Acta Biochim Biophys Sin (2009): 341–351 | © The Author 2009. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmp028.
Advance Access Publication 3 April 2009

Review

The role of autophagy in sensitizing malignant glioma cells to radiation therapy

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Malignant gliomas represent the majority of primary brain tumors. The current standard treatments for malignant gliomas include surgical resection, radiation therapy, and chemotherapy. Radiotherapy, a standard adjuvant therapy, confers some survival advantages, but resistance of the glioma cells to the efficacy of radiation limits the success of the treatment. The mechanisms underlying glioma cell radioresistance have remained elusive. Autophagy is a protein degradation system characterized by a prominent formation of double-membrane vesicles in the cytoplasm. Recent studies suggest that autophagy may be important in the regulation of cancer development and progression and in determining the response of tumor cells to anticancer therapy. Also, autophagy is a novel response of glioma cells to ionizing radiation. Autophagic cell death is considered programmed cell death type II, whereas apoptosis is programmed cell death type I. These two types of cell death are predominantly distinctive, but many studies demonstrate a cross-talk between them. Whether autophagy in cancer cells causes death or protects cells is controversial. The regulatory pathways of autophagy share several molecules. PI3K/Akt/mTOR, DNA-PK, tumor suppressor genes, mitochondrial damage, and lysosome may play important roles in radiation-induced autophagy in glioma cells. Recently, a highly tumorigenic glioma tumor subpopulation, termed cancer stem cell or tumor-initiating cell, has been shown to promote therapeutic resistance. This review summarizes the main mediators associated with radiation-induced autophagy in malignant glioma cells and discusses the implications of the cancer stem cell hypothesis for the development of future therapies for brain tumors.

Keywords autophagy; glioma cell; radiation; PI3K/Akt/mTOR; DNA-PK

Received: November 1, 2008 Accepted: February 25, 2008

Introduction

Malignant gliomas are the most common primary central nervous system (CNS) tumors in adults accounting for 78% of all primary malignant CNS tumors [1]. The current standard treatments for malignant gliomas include surgical resection, radiation therapy, and chemotherapy. Despite this multimodality treatment, clinical recurrence or progression is nearly universal. The median survival of patients with gliomas is only 9–12 months [2]. Ionizing radiation (IR) is the gold-standard adjuvant treatment for malignant gliomas. Although radiation-induced apoptosis has extensively been studied over the past decade, it is not a major form of cell death (believed to account for 20%) [3]. Other forms of non-apoptotic cell death have been described, which include mitotic catastrophe, necrosis, autophagy, and senescence. Malignant glioma cells are less resistant to autophagy-related cell death than to apoptosis [4,5].

Autophagy is a so-called 'self-eating' system responsible for degrading long-lived proteins and cytoplasmic organelles, the products of which are recycled to generate macromolecules and ATP so as to maintain cellular homeostasis [6]. This ability makes autophagy a good candidate for a survival mechanism in response to several stresses, such as damaged mitochondria, protein aggregation, pathogens, and nutrient starvation [6, 7]. However, several recent studies suggest that autophagy also functions as a pro-death mechanism at the cellular level [7–10]. Autophagy has also been noted in neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases [7,11], and in dying cells during development and tissue remodeling [12]. Recently, interest in autophagy has been renewed among oncologists, because different types of cancer cells undergo autophagy in response to anticancer therapies.

IR also induces autophagy in some types of cancer cells including malignant glioma cells [13–15].

Whether autophagy represents a survival mechanism or rather contributes to cell death remains uncertain. The role of autophagy in cancers treated with chemotherapy or irradiation is a topic of intense debate and may, depending on the circumstances, have diametrically opposite consequences for the tumor. The outcome of autophagy observed in malignant glioma cells after IR is not straightforward. On some occasions, autophagy induces death of damaged cells; in others, autophagy plays a protective role rather than a death program. For example, loss of DNA-associated protein kinase radiosensitizes malignant glioma cells by inducing autophagic cell death [16]. In contrast, however, inhibition of autophagy radio-sensitized malignant glioma cells, which are very resistant to radiation [15]. Autophagy is a dynamic multistep process that can be modulated at several points, both positively and negatively [17]. Some findings suggest that inhibition of autophagy at different stages may yield different outcomes. 3-Methyladenine (3-MA) [18] is a phosphatidylinositol 3-kinase (PI3K) inhibitor whereas bafilomycin A1 is an inhibitor of H^+-ATPase. 3-MA inhibits the formation of autophagy at its early stage whereas bafilomycin A1 attenuates acidification of vacuoles, resulting in the inhibition of the fusion of autophagosomes and lysosomes at the late stage [19]. Inhibition of an early stage of autophagy by 3-MA rescues cancer cells from death, whereas inhibition of a late stage of autophagy by bafilomycin A1 induces apoptosis in the same malignant glioma cell types treated with temozolomide (TMZ) [20]. However, both 3-MA and bafilomycin A1 radio-sensitize malignant glioma cells (U373-MG) [15].

