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Targeting autophagy for combating chemoresistance and radioresistance in glioblastoma

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Abstract

Autophagy is an evolutionarily conserved catabolic process that plays an essential role in maintaining cellular homeostasis by degrading unneeded cell components. When exposed to hostile environments, such as hypoxia or nutrient starvation, cells hyperactivate autophagy in an effort to maintain their longevity. In densely packed solid tumors, such as glioblastoma, autophagy has been found to run rampant due to a lack of oxygen and nutrients. In recent years, targeting autophagy as a way to strengthen current glioblastoma treatment has shown promising results. However, that protective autophagy inhibition or autophagy overactivation is more beneficial, is still being debated. Protective autophagy inhibition would lower a cell's previously activated defense mechanism, thereby increasing its sensitivity to treatment. Autophagy overactivation would cause cell death through lysosomal overactivation, thus introducing another cell death pathway in addition to apoptosis. Both methods have been proven effective in the treatment of solid tumors. This systematic review article highlights scenarios where both autophagy inhibition and activation have proven effective in combating chemoresistance and radioresistance in glioblastoma, and how autophagy may be best utilized for glioblastoma therapy in clinical settings.

Keywords

Autophagy; Cell Death; Chemoresistance; Glioblastoma; Radioresistance

Introduction

Glioblastoma multiforme, also simply known as glioblastoma, is the most common and aggressive primary brain tumor in adults, with a yearly occurrence of about 4 per 100,000 people and a median survival of about 14.6 months [1]. The poor prognosis of almost all

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Conflict of interest

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glioblastoma patients demonstrates the diverse nature of this malignant disease and highlights the need for novel treatment strategies that can consistently enhance patient survival times. Since glioblastoma is derived mainly from abnormal astroglia, which are star-shaped cells with extended processes throughout the brain, it is almost impossible for surgery alone to remove all glioblastoma cells with their entire cellular network. A few remaining glioblastoma cells, which infiltrate into the nearby brain tissue, can readily cause recurrence of the tumor. Thus, glioblastoma is practically an incurable brain tumor. The growth or regrowth of glioblastoma occurs very rapidly due to its resistance to chemotherapy and radiotherapy. Sixty-one percent of these tumors are found in the cerebral hemispheres, but they can occur anywhere throughout the central nervous system [2]. These tumors are accompanied by edema and inflammation and they have poorly-defined borders along with vast infiltration; which makes complete surgical resection generally unattainable [3]. Glioblastoma stem cells (GSCs), often remaining in the brain tissue after surgical resection, are highly resistant to chemotherapy and radiotherapy; therefore, GSCs rapidly regrow the tumor [4]. Autophagy, which is a cellular recycling mechanism, confers strong chemoresistance and radioresistance to GSCs that can cause regrowth of glioblastoma, making its standard treatments almost always unsuccessful.

After surgical resection, glioblastoma patients are treated with radiotherapy combined with the adjuvant chemotherapy Temozolomide (TMZ); total treatment lasting approximately 30 weeks [1]. TMZ (compound **1**) is an alkylating agent that becomes unstable at physiological pH, thus prompting conversion of TMZ into 3-methyl-(triazene-1-yl)-imidazole-4-carboxamide (MITC, compound **2**) (Fig. 1), which acts as an active alkylating agent readily donating a methyl group to purine bases in cellular DNA (N7-guanine, O6-guanine, and N3-adenine). MITC further reacts with water to liberate 5-aminoimidazole-4-carboxamide (AIC, compound **3**) and the highly reactive methyldiazonium cation (compound **4**) (Fig. 1). The active species methyldiazonium cation preferentially methylates cellular DNA at N7 position of guanine (compound **5**) in guanine rich regions (N7-MeG; 70%) but it also methylates N3 position of adenine (N3-MeA; 9%) and O6 position of guanine (O6-MeG; 6%) contributing significantly to DNA alkylations to produce TMZ cytotoxicity [5]. It should be noted that N7-methylguanine is relatively less cytotoxic due to activation of the nucleotide excision repair pathway that removes these lesions without harming the DNA. Mainly, O6-methylguanine causes the cytotoxic effects of TMZ. The mismatch repair enzymes trying to excise O6-methylguanine lesions produce single-strand and double-strand DNA breaks, which trigger activation of apoptotic pathways.

Genomic studies showed that glioblastoma arises from defects in three main molecular signaling pathways involving p53, Rb, and phosphoinositide 3-kinase (PI3K) [6]. After approximately a year into treatment, glioblastoma becomes resistant to TMZ. Most of the investigations indicate that the greatest genomic predictor of glioblastoma resistance to TMZ therapy and overall low survival is due to induction of O6-methylguanine-DNA methyltransferase (MGMT) [5]. Because MGMT is a DNA repair enzyme, it removes methyl groups attached to O6-guanine residues in DNA, along with other alkyl groups. This DNA repair mechanism counteracts the cytotoxic effects of TMZ. The brain is protected through, use of the blood-brain-barrier (BBB), by resisting permeability to many invading

pathogenic agents and also chemotherapeutic agents [7]. So far, TMZ is one of the only potential chemotherapeutic agents capable of passing through the BBB, and its DNA alkylating activity is one of the only mechanisms for medically combating glioblastoma growth [8].

Due to the bleak outcome accompanying the most cases of glioblastoma diagnoses, alternative therapeutic pathways are being aggressively explored. A common alternative approach is the use of Bevacizumab (Avastin®), an antibody that blocks the epidermal growth factor receptor (EGFR), a transmembrane receptor protein in humans, to prevent tumor vascularization [9]. Recent clinical trials have shown that Bevacizumab (Avastin®) is capable of increasing progression-free survival in glioblastoma patients, but it has no notable effect on improving survival of newly diagnosed glioblastoma patients [10]. Other alternative therapies include use of anti-epileptic drugs, histone deacetylase inhibitors (HDACi), and nanocarriers formulated with various drug combinations [1,3,9]. Unfortunately, most of these trials have had few positive outcomes on glioblastoma patients. In recent years, understanding and modulation of the autophagy pathway appear to be a promising treatment avenue for future cancer therapies. Although the autophagy pathway itself has been well defined, the most efficient use of its seemingly contradictory effects is still highly debated and will be the focus of this article.

