suggested that Beclin 1 played a cytoprotective role, facilitating recovery at an early stage of autophagy. In salivary gland ACC, inhibition of Beclin 1 by siRNA or 3-MA decreased autophagy, which enhanced the cytotoxicity of cisplatin in ACC-M cells.

MicroRNAs (miRNAs), a class of endogenous RNAs, consisting of 19–25 nucleotides of non-coding RNA, have been shown to regulate autophagy, which leads to chemoresistance. Decreased levels of miR-30d may affect cisplatin in SW 1736 and 8305C human anaplastic thyroid carcinoma (ATC) cell lines mediated by overexpression of Beclin 1 and induction of autophagy. miR-30d negatively modulated Beclin 1 expression in ATC cells, and an miR-30d mimic significantly enhanced the sensitivity of ATC cells to cisplatin; however, induced expression of Beclin 1 rescued the downregulation of autophagy induced by the miR-30d mimic.

In Hep-2 laryngeal carcinoma cells, overexpression of Beclin 1 after transfection with Beclin 1 did not affect cellular proliferation and apoptosis. After cisplatin treatment, overexpression of Beclin 1 decreased survival and increased apoptosis, and increased the sensitivity of Hep-2 cells to cisplatin as a result of Bcl-2-regulated autophagy. Beclin 1-mediated autophagy might protect the growth of laryngeal carcinoma Hep-2 cells and increase resistance to cisplatin treatment, whereas inhibition of Beclin 1 by siRNA could increase the cytotoxic sensitivity to cisplatin treatment by promoting cisplatin-mediated apoptosis via upregulation of caspase-3 and caspase-9.

Radioresistance: HMBG1 binding to Beclin 1, assessed by co-immunoprecipitation, was increased in SCC15 radioresistant oral squamous carcinoma cells. HMBG1 is an important inducer of autophagy. When HMBG1 interacts with Beclin 1, it promotes the formation of a Beclin 1-PI3KIII complex to increase the level of autophagy, which may lead to chemoradioresistance. Low power laser irradiation (LPLI) induced Beclin 1-mediated autophagy in OECM-1 and Ca9-22 oral cancer cells, resulting in LPLI resistance. LPLI induced the formation of reactive oxygen species (ROS) and RelA, which is a major member of the canonical NF-kB pathway, and which elicited BECN1 gene expression. Furthermore, ROS might promote the DNA binding activity of RelA-enhancing Beclin 1 expression in oral cancer cells.

Targeted Beclin 1 as an Adjuvant for Other Anti-Cancer Therapies

Some therapeutic agents and sensitizers may regulate Beclin 1 expression to affect autophagy-inducing apoptosis of head and neck cancer cells.

Taxol: Inhibition of Beclin 1 by siRNA decreased autophagy to improve the effect of Taxol on 5-8F/Taxol Taxol-resistant NPC cells. A possible mechanism may be that Beclin 1 siRNA upregulates caspase-1 and IL-1β mRNA levels, as well as the levels of cleaved gasdermin D (GSDMD) and activated caspase-1. GSDMD is a canonical pyroptosis executor. Pyroptosis, a novel programmed cell death process that is initiated by inflammatory caspases, was downregulated by autophagy by suppressing the caspase-1/GSDMD pathway. In another study, Taxol induced autophagy in NPC CNE-1, HNE-2, CNE-1/Taxol, and HNE-2 Taxol cells. Inhibition of autophagy by 3-MA and Beclin 1 siRNA increased the effect of Taxol on NPC Taxol-resistance in CNE-1/Taxol and HNE-2/Taxol cell lines. In this study, inhibition of autophagy enhanced the Taxol sensitivity of NPC cells mainly through increased apoptosis via promotion of cleaved caspase-3 expression.

Brazilin: Brazilin is a purified natural product from sappan wood (Caesalpinia sappan L), which has antiarcinogenic activity in human head and neck squamous cell carcinoma (HNSCC) Cal27 cells. Brazilin might induce apoptosis and autophagy. Beclin 1 siRNA sensitized Ca127 cells to brazilin-induced apoptotic cell death by a mechanism involving activation of the NF-kB p65 pathway, showing that Beclin 1 had a cytoprotective effect.

Tetrandrine: Tetrandrine is a bisbenzylisoquinoline alkaloid, which induces oral cancer CAL 27 cell death by inducing apoptosis and autophagy. Inhibition of Beclin 1 and Atg5 by siRNA decreased the cytotoxic activity of tetrandrine.

Luminacin: Luminacin is a metabolite from marine Streptomyces species, which inhibits cell growth and decreases the viability, migration, and invasive activities of HNSCC cell lines (SCCQLL1, SCC15, SCC25, SCC1483, MSKQLL1, and HN6). The results of this study showed that cytotoxicity induced by Tetrandrine was not caused by apoptosis or necrosis, but by autophagy through increased levels of Beclin 1 and LC3BI/II.