Whether radiation-induced autophagy in malignant glioma cells causes death or protects cells is controversial. In multiple studies, autophagy has been inhibited pharmacologically or genetically, resulting in contrasting outcomes—survival or death—depending on the specific contexts. This fact may encourage us to better understand the nuances of how autophagy in response to radiation affects malignant glioma development, progression, and treatment so that we can use this information to prevent and more effectively treat malignant glioma. Recent studies have demonstrated the existence of a small fraction of glioma cells endowed with features of primitive neural progenitor cells and tumor-initiating function. Such cells have been defined as glioma stem cells [21–25]. Taken together, these accumulating data may lead to develop a new therapy to radio-sensitize malignant glioma cells by modulating autophagy.

Cells Death/Survival Signal Pathways in IR-Induced Autophagy

The signaling pathway composed of PI3K, protein kinase B (Akt), and mammalian target of rapamycin (mTOR) is a cell survival pathway that is important for normal cell growth and proliferation [26]. This pathway has also been implicated in tumorigenesis [27] and is becoming an important target for cancer treatment [28, 29]. The PI3K/Akt pathway is known to be activated by radiation. It is widely known that mTOR, a downstream effector of Akt, plays a critical role in regulating autophagy in cells from yeast to mammalian cells [30,31]. mTOR inhibits autophagy predominantly by activating a downstream molecule, p70S6 kinase (p70S6K).

Phosphatidylinositol 3-kinase/Protein Kinase B

Some findings strongly support inhibition of the PI3K/Akt/mTOR pathway as a promising strategy for treatment of malignant gliomas both as a single agent and as a radio-sensitizer [32,33]. Using clonogenic assays, the PI3K inhibitor LY294002 has been shown to sensitize prostate cancer cells, breast cancer cells, and malignant glioma cells to radiation [34–36]. The LY294002 and UCN-01(7-hydroxystaurosporine, Akt inhibitor) synergistically augmented the effect of rapamycin (an inducer of autophagy, inactivating mTOR [37]) in all of the three malignant glioma cell lines, U87-MG, T98G, and U373-MG. The treatment combining an mTOR inhibitor with the PI3K or Akt inhibitor can overcome the resistance of tumor cells to an mTOR inhibitor by stimulating autophagy [38]. Akt inhibitor was not shown to induce apoptosis in leukemia cells except in combination with other chemotherapeutic drugs or radiation [39]. It also showed cytotoxicity, and induced autophagy in malignant glioma U87-MG and radio resistant U87-MG cells with a constitutively active form of epidermal growth factor receptor (U87-MGΔEGFR). Furthermore, Akt inhibitor radio-sensitized both cell types by enhancing autophagy instead of apoptosis [40].

Mammalian Target of Rapamycin

In the presence of Rad001 (everolimus, an mTOR inhibitor), both autophagy and the sensitization to radiation were enhanced in Bax/Bak$^{-/-}$ DKO MEF cells, demonstrating that inhibition of pro-apoptotic proteins and induction of autophagy sensitizes cancer cells to radiation therapy [41]. In MCF-7 cells, radiation leads to inhibition of the mTOR pathway with the consequent development of autophagy, mitochondria hyperpolarization and decreased the level of the translation initiation factor eIF4G. Administration of radiation together with rapamycin showed that inhibition of the mTOR pathway in irradiated cells, unlike in non-irradiated ones, increases both p53 phosphorylation and cell death. Radiation-induced inactivation of the mTOR pathway was regarded as an underlying mechanism of radiation-induced autophagy in the human breast cancer cell line MCF-7 [42].

These findings suggest that autophagy is one of anticancer effects to inhibit the PI3K/Akt/mTOR signaling. However, how radiation leads to inactivation of the PI3K/Akt/mTOR pathway remains to be determined. How autophagy is mediated by inhibition of the PI3K/Akt/mTOR pathway, how they relate to each other and what is their contribution in the overall observed increase in the sensitivity to radiation are important questions that need to be answered.