Autophagy as a double-edged sword in cancer therapy

Autophagy is a conserved, cellular catabolic process that gives cells rapid adaptive advantage to stressful or hostile environments. In healthy cells, autophagy occurs at a basal level to maintain homeostasis and remove damaged organelles in the cells [11]. But when triggered by environmental stressors such as high heat, nutrient starvation, or hypoxia, autophagy gives cells the ability to degrade nonessential or damaged proteins and organelles in order to reutilize the resulting amino acids for more essential cellular processes [12]. In cancer therapy, this adaptive property presents itself as a double-edged sword. On one hand, autophagy can desensitize cells to stressful situations such as chemotherapy or nutrient starvation, as seen in many tumor microenvironments. This is known as protective autophagy [13]. On the other hand, prolonged autophagy can result in an unwarranted form of cell death similar to necrosis, called autophagic cell death [14]. A passionate debate still remains unresolved whether it is essential to inhibit autophagy so as to decrease cellular defenses; or it is beneficial to upregulate autophagy in hopes of achieving abundant autophagic cell death in specific cancers.

In mammals, there are three types of autophagy, all of which have the goal of delivering proteins and organelles to autophagosomes for lysosomal degradation. The first type is microautophagy, which involves direct sequestration of organelles into the lysosome through invaginations existing in the lysosomal membrane. The second type is chaperone-mediated autophagy (CMA) [15], which is a highly specific form of autophagy that targets cytosolic proteins containing a specific pentapeptide motif. The targeted proteins are unfolded by chaperones and transported directly to the lysosome for degradation. The third type is macroautophagy, which is the most studied and hereby referred to as autophagy. It plays a constant role in the recycling of the damaged or unneeded organelles and proteins in healthy,

viable cells. Autophagy can also be triggered to act as a protective mechanism in stressful environments [16]. In the progressive phase of growth, glioblastoma combats stressful microenvironments by using autophagy to recycle the damaged proteins and organelles to provide the tumor with cellular building blocks (e.g., amino acids, carbohydrates, nucleic acids) for survival and sustained growth. The manipulation and utilization of autophagy may provide an avenue for natural sensitization of the radioresistant and chemoresistant tumors, such as glioblastoma, to the currently developed novel treatment strategies.

Molecular mechanism of protein degradation in autophagosome through macroautophagy

Autophagy is tightly regulated at the molecular level. It consists of four distinct stages: (a) induction, (b) autophagosome formation, (c) lysosome fusion, and (d) degradation (Fig. 2). Although autophagy in mammals was discovered 50 years ago, its mechanism of action has only been uncovered within the last decade. Upon exposure to autophagy-inducing stimuli, such as high heat, nutrient starvation, oxidative stress, or hypoxia, phagophore formation is initiated. Initiation relies on two autophagy protein complexes: (i) PI3K-NI (Vps34), Beclin-1 (Atg6), Atg14L, and p150 (Vps15); and (ii) the serine-threonine kinase ULK1 (Atg1) [17]. ULK1 is an essential component of downstream of autophagosome formation and it promotes adherence of Vps34 to the newly forming phagophore. Vps34 binds to p150, which then associates with Beclin-1. Beclin-1 allows additional binding of Atg14L to the complex. ULK1, Vps34, p150, Beclin-1, and Atg14 are all essential for the induction of autophagy [18]. Elongation of the phagophore and subsequent double-membrane autophagosome formation is catalyzed by Atg7 and involves two distinct ubiquitin-like conjugation pathways. The first pathway is Atg5-Atg12 that combines with Atg16L, which associates with the outer membrane of the autophagosome. In the second pathway, LC3B (Atg8) is cleaved by Atg4 to form LC3B I. LC3B I is then activated by Atg7 and conjugated with phosphatidylethanolamine (PE) to generate a [LC3B-I-PE] complex, which then creates LC3B II. LC3B II, dependent on the [Atg5- Atg12-Atg16L] complex, is then bound to both the inner and outer membranes of the autophagosome. Upon complete formation of the autophagosome, the [Atg5-Atg12-Atg16L] complex dissociates from the outer membrane while LC3B II remains covalently attached to the autophagosome. This allows LC3B II to be a reliable biomarker of autophagosome formation within a cell population [14]. Upon completion of the autophagosome, LC3B II on the outer membrane is cleaved from PE by Atg4 and recycled into the cytosol. A lysosome then fuses with the autophagosome, forming the autolysosome. Lysosome fusion exposes the phagocytosed cellular components to the hydrolases, which are responsible for massive degradation of the damaged or unwanted proteins into amino acids. These amino acids are then transported into the cytoplasm where they are reused for biosynthesis of functional proteins and enzymes, which are essential to cell viability [17].

Inhibiting protective autophagy in glioblastoma

Throughout the evolutionary history, cells have developed multiple protective mechanisms to ensure their survival. The mammalian autophagy mechanism has been highly conserved

throughout species, highlighting its importance for development and survival of organisms. Studies performed on eukaryotes demonstrated this importance by showing terminal defects in embryogenesis in organisms with ATG gene knockouts [19]. As an organism develops, autophagy encompasses the role of regulating homeostasis. In order to remove damaged cellular components, autophagy occurs at a basal level throughout the body. Autophagy also plays an important role in preventing kidney damage during nephro-ischemic events or Cisplatin-induced nephrotoxicity [11]. In glioblastoma, upregulation of autophagy runs rampant due to the dense nature and rapid expansion of this solid tumor in the brain. Hypoxia and nutrient starvation are the primary inducers of autophagy in glioblastoma because rapid tumor growth does not allow the vasculature to supply sufficient nutrients to the tumor site [20]. This presents an issue where protective autophagy may play a role in enhancing chemoresistance and radioresistance in glioblastoma.

Recently, a study investigating the treatment of glioblastoma with Bevacizumab (Avastin®), a blocker of EGFR [21], found that Bevacizumab-induced autophagy contributed to glioblastoma chemoresistance. Inhibition of protective autophagy through the use of Chloroquine increased the sensitivity of glioblastoma to Bevacizumab in vitro [22]. The investigators emphasized the need for further studies into pharmacological inhibition of autophagy as a way to potentiate the therapeutic effects of Bevacizumab.

Chloroquine for inhibiting autophagy in glioblastoma

Many studies have been performed that investigate the impact autophagy has on combating glioblastoma. The first step in this process is finding a way to inhibit autophagy in order to observe its subsequent effects on apoptosis. The most proficient method of inhibiting autophagy is the use of Chloroquine (CQ). Typically an anti-malarial drug, CQ can also inhibit autophagy by becoming imbedded in the autolysosome, thereby inhibiting its degradative enzymatic function [23]. Although the exact mechanism is unknown, CQ exists as a weak base at physiological pH, which allows it to traverse the nonpolar lipid bilayers of the cells. When CQ contacts the acidic vesicular organelles (AVOs) formed by the autolysosome, CQ becomes protonated (CQH⁺) and thus hinders its ability to exit the cells. This buildup of CQH⁺ lessens the acidity of the autolysosome and hinders its degradative properties [11]. Experiments using CQ in combination with TMZ, a potent autophagy upregulator, on glioblastoma showed autophagy inhibition enhanced the cytotoxicity of TMZ by evidence of increased cleavage of poly (ADP- ribose) polymerase (PARP), a DNA repair enzyme [23]. This directs to the idea that autophagy is acting as a protective mechanism in glioblastoma, and that inhibition of autophagy can enhance chemosensitivity in TMZ-resistant cells. It has also been demonstrated that autophagy inhibition by CQ can increase radiosensitivity in glioblastoma [24]. It has been shown that radiotherapy on the U87MG glioblastoma initiating cells (GICs) caused an increase in autophagy; thereby acting as a protective mechanism to help desensitize these cells to treatment [25]. When the GICs were treated in combination with radiation and CQ, combination therapy worked synergistically for induction of apoptosis, indicating that autophagy inhibition contributed to enhancement of radiosensitization [25].

