Targeting autophagy in neuroblastoma

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ABSTRACT

Background Neuroblastoma (NB) is the most common extracellular solid tumor among children accounting for serious mortality. Macroautophagy, a common housekeeping mechanism to maintain cellular homeostasis in eukaryotic cells, is involved in tumorigenesis and chemoresistance in a spectrum of cancers. Autophagy is controlled by a highly regulated set of molecular mechanisms, and it involves evolutionarily conserved genes called autophagy related (ATG) genes. This process is typically divided into several distinct stages: initiation, nucleation of the autophagosome, expansion and elongation of the autophagosome membrane, closure and fusion with the lysosome. The process is completed with degradation of intravesicular contents. Initiation begins with the formation of phagophore, which is activated by the negative regulation of serine/threonine kinase (mammalian target of rapamycin (mTOR)). Following the formation of autophagosomes, ulk1-atg13-fip200-atg101 protein kinase complex and two ubiquitin-like binding systems, namely atg12-atg5/atg16 complex and LC3 complex, serve to complete the autophagosome. Eventually, the autophagosome fuses with the lysosome, the contents within are degraded and decomposed into macromolecular precursors to fuel metabolic pathways. The function of autophagy in neurodegenerative disease, microbial infection, intestinal inflammation, senescence, cancer and other physiological or pathological phenomena are widely discussed.

INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumor in children, accounting for 6%–10% of malignant tumors, as well 15% of the mortality rate in childhood cancer. It is urgent to develop more effective treatments for elderly patients in advanced stages. Studies have shown that macroautophagy (hereafter referred to as autophagy), a universal adaptive response in eukaryotic cells, serves to maintain cell homeostasis under stressful situations. However, under certain circumstances autophagy can act as a death program to induce cell death, which provides a new perspective for tumor treatment.

The role of autophagy in the development of NB and its applied value are under discussion. Here, we summarized recent researches regarding autophagy in NB and attempted to bring further understanding in the treatment of NB.

Autophagy is a common housekeeping mechanism to maintain cellular homeostasis in eukaryotic cells. Under exogenous stimuli (such as nutrient deprivation, hypoxia, oxidative stress, infection, etc), the formation of double membraned vesicles called autophagosomes begins. Autophagosomes engulf cellular proteins or organelles for delivery to the lysosome and in turn produce into amino acids, nucleotides and other nutrients for the cell. However, once this degradation process becomes continuous and excessive, it can serve as a cellular programmed death mechanism. Autophagy is controlled by a highly regulated set of molecular mechanisms, and it involves evolutionarily conserved genes called autophagy related (ATG) genes. This process is typically divided into several distinct stages: initiation, nucleation of the autophagosome, expansion and elongation of the autophagosome membrane, closure and fusion with the lysosome. The process is completed with degradation of intravesicular contents. Initiation begins with the formation of phagophore, which is activated by the negative regulation of serine/threonine kinase (mammalian target of rapamycin (mTOR)). Following the formation of autophagosomes, ulk1-atg13-fip200-atg101 protein kinase complex and two ubiquitin-like binding systems, namely atg12-atg5/atg16 complex and LC3 complex, serve to complete the autophagosome. Eventually, the autophagosome fuses with the lysosome, the contents within are degraded and decomposed into macromolecular precursors to fuel metabolic pathways. The function of autophagy in neurodegenerative disease, microbial infection, intestinal inflammation, senescence, cancer and other physiological or pathological phenomena are widely discussed.

The development of cancer is a multistep and multifactor mediated process, which can be delicately interpreted as having several hallmarks. Based on genetic diversity rising from genome instability, cancer cell itself embodies sustaining proliferation, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, resetting energy metabolism and evading immune destruction. Beyond itself, the tumor takes advantage of surrounding normal cells to create its microenvironment, which fosters the tumor and protects it from eliminating.

The role of autophagy in cancer has been discussed worldwide. The following overview focuses on how autophagy manifests in NB and on applicability of autophagy.