Inhibition of the DNA-PK Radio-Sensitizes Glioma Cells (By Inducing Autophagy)

DNA repair is one of the main reasons for the resistance to IR. The main deleterious damage induced by IR is DNA-double-strand breaks (Dsbs). They can lead to fragmentation, translocation, misrepair, and loss of chromosomes. Such genotoxic events activate a number of signaling pathways that serve to activate DNA repair process and cell cycle arrest, or trigger cells into apoptosis. The major mechanism underlying the repair of DNA-Dsbs in mammalian cells is non-homologous end-joining [43,44] and requires the DNA-PK (DNA-dependent protein kinase). DNA-PK is a serine–threonine protein kinase consisting of three subunits: a 450,000-Da catalytic subunit (DNA-PKcs), a heterodimeric complex composed of the proteins Ku70 (70,000 Da) and Ku80 (86,000 Da). Ku binds to both ends of a double-strand break and recruits DNA-PKcs to the DNA end.

Cells lacking DNA-PK activity as a result of mutation in any of the subunits are deficient in the rejoining of radiation-induced DNA-Dsbs and are radiosensitive in the clonogenic assay [45,46]. In general, IR induces apoptosis and cell cycle arrest. The human glioma cell line M059J lacking the catalytic subunit of DNA-PK and its DNA-PKcs proficient counterpart M059K both display radiation-induced apoptosis. The apoptotic course differs between the two cell lines and is dependent on the quality of IR [47]. The cell cycle distribution was investigated after exposure to ^{60}Co photons or accelerated nitrogen ions (^{14}N) to elucidate the further different responses of M059J and M059K cells with regard to cell cycle perturbations [48]. Typically, IR induces DNA damage, which generates a complex cascade of events leading to cell cycle arrest, transcriptional and post-transcriptional activation of a subset of genes including those associated with DNA repair, and triggering of apoptosis. On the other hand, non-apoptotic cell death, autophagy, has recently attracted attention as a novel response of cancer cells to chemotherapy and IR. DNA damage does not induce apoptosis in DNA-PKcs$^{-/-}$ cells [49,50]. Low-dose IR induced massive autophagic cell death in M059J cells. Most M059K cells survived, and proliferated although a small portion of the cells underwent apoptosis. The treatment of M059K cells with antisense oligonucleotides against DNA-PKcs caused radiation-induced autophagy and radio-sensitized the cells. Furthermore, antisense oligonucleotides against DNA-PKcs radio-sensitized other malignant glioma cell lines with DNA-PK activity, U373-MG and T98G, by inducing autophagy [16].

DNA-PKcs is a member of the PI3K-like family [45], which has a catalytic domain homologue to PI3K. Other members of the PI3K-like family are ataxia-telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and mTOR. ATM and ATR are associated with the control of cell cycle checkpoints in response to DNA damage [51]. mTOR is a modulator of autophagy. The targets that DNA-PKcs phosphorylates include DNA-PKcs [45], two Ku subunits [52], XRCC4 [53], p53 [54], MDM2 [55], and c-Abl [56]. The phosphorylation of DNA-PKcs, Ku subunits, and XRCC4 is associated with DNA repair, whereas that of p53, MDM2, and c-Abl induces apoptosis. The different roles played by these targets are, therefore, consistent with the notion that DNA-PK has dual roles in DNA damage: one is to sense DNA damage and repair it and the other is to induce apoptosis [16]. Specifically, in response to DNA damage, the cell first tries to repair the damage and survive. However, if the cell cannot repair the damage, it undergoes apoptosis and avoids passing damaged DNA

to its progeny cells. Furthermore, the mTOR/p70S6K pathway was suppressed by IR and autophagy was induced in M059J cells [16].

Thus, it is tempting to speculate that DNA-PKcs plays a key role not only in the induction of apoptosis but also in the inhibition of autophagy. The specific inhibition of DNA-PKcs may be promising as a new therapy to radiosensitize malignant glioma cells by inducing autophagy. This information is important for the selection of treatment for glioblastoma resistant to conventional radiation therapy.