But despite the ability for CQ to inhibit autophagy and its potential therapeutic benefits in cancer therapy, CQ has also been shown to produce harmful effects that do not make it an ideal candidate for future synergistic cancer therapies. At high doses, CQ may produce side effects such as cardiac disturbances, visual defects, and kidney damage. It has been suggested that the broad-range autophagy inhibition by CQ can lead to increased nephrotoxicity caused by chemotherapy with TMZ [11]. This was demonstrated in proximal tubule-specific autophagy-deficient mice treated with Cisplatin. In comparison with control mice, the autophagy-deficient mice displayed severe Cisplatin-induced acute kidney injury (AKI), indicating that autophagy played an important role in protecting kidney function when undergoing chemotherapy treatment [26]. Therefore, despite CQ providing a clear model on the effects of autophagy inhibition in cancer therapy, its cytotoxic properties has promoted the investigation into more region-specific autophagy inhibitors.

Novel autophagy inhibitors in the treatment of glioblastoma

Recent evidence points to the theory that autophagy inhibition when cells are exposed to stressful stimuli can prove beneficial in glioblastoma therapy (Table 1). TMZ, a potent autophagy inducer as mentioned above, may have its anti-cancer effects enhanced by inhibiting autophagy on its target cells [11]. As a result, many synergism studies have been conducted in the last decade to investigate drug effects on inhibiting autophagy and its subsequent impact on apoptosis through the use of RNA-interference (RNAi), previously established autophagy inhibitors, and even bioflavonoids [23,27–31]

One example of a powerful autophagy inhibitor is 3-Methyladenine (3-MA). It inhibits autophagy by preventing autophagosome formation through the inhibition of class III PI3K. A study aimed at assessing the apoptosis mechanism of Bortezomib, a 26S proteasome inhibitor, in glioblastoma used 3-MA and Atg7 siRNA to investigate the effects of autophagy inhibition on apoptosis [29]. They demonstrated that Bortezomib induced autophagy in glioblastoma, and simultaneous inhibition of autophagy in the treated population caused an increase in apoptosis by evidence of increased caspase-3 activation [29]. Results provide additional evidence that inhibition of pre-established autophagy can enhance the apoptotic capacity of anti-cancer drugs.

To further investigate the effects of autophagy inhibition in anti-cancer therapy, studies aimed at more mechanistic-specific inhibition have been performed (Table 1). Ma et al. have treated human glioblastoma cells with Cisplatin (Platinol®) and analyzed how the Cisplatin-induced long non-coding RNA AC023115.3 plays a role in induction of apoptosis. The results showed that AC023115.3 increased the chemosensitivity of human glioblastoma cells to Cisplatin by inhibiting protective autophagy through sequestration of miR-26a, a microRNA highly upregulated in glioblastomas, which is thought to target the tumor suppressor phosphatase and tensin homolog (PTEN) transcripts, leading to an increase in activity of the glycogen synthase kinase 3 β (GSK3 β) pathway [27]. GSK3 β is a serine-threonine kinase that has a wide range of biochemical functions including Inflammation regulation, glycogen synthesis, and autophagy inhibition [32]. Another study used the autophagy inhibitor miR-30e in combination with proanthocyanidin (PAC) to demonstrate that autophagy inhibition through inactivation of Beclin-1 can enhance a drug's anti-cancer

capabilities in GSCs and glioblastoma SNB19 cells [30]. Treatments of GSCs and glioblastoma SNB19 cells with a single-dose of the autophagy inhibitor miR-30e showed approximately 15–20% apoptosis, while cells treated with a single-dose of PAC showed approximately 20–30% apoptosis. In combination, between 33–49% apoptosis was observed across both cell lines. By inhibiting protective autophagy, the anticancer capabilities of PAC in human GSCs and glioblastoma SNB19 cells were increased by 55% and 64%, respectively [30].

In addition to common chemotherapy drugs and autophagy suppressing microRNA, naturally occurring flavonoids have also been shown to enhance their anti-cancer capabilities by inhibiting protective autophagy in their target tissues. Flavonoids are plant-derived chemical compounds characterized by their double phenyl ring structure that play many roles in plant function and are abundantly distributed amongst common food sources [33]. An example of their potent anti-cancer effects was demonstrated by treating human U87MG (wild-type p53) and T98G (mutant p53) cell lines [31] with Luteolin (LUT), a flavonoid known to inhibit autophagy, and Silibinin (SIL), another flavonoid that has shown anti-cancer effects in a variety of cancers. The results showed that inhibition of Rapamycin-induced autophagy through the synergistic effects of LUT and SIL in combination allowed for over a 100% increase in apoptosis across two types of human glioblastoma cell lines [31].

The substantial evidence showing the increased chemosensitization through autophagy inhibition gives insightful clues into how autophagy can most effectively be used in cancer therapies. In tumor microenvironments, cells have been shown to use protective autophagy to help survive conditions such as hypoxia, nutrient starvation, or anti-cancer treatments with drugs such as TMZ. By inhibiting therapy resistant tumors such as glioblastoma, natural defense mechanism to hostile environments, a better response may be observed to existing treatments and open the door to greater exploration into novel therapies.

Activation of autophagic cell death in glioblastoma

The core function of autophagy is the programmed lysosomal degradation process of damaged or excess proteins and organelles to help maintain homeostasis in cells [28]. Under basic growing conditions, autophagy is essential to cell survival and it maintains consistent low-level activation. When exposed to hostile environments such as nutrient starvation, autophagy is upregulated as a protective mechanism to prevent necrotic cell death [11,23,27]. However, it has also been widely demonstrated that excess levels of autophagy can induce type II programmed cell death (Table 2), which is also known as autophagic cell death [34]. This is evident through experiments showing Bax/Bak knockouts undergoing caspase-independent cell death, largely through the presence of abundant autolysosomes and simultaneous activation of phosphorylated c-Jun N-terminal kinase (p-JNK) [34]. Both of which are believed to be necessary in order to initiate autophagic cell death. In subsequent experiments, autophagy genes such as Atg5 and Beclin-1 (Atg6) were silenced, and any evidence of autophagic cell death was abolished [14]. Using this concept, many studies have been conducted that have investigated the therapeutic potential of initiating autophagic cell death in different cancer populations. In glioblastoma, autophagic cell death (Table 2) has

been seen throughout chemotherapy with TMZ and radiotherapy, and with alternative therapies such as HDACi and other anti-cancer drugs [34].