Autophagy and apoptosis in NB
First, a massive increase in the number of autophagic vacuoles and damaged mitochondria
were found in aggressive human NBs associated with proliferation rate and MYCN gene amplification.\(^\text{16}\) As for the activation of autophagy in NB, it can be attributed to several upstream signals. For example, β2-adrenergic receptor is significantly increased in clinical samples of NB and is responsible for NB cell growth via autophagy.\(^\text{17}\) Under these scenarios, autophagy acts as a survival mechanism and targeting related genes or the process itself seems reasonable. For instance, miR-34a\(^\text{18}\) and miR-20a-5p\(^\text{19}\) could inhibit autophagy through targeting specific ATG genes resulting in suppressed proliferation and apoptosis in NB. Inhibition of unc-51 like autophagy kinase 1 (ULK1), an essential autophagy kinase, with SBI-0206965 or a dominant-negative mutant of ULK1 could reduce growth and metastasis in NB cell and xenograft tumors.\(^\text{20}\) Pharmacological inhibition of autophagy with 3-methyladenine also could reduce tumor growth.\(^\text{17}\)

Other than this, autophagy is associated with chemoresistance in NB. Anaplastic lymphoma kinase (ALK) inhibitor entrectinib induces apoptosis and autophagy in NB cells. Abrogation of autophagy by chloroquine (CQ) significantly increases the toxicity of entrectinib.\(^\text{21}\) Geneside compound K,\(^\text{22}\) genistein,\(^\text{23}\) GANT-61\(^\text{24}\) and radiation\(^\text{25}\) exert similar effects towards NB cells for inhibiting autophagy augments apoptosis. However, opposing effects of autophagy on the ability of tumor cells to undergo apoptosis were found, and the underlying mechanisms were uncertain and poorly understood.\(^\text{26}\) AZD8055, a potent dual mTORC1- mTORC2 inhibitor, effectively inhibits cell growth and induceds autophagy and apoptosis in NB cells,\(^\text{27}\) so are rapamycin\(^\text{28}\) and onconase.\(^\text{23}\) The effects of autophagy in those contexts need to be further determined.

**Autophagy and histone modification in NB**

Abnormal DNA methylation in cancer cells always leads to aberrant genetic expression patterns.\(^\text{29}\) In NB, the DNA methylation of CASP8,\(^\text{30}\) RASSF1A,\(^\text{31}\) HIST1H3C and GNAS is most described and associated with risk factors, such as MYCN amplification, age at diagnosis, tumor stage and survival rate.\(^\text{32}\) G9a is a methyl-transferase of histone H3K9. The Xiaoxue Ke team exposed NB cells to the G9a inhibitor BIX01294, which could block the proliferation of cells and induce autophagy in cells.\(^\text{33}\) However, the role of autophagy in this circumstance needs to be further determined.

Inhibition of histone deacetylases (HDACs) by HDAC inhibitors (HDACis) could induce autitumor effects, including cell-cycle arrest, differentiation, and apoptosis, in a broad spectrum of solid tumors, especially in high-risk embryonal tumors.\(^\text{34}\) HDAC10 is overexpressed in patients with high-risk NB and correlated with poor overall survival. HDAC10 protects cancer cells from cytotoxic agents by inducing autophagy.\(^\text{35}\) No matter targeting HDAC10 or autophagy, it seems reasonable to improve treatment response. HDAC6 inhibitor C1A has been evaluated as an autophagy inhibitor, and Myc-positive NB shows marked cell growth inhibition in response to C1A via autophagy pathways.\(^\text{36}\) Tichostatin A, HDACi decreases cell viability in NB cells via G2/M-phase arrest, apoptosis and autophagy. Autophagy induction accompanies apoptosis in MYCN-positive NB cells, while autophagy precedes apoptosis and acts as a protective mechanism in MYCN-negative cells.\(^\text{34}\)

**Autophagy and angiogenesis in NB**

NB is characterized by abundant vascularization leading to rapid metastasis, and angiogenesis plays a vital role in its progression.\(^\text{37}\) In this case\(^\text{38}\) an increased expression of gastrin-releasing peptide (GRP) and its receptor is found in NB. Inhibition of GRP receptor using short hairpin RNAs (shRNA) or a specific antagonist, RC-3095, enhances autophagy-mediated degradation of GRP and subsequent inhibition of angiogenesis in NB.\(^\text{38}\) Therefore, activation of autophagy may provide a new anti-tumor method targeting NB.