Tumor Suppressor Genes Modulate the Glioma Cellular Sensitivity to Radiation

p53 gene

Some studies imply that the tumor suppressor genes contribute to autophagy, like apoptosis and cell cycle arrest. A tumor suppressor gene, *p53*, is mutated in ~50% of all tumors [57] and is known as an important determinant of DNA-damage-induced apoptosis. Recently, a direct link between p53 and autophagy has been suggested [58]. p53 is a central signal integrator of stress, such as DNA damage, hypoxia, and oncogenic activation. These types of stresses could activate p53. As depicted in **Fig. 1**, DNA damage following IR leads to activation of p53. p53 was demonstrated to inhibit the mTOR pathway via activation of AMP-activated kinase (AMPK) [59]. AMPK in turn inhibits mTOR via upregulation of the *PTEN* and *TSC2* genes [59].

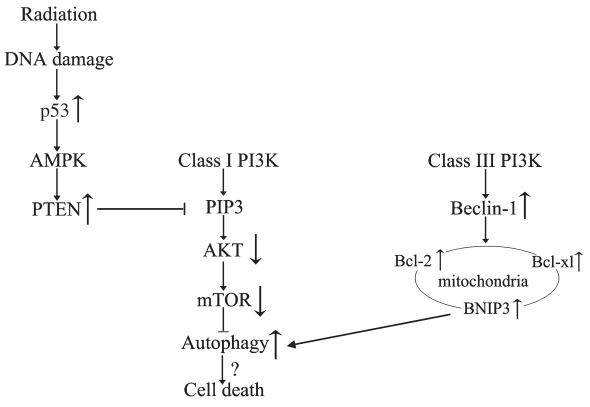

Fig. 1 Schematic model illustrating possible signal pathways that regulate radiation-induced autophagy in malignant glioma cells PI3K/AKT/mTOR, p53, PTEN, Beclin-1, BNIP3, and mitochondrial damage may play important roles. Their inter-relationship and differential contribution to the effect of radiotherapy to glioma has yet to be determined.

It has been shown that autophagy can be modulated by p53 [59]. Furthermore, damage-regulated autophagy modulator (DRAM), a *p53* target gene encoding a lysosomal protein that induces autophagy, is an effector of p53-mediated death. The discovery of DRAM suggests that induction of autophagy by p53 via DRAM contributes to apoptotic cell death [58]. However, apoptosis upon irradiation contributes only minor to the therapeutic effect in solid tumor cells [60, 61]. The level of p53 does not influence the formation of autophagic vesicles upon irradiation because there is no difference in accumulation of autophagosomes among HTB43 pharyngeal cancer, MDA-MB-231 breast cancer, and HTB35 cervical squamous cell carcinoma cells with mutated p53, or A549 lung cancer cells with wild-type p53 and A549 cells in which p53 function was blocked by activation of an Ecdysone-inducible mutated p53 (mtp53) construct [62].

PTEN gene

Another tumor suppressor gene *PTEN* (phosphatase and tensin homologue on chromosome 10) also induces autophagy [63]. PTEN modulates the cell cycle, cell survival, and cell growth by inhibiting the PI3K/Akt pathway. PTEN dephosphorylates the second messenger PIP3, interrupting PI3K activation of Akt and decreasing overall flux through the PI3K pathway [64]. PTEN has been shown to promote autophagy in HT-29 colon cancer cells [63]. Moreover, the prostate cancer cell lines $PTEN^{-/-}$ PC-3 and $PTEN^{+/+}$ DU145 became more vulnerable to irradiation after treatment with RAD001 (mTOR inhibitor), with the PTEN-deficient PC-3 cell line showing the greater sensitivity. This increased susceptibility to radiation is associated with induction of autophagy [65]. Mutation of PTEN has also been observed in malignant glioma cells [66,67]. These data implicate that PTEN may play a role in radiation-induced autophagy in malignant glioma cells.

Beclin 1 gene

Beclin 1, the mammalian orthologue of the yeast *Apg6*/*Vps30* gene, plays a role in two fundamentally important cell biological pathways: autophagy and apoptosis. Beclin 1 is a major determinant in the initiation of autophagy [68–70]. Beclin-1 interacts with Bcl-2/Bcl-xL and forms part of a class III PI3K complex playing an important role in production of PI-3-phosphate. The Beclin-PI3K complex is located at the cytosol and the trans-Golgi network. This may be essential for sorting autophagosomal components and lysosomal proteins [68,71–73]. In fact, Beclin 1 is mono-allelically deleted

in human breast, ovarian, gastric, and prostate cancers and is expressed at reduced levels in those tumors [13,68,74,75]. The expression of Beclin 1 protein and its mRNA were found decreased also in a series of human brain tumors including glial and non-glial neoplasms, which had previously been demonstrated in a few experimental studies, both in spontaneous and in therapy-induced autophagy [76]. Further studies are needed to highlight Beclin 1 function in radiation-induced autophagy in malignant glioma cells.