TMZ as chemotherapy for autophagic cell death in glioblastoma

TMZ is a DNA alkylating agent that is currently used as the first-line treatment of glioblastoma after surgical resection. TMZ is administered orally with simultaneous radiotherapy for approximately 30 weeks. Unfortunately, most patients become resistant to TMZ due to the action of MGMT, which counteracts the cytotoxic effects of TMZ [1]. Therefore, a considerable effort has been put towards finding more effective and less resistance-prone glioblastoma therapies. However, to date, few therapies have been developed that supersede the efficacy of TMZ. This highlights the importance of finding methods to enhance the anti-tumor effects of TMZ. One prominent avenue is to utilize TMZ-induced autophagy to enhance glioblastoma chemosensitivity.

It has been widely proven that TMZ induces autophagy when treating glioblastoma [11,23,35]. Studies have shown that TMZ induces autophagy by causing DNA damage that activates ataxia-telangiectasia (ATM). This promotes the downstream phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and subsequent activation of ULK1, which then induces autophagy [36]. However, the argument persists on whether it is more beneficial to inhibit protective autophagy [11] or enhance it to increase autophagic cell death when treating glioblastoma [37]. A recent study demonstrated that TMZ cytotoxicity was enhanced in both human GSCs and U87MG glioblastoma cells by simultaneous treatment with endothelial monocyte-activating polypeptide-II (EMAP-II); which is a pro-inflammatory cytokine [38]. The investigators argue that the uncontrolled autophagy induced by both TMZ and EMAP- II allowed for TMZ to have a greater effect on the MGMT-positive GSCs by permitting progression into G₂/M cell cycle arrest, which was unable to be achieved in the TMZ-only treated GSCs [38]. Another study using the mechanistic target of rapamycin (mTOR) inhibitor RAD001 in combination with TMZ and γ -radiation on U87MG glioblastoma cells showed a drastic decrease in cell viability with the addition of RAD001. It is interesting to note that after the RAD001 combination treatment, most of the cell death occurred via autophagy and not apoptosis. This change in the cell death mechanism allowed for a drastic increase in radiosensitivity [39]. Although TMZ treatment alone does not induce sufficient amounts of autophagic cell death, both studies are showing that drug combinations with TMZ and radiation treatment allows autophagy to be used as an alternative cause of cell death in TMZ-resistant glioblastoma cells. Since standardized glioblastoma treatment uses a combination of both radiation and TMZ, utilizing the resulting autophagy may allow for increased tumor regression and ultimately greater patient survival.

Radiotherapy for autophagic cell death in glioblastoma

Most modern glioblastoma treatments utilize radiotherapy as one of the primary methods for shrinking existing tumors or eliminating remnants of a tumor after surgery. Ionizing radiation uses charged particles delivered to the tumor site with either an external beam or internally planted radioactive seeds to cause DNA damage or genetic mutations that cause

instability and ultimately result in cell death. Radiation has a damaging effect on both cancerous and healthy cells, but cancer cells remain more susceptible to radiation because their rapid proliferation does not allow time for DNA repair mechanisms to completely take action [40]. In glioblastoma, radiation is introduced with simultaneous TMZ treatment soon after surgical resection [1]. Since almost all glioblastoma cells are impossible to completely remove with surgery due to their diffuse progressive nature, radiation is used in an attempt to eliminate as much remaining tumor as possible. Radiation causes cell death through a variety of mechanisms, with less than 20% of cell death caused by apoptosis [41]. Other forms include errors in mitosis, necrosis, senescence, and autophagy [40]. Early studies show that autophagy is ultimately the preferred form of cell death in glioblastoma cells following radiation treatment [11]. Since it is well-known that radiation is a powerful autophagy-inducing stimulus, it is important to investigate how radiation-induced autophagy promotes cell death and how this can be most efficiently utilized in glioblastoma treatment.

Since radiotherapy is often coupled with chemotherapy during cancer treatment, many experiments have been conducted to uncover the most effective way to utilize these two therapies in the treatment of glioblastoma (Table 2). However, the debate about autophagy induction vs. autophagy inhibition still remains. A study was performed using a combination of TMZ, ionizing radiation (IR), and the PI3K inhibitor GDC-0941 to study the effects of PI3K inhibition on increasing radiosensitization and reducing chemoresistance in glioblastoma cells [42]. This study demonstrated that GDC-0941, a known autophagy inducing agent, drastically increased the sensitization of glioblastoma to the dual (TMZ + IR) treatment [42]. This directs to the idea that autophagic cell death can positively contribute to radiosensitization in glioblastoma. On the other hand, another experiment using radiation therapy (RT) and the DNA intercalating agent/topoisomerase II inhibitor Vosaroxin showed that autophagy inhibition caused by Vosaroxin increased the radiosensitivity of glioblastoma cells [43]. The investigators hypothesized that the inhibition of RT-induced autophagy allowed for the increase in apoptosis [43]. However, is it still unknown whether the cytotoxic effects of Vosaroxin or RT were the causes of the increased apoptosis. The conflicting results of these studies point to the theory that any drastic change in autophagy levels can produce positive effects in glioblastoma therapy.

Alternative inducers of autophagic cell death

In addition to TMZ and radiation-induced autophagic cell death, many alternative glioblastoma therapies have also been shown to provide anti-cancer properties through increased autophagy. One example is Itraconazole, a broad-spectrum antifungal agent that has shown evidence of having anti-cancer capabilities as an mTOR inhibitor. Itraconazole-9 inhibited glioblastoma cell proliferation in vitro and in vivo through a marked increase in autophagy [44]. This was further demonstrated by showing that autophagy inhibition completely reversed the anti-proliferative properties of Itraconazole-9 [44]. Icaritin-8, a compound used in Chinese herbal medicine, demonstrated the therapeutic potential of autophagic cell death coupled with apoptosis. The investigators found that Icaritin-8 caused apoptosis through both the intrinsic and extrinsic pathways of apoptosis while also inducing autophagy through inhibition of mTOR. When autophagy was inhibited by 3-MA, cell death was reduced indicating that increased autophagy played a role in cell death. Li et al. have

demonstrated how autophagy can synergistically enhance apoptosis by degrading inhibitor of apoptosis proteins (IAPs) and Fas-associated phosphate 1 (FAP-1), but can also inhibit apoptosis by acting as a protective mechanism [37]. Finally, a study targeting nucleolin, a ribonucleoprotein that is highly expressed in many cancer types, gave another example of autophagic cell death [45]. Benedetti et al. treated glioblastoma cells with the nucleolin inhibitor N6L and found little evidence of apoptosis. Rather, they found high amounts of autophagy-induced cell death through a decrease in cytoplasmic p53 and increase in LC3 II [45].