**Autophagy and metabolism in NB**

Hagenbuchner et al found that high-risk NB was often characterized by a gain of chromosome 17q, which led to overexpression of the antiapoptotic protein BIRC5/Survivin. In this circumstance NB cells take advantage to transform its metabolism pattern from oxidative phosphorylation to aerobic glycolysis and resist cell death. Aerobic glycolysis inhibitor 2-DG can induce autophagy to degrade BIRC5/Survivin in those NB cells and induce apoptosis.\(^\text{39}\) In another study, 2-DG can trigger endoplasmic reticulum stress in human neuroblastoma SK-NE-BE(2) cells to induce apoptosis together with autophagy. Interestingly, autophagy in this scenario favors cell from apoptotic destiny.\(^\text{40}\)

**Autophagy and other hallmarks in NB**

Ganglioside GD2 is ubiquitously present on the surface of NB cells\(^\text{41}\) and targeting its antibody Ch14.18 (also known as dinutuximab) has been recognized as the most effective immunotherapy to date for pediatric solid tumors.\(^\text{42}\) Inhibiting the autophagy induced by GD2-specific 14G2a monoclonal antibody in IMR-32 NB cells with CQ augments the counterpart apoptosis.\(^\text{43}\) The high mobility group box 1 (HMGB1) is an inflammatory factor that is reported to be increased in NB. HMGB1-induced autophagy in its surrounding Schwann cells through classical pathway (naming Beclin1-dependent) contributes to the proliferation of NB cells.\(^\text{44}\) Furthermore, HMGB1-mediated autophagy also promotes chemoresistance in NB cells, which implicates the protective role of autophagy in the development of NB.\(^\text{45}\) Research shows that honokiol can induce autophagy and reduce migration in NB, while whether autophagy can reduce migration needs to be elucidated in further researches.\(^\text{46}\)

**Autophagy in the treatment and prognosis of NB**

The current risk classification strategy for NB is based on the International Neuroblastoma Risk Group (INRG) classification system which determines patients’ treatment
outcome and overall prognosis. Evidence has shown autophagy is associated with high-risk NB especially with MYCN gene amplification. Thus, it is reasonable to evaluate correlation between autophagy and other NB prognostic factors. The fact that 2% of patients with NB will undergo spontaneous regression is interesting, and the potential mechanism is mostly focused on the function of telomerase or Trk pathways. Future studies are needed to explore the role of autophagy under those circumstances on spontaneous regression of patients with NB. The INRG classification system has not involved NB genome, transcriptome, and epigenome, while these genetic information have participated in more precise prognostication. Mutations of genes that seem as autophagy-dependent oncogenes in the RAS pathway are often associated with high levels of autophagy in pancreatic cancer, while mutations of ALK were found in rare familial NBs and 10%-15% of sporadic NBs. Also, amplified MYCN is found in 20% of cases which is correlated with a highly aggressive subtype. In MYCN-positive NBs, autophagy was overexpressed and contributed to chemoresistance towards certain anticancer drugs. With those facts we can assume that high-risk NB is an autophagy-dependent tumor, which means that autophagy inhibition therapy is desirable. Interestingly, Sarah et al found that poor outcomes usually occurred in the presence of telomere maintenance, whereas patients with NB without activation of telomere maintenance mechanisms (TMMs) had excellent outcomes. Telomere maintenance is induced in NB mostly through activated MYCN or MYC or telomerase reverse transcriptase gene rearrangements, or mechanisms triggering ALT activation. Under the activation of TMMs, patients with NB with additional mutations of genes in the ALK–RAS or p53 pathway have an inferior prognosis than those with TMM-negative NB. Following this novel mechanistic classification of NB, autophagy in TMM-positive NB could be monitored to explore whether targeting autophagy in this group could improve treatment outcome. Subsequently, therapies targeting RAS-ALK pathway or telomerase or the ALT pathway seem reasonable and the effect of therapy-inducing autophagy needs to be determined. In general, inhibiting autophagy in high-risk NB is encouraging especially when the utilization of CQ or hydroxychloroquine as autophagy inhibitors in clinical trials has been demonstrated to be safe for cancer therapy.

CONCLUSION

NB is the most common extracranial solid tumor in children, and autophagy can act as a protective role in tumorigenesis and treatment resistance. The majority of studies focusing on autophagy regarding NB (especially high-risk NB), imply a pro-oncogenic effect. Thus, inhibiting autophagy in the treatment of NB seems reasonable. Further researches are needed to explore the application of autophagy in treatment and prognosis of NB.

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