Puquitinib mesylate: Puquitinib mesylate (XC-302) is a molecular-targeted drug, which directly suppresses the
activity of PI3K. XC-302 inhibits the viability of and induces autophagy in NPC CNE-2 cells. Beclin 1 siRNA or 3-MA significantly promoted the survival and cloning of CNE-2 cells. The authors suggested that induced autophagy may be a survival disadvantage of NEC-2 cells.\textsuperscript{114}

Areca nut extract (ANE): Although the Areca nut is a common carcinogen, ANE and its 30–100 kDa fraction (ANE 30–100K) significantly induced autophagic cell death in both normal and malignant cells.\textsuperscript{115} Inhibition of Beclin 1 by an shRNA fragment as well as knockdown of ATG5 improved ANE30–100k-induced autophagy in head and neck cancer OECM-1/CE81T/VGH/Jurkat T and OE CM-1/SCC25/SCC-15 cells. Inhibition of Beclin 1 also induced apoptosis, resulting in cell death.\textsuperscript{116}

Erlotinib: Erlotinib is an inhibitor of the EGFR. It decreased cytotoxicity over time in both FaDu and Cal27 HNSCC cells and induced autophagy. CQ, Beclin 1 siRNA, or Atg5 siRNA combined with erlotinib increased the sensitivity to erlotinib in both FaDu and Cal27 HNSCC cells.\textsuperscript{117}

Parthenolide: Parthenolide inhibits growth of MDA-T32 thyroid cancer cells and mouse xenograft tumors in vivo through increased expression of the autophagic proteins, LC3-II and Beclin 1.\textsuperscript{118}

Polygonum cuspidatum (Hu Zhang), which is a traditional Chinese medicine, reduces the viability of oral cancer cisplatin-resistant CAR cells by simultaneously inducing autophagy by increasing protein levels of ATGs, Beclin 1, and LC3-II, resulting in apoptosis.\textsuperscript{119}

Buddleja officinalis: \textit{Buddleja officinalis} (BO) extract, which is a sensitizer to photodynamic therapy, may induce cell death of FaDu hypopharyngeal carcinoma cells.\textsuperscript{120} The study showed that BO upregulated autophagy by increasing ATG5, Beclin 1, and LC3-II expression levels and decreased mTOR expression and promoted apoptosis by increasing Bax protein expression and decreasing Bel-2 expression. This study also showed that crosstalk occurred between autophagy and apoptosis, and that activated autophagy occurred prior to activated apoptosis.\textsuperscript{120}

Flavokawain B (FKB): Flavokawain B is a natural kava chalcone, which inhibits growth, migration, and invasion, and induces apoptosis of Tca thyroid cancer cells. During cytotoxicity of FKB, FKB induced autophagy by increasing LC3B-II and Beclin 1 expression and decreasing p62 expression.\textsuperscript{121} In this study, Beclin 1 was activated by activating AMPK in a similar manner as in other studies. The antitumor effect of FKB was improved by inhibition of autophagy through 3-MA or cotreatment with Beclin 1 siRNA and ATG5 siRNA.\textsuperscript{121}

CZ415: CZ415 is a highly selective oral mTOR kinase inhibitor, which may have an anticancer effect on HNSCC cell lines (SCC-9, AQ20B, and A253) through inhibition of cell survival and proliferation. In this process, CZ415 upregulated Beclin 1, ATG5, ATG7, and LC3B-II, and degraded p62, resulting in autophagy.\textsuperscript{122} Beclin 1 knockdown by shRNA increased the CZ415-induced anticancer activity. In thyroid carcinomas, inhibition of autophagy by Beclin 1 siRNA or 3-MA increased the sensitivity of TPC-1 cells to CZ415.\textsuperscript{123}

Inhibition of Beclin 1 may increase the antitumor effect obtained by suppressing long non-coding RNA (lncRNA). The lncRNA HOX transcript antisense RNA (lncRNA HOTAIR), which is localized in the endonuclear region, is a functional RNA molecule that contains more than 200 nucleotides and is upregulated in many cancers. HOTAIR siRNA inhibited proliferation, migration, and invasion of oral SCC CAL-27 cells by decreasing the level of autophagosomes and the expression of Beclin 1, ATG5, ATG7, and LC3B-II.\textsuperscript{124}

Conclusions and Future Prospects

The pathogenesis, occurrence, development, and optimal treatment of head and neck cancers are still unclear. It has been shown that Beclin 1 is involved in carcinogenesis of head and neck cancers. There are multiple mechanisms involved in Beclin 1 carcinogenesis of head and neck cancers, including Beclin 1-mediated autophagy, which limits DNA damage and chromosomal instability, while impairment of both autophagy and apoptosis increases necrosis and inflammation. Increased levels of inflammatory cytokines are correlated with cancer development; and chronic bile acid stimuli decrease Beclin 1 expression, resulting in promotion of genomic instability and cancer progression. Abnormal Beclin 1 expression was associated with chemo-radiotherapy resistance in head and neck cancer. In combination with our previous studies,\textsuperscript{125–128} we will continue to investigate the role of Beclin 1-mediated autophagy in laryngeal carcinoma as well as the role of Beclin1 in glucose transporter-1 (GLUT1)-mediated radioresistance. Furthermore, future studies should be designed to test the effects of Beclin1 activity on GLUT1 expression as a pathway to improve the radiosensitivity of laryngeal carcinoma.

Disclosure

The authors report no conflicts of interest in this work.
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