Clinical applications of autophagy inhibition

Despite the extensive research that has been conducted on autophagy in glioblastoma, few clinical trials assessing its therapeutic potential have been conducted. A Phase II clinical trial in the Netherlands (NCT02432417) aims to investigate how autophagy inhibition with CQ can benefit glioblastoma patients currently being treated with TMZ [46]. The trial is an expansion of a similar trial conducted in Mexico involving 30 patients, with half receiving supplementary CQ along with standard therapy, with the other half receiving a placebo. Albeit a small sample size, the results showed a median survival time of 24 months in the CQ-treated patients and a median survival time of 11 months in the placebo treated glioblastoma patients [46].

Another Phase I/II clinical trial treated glioblastoma patients with hydroxyl CQ (HCQ) in combination with radiotherapy and TMZ treatment [35]. By using quantitative electron microscopy and immunoblotting, the investigators were able to effectively monitor intra-tumor autophagy levels. The goal of the trial was to assess the maximum tolerated dose of HCQ while glioblastoma was undergoing standard therapy. The study concluded that at 800 mg HCQ, 3/3 patients experienced adverse events including Grade 3 and 4 neutropenia, thrombocytopenia, and one with sepsis. The maximum tolerated dose was 600 mg HCQ. However, 600 mg HCQ was not high enough to consistently produce sufficient levels of autophagy inhibition. The investigators have urged the development of less toxic autophagy inhibitors [35].

HCQ is currently the only clinically approved autophagy inhibitor [47]. This may explain why almost all clinical trials targeting autophagy in cancer are aiming to inhibit autophagy through HCQ. But despite its proven ability to disrupt autophagy, HCQ causes an abundance of adverse side effects to where the benefits likely will not outweigh the risks [35]. However, there are many other compounds that have shown autophagy inhibition in vitro [48–51]. These compounds, in addition to many others, have the potential to deliver therapeutic autophagy inhibition with less adverse side effects.

Clinical applications of autophagy activation

Despite its therapeutic potential being widely demonstrated in vitro, no clinical trials have been conducted investigating the benefit of autophagy activation in glioblastoma while undergoing standardized chemotherapy and radiotherapy. This may be due to the lack of clinically approved autophagy activators. However, many compounds have been shown to

activate autophagy in different types of cancers, in addition to their primary therapeutic effect [52–55]. These compounds include Metformin in endometrial cancer [52], Obatoclax - a small molecule targeting Bcl-2, in lymphoblastic leukemia [53], natural alkaloid agents [55], and the antidepressants Maprotiline and Fluoxetine in Burkitt's lymphoma [54].

Rapamycin, an mTOR inhibitor and potent autophagy inducer [27,56], was clinically approved for treatment in renal cell carcinoma [57]. A Phase III clinical trial using the rapalog Temsirolimus in treating mantle cell lymphoma showed positive results, partly, due to overactivation of autophagy [58]. Similar results were seen in vitro with glioblastoma cells [39]. Despite these results, there must be more investigation into the potential benefit of autophagy inducers in treating glioblastoma from a clinical point of view.

Conclusion

Since the discovery of mammalian autophagy and its link to cancer, there has been a debate as to what is the best way to utilize its seemingly opposite properties. At its core, autophagy exists as the evolutionary conserved lysosomal degradation process used for routine cellular maintenance. Many studies have demonstrated that autophagy is an essential part of mammalian development and continued survival [19]. However, when cells are exposed to hostile stimuli such as nutrient starvation, ROS, hypoxia, or cancer stimuli; cells utilize autophagy as a protective mechanism to maintain their vitality [59]. Protective autophagy allows for the degradation and subsequent recycling of proteins and organelles unnecessary for survival, including proteins responsible for facilitating apoptosis [14]. On the other hand, hyperactive autophagy can cause excess cellular degradation and ultimately lead to autophagic cell death [43]. Many scientists claim it is more beneficial to inhibit protective autophagy in order to make naturally chemoresistant cancers, such as glioblastoma, more susceptible to therapy [30]. Others claim excess autophagy provides an alternative form of cell death that can act synergistically with apoptosis [37]. While the exact role of autophagy in cancer has yet to be fully uncovered, this review article has aimed to highlight the current understanding of autophagy and how it can be best used to enhance the treatment of glioblastoma (Fig. 3).

The idea of inhibiting protective autophagy to enhance cancer therapy makes intuitive sense; and therefore, has been immensely studied. When treating glioblastoma with TMZ, drastic increases in autophagy have been observed. This indicates that the glioblastoma cells are sensing the hostile environment induced by TMZ and reacting accordingly by protecting themselves. By inhibiting this protective barrier with CQ or other autophagy-inhibiting drugs, the cytotoxic effects of TMZ were enhanced [23]. Similar results were also seen in novel anticancer drugs when paired with an autophagy inhibitor [27,29–31]. In contrast, the concept of overactivating autophagy to induce additional cell death also makes intuitive sense. Autophagy at its core is a programmed degradative process, and by increasing the activity of that process, cell viability can be greatly reduced [28]. This concept has been demonstrated in glioblastoma with a variety of therapies, including TMZ. Since many glioblastomas quickly become resistant to TMZ through an unmethylated MGMT promoter, excess autophagy has been used as an alternative form of cell death to help potentiate the anti-cancer effects of TMZ [38]. It was also demonstrated that the effectiveness of TMZ +

IR, the standard treatment for glioblastoma, was enhanced through an additional autophagy-inducing agent [42]. These results show relevant clinical application that can easily be deployed in current glioblastoma treatment.

The outcome from current autophagy research in glioblastoma and other types of cancer paints an interesting picture as to how we can best utilize autophagy. Instead of the preconceived notion that activation or inhibition of autophagy is more beneficial than the other, efforts must be refocused towards a situational approach. Autophagy levels can be measured in situ through immunohistochemistry of autophagy-specific biomarkers [60]. Depending on the in vivo conditions of the tumor microenvironment, glioblastoma cells may have varying levels of autophagy. Depending on those levels, treatments should be focused on pushing autophagy in the opposite direction. This counteracts the adaptive properties of autophagy and may activate an additional target for cell death. In difficult to treat cancers, such as glioblastoma, autophagy utilization may provide a more positive patient outlook. Glioblastoma is a unique cancer that displays a wide range of behaviors; therefore, finding effective treatment has remained difficult. This highlights the importance of continued research into autophagy to make this process useful to glioblastoma therapy. By utilizing the double-edged sword of autophagy as a supplementary tool to enhance established and novel therapies, the prevalent chemoresistance and radioresistance seen in glioblastoma may be attenuated, and a greater prognosis may be attainable for many glioblastoma patients.

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References

- [1]. Hottinger AF, Stupp R, Homicsko K. Standards of care and novel approaches in the management of glioblastoma multiforme. *Chin. J. Cancer*. 2014;33:32–39. [PubMed: 24384238]
- [2]. Davis ME. Glioblastoma: Overview of disease and treatment. *Clin. J. Oncol. Nurs*. 2016;20:1–8.
- [3]. Lee DH, Ryu H-W, Won H-R, et al. Advances in epigenetic glioblastoma therapy. *Oncotarget*. 2015;8:18577–18589.
- [4]. Bischof J, Westhoff M-A, Wagner JE, et al. Cancer stem cells: The potential role of autophagy, proteolysis, and cathepsins in glioblastoma stem cells. *Tumor Biol*. 2017;39:101042831769222.
- [5]. Messaoudi K, Clavreul A, Lagarce F. Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide. *Drug Discov. Today*. 2015;20:899–905. [PubMed: 25744176]
- [6]. Brennan CW, Verhaak RGW, McKenna A, et al. The Somatic Genomic Landscape of Glioblastoma. *Cell*. 2013;155:462–477. [PubMed: 24120142]
- [7]. Liebnier S, Dijkhuizen RM, Reiss Y, et al. Functional morphology of the blood-brain barrier in health and disease. *Acta Neuropathol*. 2018;135:311–336. [PubMed: 29411111]
- [8]. Lee DH, Ryu H-W, Won H-R, et al. Advances in epigenetic glioblastoma therapy. *Oncotarget* [Internet]. 2017;8 Available from: <http://www.oncotarget.com/fulltext/14612>.
- [9]. Lee CY. Strategies of temozolomide in future glioblastoma treatment. *Onco. Targets. Ther*. 2017;10:265–270. [PubMed: 28123308]

- [10]. Kloepper J, Riedemann L, Amoozgar Z, et al. Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. *Proc. Natl. Acad. Sci.* 2016;113:4476–4481. [PubMed: 27044098]
- [11]. Kimura T, Takabatake Y, Takahashi A, et al. Chloroquine in cancer therapy: A double-edged sword of autophagy. *Cancer Res.* 2013;73:3–7. [PubMed: 23288916]
- [12]. Huang Z, Zhou L, Chen Z, et al. Stress management by autophagy: Implications for chemoresistance. *Int. J. Cancer.* 2016;139:23–32. [PubMed: 26757106]
- [13]. Pratt J, Iddir M, Bourgault S, et al. Evidence of MTCBP-1 interaction with the cytoplasmic domain of MT1-MMP: Implications in the autophagy cell index of high-grade glioblastoma. *Mol. Carcinog.* 2016;55:148–160. [PubMed: 25640948]
- [14]. Shimizu S, Yoshida T, Tsujioka M, et al. Autophagic cell death and cancer. *Int. J. Mol. Sci.* 2014;15:3145–3153. [PubMed: 24566140]
- [15]. Anguiano J, Garner T, Mahalingam M, et al. Chemical modulation of chaperone-mediated autophagy by retinoic acid derivatives. *Nat Chem Biol.* 2013;9:374–382. [PubMed: 23584676]
- [16]. Parzych KR, Klionsky DJ. An Overview of Autophagy: Morphology, Mechanism, and Regulation. *Antioxid. Redox Signal.* 2014;20:460–473. [PubMed: 23725295]
- [17]. Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* 2010;22:124–131. [PubMed: 20034776]
- [18]. Weathers SPS, Gilbert MR. Toward Personalized Targeted Therapeutics: An Overview. *Neurotherapeutics.* 2017; 14:256–264. [PubMed: 27995437]
- [19]. Mizushima N, Levine B. Autophagy in mammalian development and differentiation. *Nat. Cell Biol.* 2010;12:823–830. [PubMed: 20811354]
- [20]. Jawhari S, Bessette B, Hombourger S, et al. Autophagy and TrkC / NT-3 signaling joined forces boost the hypoxic glioblastoma cell survival. 2017;1–12.
- [21]. Saran F, Chinot OL, Henriksson R, et al. Bevacizumab, temozolomide, and radiotherapy for newly diagnosed glioblastoma: Comprehensive safety results during and after first-line therapy. *Neuro. Oncol.* 2016;18:991–1001. [PubMed: 26809751]
- [22]. Huang H, Song J, Liu Z, et al. Autophagy activation promotes bevacizumab resistance in glioblastoma by suppressing Akt/mTOR signaling pathway. *Oncol. Lett.* 2018; 15:1487–1494. [PubMed: 29434840]
- [23]. Golden EB, Cho H-Y, Jahanian A, et al. Chloroquine enhances temozolomide cytotoxicity in malignant gliomas by blocking autophagy. *Neurosurg. Focus.* 2014;37:E12.
- [24]. Yuan X, Du J, Hua S, et al. Suppression of autophagy augments the radiosensitizing effects of STAT3 inhibition on human glioma cells. *Exp. Cell Res.* [Internet]. 2015;330:267–276. Available from: <http://dx.doi.org/10.1016/j.yexcr.2014.09.006>. [PubMed: 25220423]
- [25]. Ye H, Chen M, Cao F, et al. Chloroquine, an autophagy inhibitor, potentiates the radiosensitivity of glioma initiating cells by inhibiting autophagy and activating apoptosis. *BMC Neurol.* [Internet]. 2016;16:178 Available from: <http://bmcneurol.biomedcentral.com/articles/10.1186/s12883-016-0700-6>. [PubMed: 27644442]
- [26]. Takahashi A, Kimura T, Takabatake Y, et al. Autophagy guards against cisplatin-induced acute kidney injury. *Am. J. Pathol.* 2012;180:517–525. [PubMed: 22265049]
- [27]. Ma B, Yuan Z, Zhang L, et al. Long non-coding RNA AC023115.3 suppresses chemoresistance of glioblastoma by reducing autophagy. *BBA - Mol. Cell Res.* 2017;1864:1393–1404.
- [28]. Sharma K, Le N, Alotaibi M, et al. Cytotoxic autophagy in cancer therapy. *Int. J. Mol. Sci.* 2014;15:10034–10051. [PubMed: 24905404]
- [29]. Zhang X, Li W, Wang C, et al. Inhibition of autophagy enhances apoptosis induced by proteasome inhibitor bortezomib in human glioblastoma U87 and U251 cells. *Mol. Cell. Biochem.* 2014;385:265–275. [PubMed: 24104452]
- [30]. Chakrabarti M, Klionsky DJ, Ray SK. miR-30e blocks autophagy and acts synergistically with proanthocyanidin for inhibition of AVEN and BIRC6 to increase apoptosis in glioblastoma stem cells and glioblastoma SNB19 cells. *PLoS One.* 2016;11:1–17.
- [31]. Chakrabarti M, Ray SK. Anti-tumor activities of luteolin and silibinin in glioblastoma cells: Overexpression of miR-7–1–3p augmented luteolin and silibinin to inhibit autophagy and induce apoptosis in glioblastoma in vivo. *Apoptosis.* 2016;21:312–328. [PubMed: 26573275]

- [32]. Ren F, Zhang L, Zhang X, et al. Inhibition of glycogen synthase kinase 3 β promotes autophagy to protect mice from acute liver failure mediated by peroxisome proliferator- activated receptor α . *Cell Death Dis.* 2016;7:e2151. [PubMed: 27010852]
- [33]. Taylor M, Ray SK. Prospects of Enhancing Anti-Cancer Activities of Guercetin the Treatment of Glioblastoma In: Watanabe H, editor. *Horizons Cancer Res.* 2017 Nova Science Publishers, Inc; 2016 p. 173–192.
- [34]. Chiao MT, Cheng WY, Yang YC, et al. Suberoylanilide hydroxamic acid (SAHA) causes tumor growth slowdown and triggers autophagy in glioblastoma stem cells. *Autophagy.* 2013;9:1509–1526. [PubMed: 23962875]
- [35]. Rosenfeld MR, Ye X, Supko JG, et al. A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy.* 2014;10:1359–1368. [PubMed: 24991840]
- [36]. Zou Y, Wang O, Li B, et al. Temozolomide induces autophagy via ATM-AMPK-ULK1 pathways in glioma. *Mol. Med. Rep.* 2014;10:411–416. [PubMed: 24737504]
- [37]. Li Z, Meng X, Jin L. Icaritin induces apoptotic and autophagic cell death in human glioblastoma cells. *Am. J. Transl. Res.* 2016;8:4628–4643. [PubMed: 27904667]
- [38]. Yu O, Liu L, Wang P, et al. EMAP-II Sensitize U87MG and Glioma Stem-like Cells to Temozolomide Via Induction of Autophagy-mediated Cell Death and G2/M Arrest. *Cell Cycle.* 2017;4101:00–00.
- [39]. Josset E, Burckel H, Noel G, et al. The mTOR inhibitor RAD001 potentiates autophagic cell death induced by temozolomide in a glioblastoma cell line. *Anticancer Res.* 2013;33:1845–1851. [PubMed: 23645729]
- [40]. Baskar R, Lee KA, Yeo R, et al. Cancer and Radiation Therapy: Current Advances and Future Directions. *Int. J. Med. Sci.* 2012;9:193–199. [PubMed: 22408567]
- [41]. Tam SY, Wu VWC, Law HKW. Influence of autophagy on the efficacy of radiotherapy. *Radiat. Oncol.* 2017;12:57. [PubMed: 28320471]
- [42]. Shi F, Guo H, Zhang R, et al. The PI3K inhibitor GDC-0941 enhances radiosensitization and reduces chemoresistance to temozolomide in GBM cell lines. *Neuroscience.* 2017;346:298–308. [PubMed: 28147244]
- [43]. Luca Gravina G, Mancini A, Mattei C, et al. Enhancement of radiosensitivity by the novel anticancer quinolone derivative vosaroxin in preclinical glioblastoma models. *Oncotarget.* 2017;8:29865–29886. [PubMed: 28415741]
- [44]. Liu R, Li J, Zhang T, et al. Itraconazole suppresses the growth of glioblastoma through induction of autophagy. *Autophagy.* 2014;10:1241–1255. [PubMed: 24905460]
- [45]. Benedetti E, Antonosante A, D'Angelo M, et al. Nucleolin antagonist triggers autophagic cell death in human glioblastoma primary cells and decreased in vivo tumor growth in orthotopic brain tumor model. *Oncotarget.* 2015;6:42091–42104. [PubMed: 26540346]
- [46]. Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding Chloroquine to Conventional Treatment for Glioblastoma Multiforme. *Ann. Intern. Med.* 2006;144:337–343. [PubMed: 16520474]
- [47]. Cl Chude, Amaravadi RK Targeting autophagy in cancer: Update on clinical trials and novel inhibitors. *Int. J. Mol. Sci.* 2017;18.
- [48]. Wu YT, Tan HL, Shui G, et al. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. *J. Biol. Chem.* 2010;285:10850–10861. [PubMed: 20123989]
- [49]. Donohue E, Tovey A, Vogl AW, et al. Inhibition of autophagosome formation by the benzoporphyrin derivative verteporfin. *J. Biol. Chem.* 2011;286:7290–7300. [PubMed: 21193398]
- [50]. Ronan B, Flamand O, Vescovi L, et al. A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nat. Chem. Biol.* 2014;10:1013–1019. [PubMed: 25326666]
- [51]. Akin D, Wang SK, Habibzadegah-Tari P, et al. A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy.* 2014;10:2021–2035. [PubMed: 25483883]

- [52]. Takahashi A, Kimura F, Yamanaka A, et al. Metformin impairs growth of endometrial cancer cells via cell cycle arrest and concomitant autophagy and apoptosis. *Cancer Cell Int.* 2014;14:1–12. [PubMed: 24393431]
- [53]. Bonapace L, Bornhauser BC, Schmitz M, et al. Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *J. Clin. Invest.* 2010;120:1310–1323. [PubMed: 20200450]
- [54]. Cloonan SM, Williams DC. The antidepressants maprotiline and fluoxetine induce Type II autophagic cell death in drug-resistant Burkitt's lymphoma. *Int. J. Cancer.* 2011;128:1712–1723. [PubMed: 20503272]
- [55]. Law BYK, Chan WK, Xu SW, et al. Natural small-molecule enhancers of autophagy induce autophagic cell death in apoptosis-defective cells. *Sci. Rep.* 2014;4:1–14.
- [56]. Wang H, Li D, Li X, et al. Mammalian target of rapamycin inhibitor RAD001 sensitizes endometrial cancer cells to paclitaxel-induced apoptosis via the induction of autophagy. *Oncol. Lett.* 2016;12:5029–5035. [PubMed: 28105210]
- [57]. Kwitkowski VE, Prowell TM, Ibrahim A, et al. FDA Approval Summary: Temsirolimus as Treatment for Advanced Renal Cell Carcinoma. *Oncologist.* 2010;15:428–435. [PubMed: 20332142]
- [58]. Hess G, Herbrecht R, Romaguera J, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J. Clin. Oncol.* 2009;27:3822–3829. [PubMed: 19581539]
- [59]. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome Formation in Mammalian Cells. *Cell Struct. Funct.* 2002;27:421–429. [PubMed: 12576635]
- [60]. Martinet W, Roth L, Meyer GRY De. Standard Immunohistochemical Assays to Assess Autophagy in Mammalian Tissue. *Cells.* 2017;6:17.

Fig. 1.

Temozolomide (TMZ) and mechanism of action of TMZ for alkylation of DNA in glioblastoma cells. The chemical structure of TMZ contains an imidazole ring. It is a very small molecule (194 Da), which is promptly absorbed in the digestive tract. TMZ is a lipophilic compound and it is can go across the blood-brain barrier (BBB). In acidic pH of stomach, TMZ remains stable. But in slightly basic pH of blood and brain tissues, TMZ goes through spontaneous hydrolysis to produce the active metabolite 5-(3-methyltriazene-1-yl) imidazole-4- carboxamide (MTIC). Further, MTIC is hydrolyzed to 5-amino-imidazole-4- carboxamide (AIC) and the highly reactive methyldiazonium cation. It is the methyldiazonium cation that alkylates the purine bases (guanine and adenine) in DNA in glioblastoma cells.

Fig. 2.

The four distinct phases in mechanism of the autophagy and the corresponding molecular machinery. Purple circles represent the LC3 protein family, while red circles represent the Atg protein family. Orange ovals and the large red oval represent various intracellular structures that are degraded and recycled during autophagy. The mechanism of autophagy is explained in great detail in the text.

Fig. 3.

Schematic presentation of the spectrum of autophagy inhibition vs. autophagy activation. Therapeutic strategies show how a push towards either direction can potentiate cell death. The middle of the figure demonstrates that all cells are undergoing a baseline level of autophagy in order to maintain homeostasis. The treatment modalities depicted represent cancer therapies and environmental conditions where autophagy inhibition or its activation has been demonstrated to promote cell death. For example, by inhibiting autophagy in hypoxic conditions, additional cell death will occur. By activating autophagy during radiation therapy or when chemoresistance is rampant, autophagic cell death can act as an additional cell death pathway.

Table 1.

Autophagy inhibition as therapeutic strategy in glioblastoma

Autophagy inhibitor	Glioblastoma cell line	Treatment protocol	Results	Source
Chloroquine (CQ)	U251, LN229, U87MG	Treatment with 20 μ M TMZ and 10 μ M CQ alone or in combination for 24 hours.	TMZ in combination with CQ increased the chemosensitivity of glioblastoma cells to TMZ by inhibiting protective autophagy.	[22]
IncRNA AC023115.3	U87MG, U251MG	Knockdown of AC023115.3 $\rightarrow$ Treatment with 15 μ M Cisplatin for 24 hours.	Cisplatin treatment induced increased expression of AC023115.3 IncRNA. AC023115.3 enhanced Cisplatin-induced apoptosis through autophagy inhibition.	[26]
3-MA or Atg7 siRNA	U87MG, U251	Treatment with 0, 5, 10, and 15 μ M Bortezomib for 24 hours $\rightarrow$ 5 mM 3-MA for 24 hours or Atg7 siRNA for 24 hours.	Bortezomib induced apoptosis and autophagy in glioblastoma cells. Subsequent autophagy inhibition with either 3-MA or Atg7 siRNA resulted in a further decrease in cell proliferation and increase in apoptosis.	[28]
miR-30e	GSCs, SNB19	Autophagy induced with 2 mM sodium sulfite treatment for 24 hours $\rightarrow$ 50 nM miR-30e for 12 hours $\rightarrow$ 150 μ M PAC for 24 hours.	Combination of miR-30e and PAC synergistically induced apoptosis in glioblastoma cells through both the intrinsic and extrinsic pathways of apoptosis by inhibiting AVEN, BIRC6, and Beclin-1 activation.	[29]
Luteolin (LUT)	U87MG, T98G	Autophagy induced with 200 nM RAPA for 24 hours $\rightarrow$ 20 μ M LUT and 50 μ M SIL for 24 hours.	Combination treatment with LUT and SIL inhibited RAPA-induced autophagy by suppressing PKC α and Beclin-1, resulting in increased apoptosis through overexpression of the tumor suppressor miR-7-1-3p.	[30]

Table 2.

Radiotherapy and chemotherapy for autophagy activation for combating glioblastoma

Autophagy activator	Glioblastoma cell line	Treatment protocol	Results	Source
<p>8</p>	U87MG	Treatment with 0, 5, 10, and 20 μ M Icaritin for 24, 48, and 72 hours.	Icaritin induced caspase-dependent apoptosis and autophagy through ROS generation, activation of AMPK, and inhibition of mTOR.	[36]
U87MG and C6	Treatment with DMSO (control), 2, and 5 μ M Itraconazole for 36 hours.	Itraconazole inhibited cell proliferation in U87MG and C6 cells in vitro and in vivo. Itraconazole also induced autophagy by inhibiting cholesterol trafficking, which resulted in inhibition of Akt-mTOR signaling $\rightarrow$ autophagy activation.	[43]
LN229, U251, SNB19, SF268, T98G, U138, U373, U118, LN18, A172, U87MG, SW1783, D54, BT12M, GSCs-5, GSCs-7	Glioblastoma cells were treated with 4 Gy of RT + the IC ₂₀ concentration of Vosaroxin according to the particular cell line for 24 hours.	Vosaroxin + RT showed significant anti-cancer activity in vitro and in vivo. Treatment induced apoptosis, autophagy, and necrosis.	[42]
U87MG	Treatment with 10 μ M RAD001 + 20 μ M TMZ either alone or in combination for 3, 6, 9, or 12 days.	Combination treatment with RAD001 and TMZ resulted in low levels of apoptotic cell death; but rather, high amounts of autophagic cell death.	[38]
U87MG and GSCs	Treatment with either DMSO (control), 0.5 nM EMAP-II, 100 μ M TMZ, or EMAP-II + TMZ for 72 hours.	TMZ + EMAP-II treatment resulted in lower cell viability than with TMZ-only treatment by lowering the IC ₅₀ . EMAP-II enhanced levels of autophagy through inhibition of mTOR.	[37]
T98G, SHG44, and A172	Cells were treated with 50 μ M TMZ, 2 μ M GDC-0941, 6 Gy IR, or the combination of each.	GDC-0941 alone promoted apoptosis in vitro. When combined with TMZ and IR, GDC-0941 promoted additional apoptosis, augmented autophagy, and inhibited cell migration.	[41]