Other tumor suppressor molecules associated with autophagy include BNIP3 and death-associated protein kinase (DAPK). The pro-cell death Bcl-2 family member BNIP3 (Bcl-2/adenovirus E1B 19 kDa interacting protein 3) is known to induce autophagy and cell death. It was shown that BNIP3 plays a central role in As_2O_3-induced autophagic cell death in malignant glioma cells [77]. Recently, BNIP3 was reported to induce autophagy, while expression of BNIP3 siRNA or a dominant-negative form of BNIP3 reduced hypoxia-induced autophagy in two glioma cell lines (U87 and U373) [78]. DAPK and DAPK-related protein kinase-1 proteins are calcium/calmodulin-related serine/threonine death kinases that induce both apoptotic and autophagic cell death in cancer cells [79].

The interrelationship between these tumor suppressors and their differential role in modulating the glioma cellular sensitivity to radiation remain to be determined. More studies will be necessary to clarify how to best manipulate these pathways before such new therapies can be developed.

The Glioma Genome

Cancer is a disease of genome alterations. A statistical approach called Genomic Identification of Significant Targets in Cancer (GISTIC) is applied to a newly generated, high-resolution data set of chromosomal aberrations in 141 gliomas. Focusing on chromosome 7 (chr7), focal high-level amplification at the epidermal growth factor receptor (*EGFR*) gene is associated with the activation of EGFR itself whereas broad lower-level amplification of the whole chromosome often activates the MET axis by increasing the dosage of both MET and its ligand hepatocyte growth factor (HGF) [80]. Co-expression of EGFR deletion mutant variant III (EGFRvIII) and PTEN by glioblastoma cells are associated with responsiveness to EGFR kinase inhibitors [81]. To identify the genetic alterations in gliomas, Parsons *et al.* [82] sequenced 20,661 protein coding genes, determined the presence of amplifications and deletions using high-density oligonucleotide arrays, and performed gene expression analyses using next-generation sequencing technologies in 22 human tumor samples. This comprehensive analysis led to the discovery of a variety of genes that were not known to be altered in gliomas. McLendon *et al.* [83] reported the interim integrative analysis of DNA copy number, gene expression, DNA methylation aberrations in 206 glioblastomas and nucleotide sequence aberrations in 91 of the 206 glioblastomas. They provide new insights into the roles of ERBB2, NF1, and TP53, uncovers frequent mutations of the phosphatidylinositol-3-OH kinase regulatory subunit gene *PIK3R1*.

These analyses provide a network view of the pathways altered in the development of glioblastoma. The remaining events likely point to cancer-related genes and other functional elements that remain to be discovered. This may substantially increase the sensitivity to radiation in glioma.

Mitochondrial Damage in IR-Induced Autophagy

Mitochondria can perform multiple cellular functions including energy production, cell proliferation, and apoptosis. However, the role of mitochondrial damage in autophagy is not clear. In fact, low levels of mitochondrial membrane permeabilization and depolarization can trigger sequestration and autophagy of damaged mitochondria [84,85]. A post-mitochondrial caspase cascade is delayed as a result of early disposal of damaged mitochondria within autophagosomes [86]. Mitochondrial transmembrane potential dissipate to some extent in autophagy induced by radiation, TMZ, and arsenic trioxide in malignant glioma cells [15,20,77]. Another study clearly shows that superoxide anion generated by selenite triggers mitochondrial damage and subsequent autophagy, leading to irreversible cell death in glioma cells [87]. Further investigation is indicated to examine the mechanism of mitochondrial damage in IR-induced autophagy in malignant glioma cells.

Lysosome in IR-Induced Autophagy

Autophagy is a lysosome-dependent degradative pathway frequently activated in tumor cells treated with chemotherapy or radiation. IR-mediated cell damage is, *in vitro* and perhaps *in vivo*, a consequence of intralysosomal iron-catalyzed oxidative processes leading to

lysosomal rupture with release of hydrolytic enzymes and redox-active iron [88]. Lysosomes have an important role in injury of cells and tissues. In radiation response, autophagy is increased in some cases and lysosomes are responsible for regulating the degradation of the phagocytotic vacuoles. Tumor invasion and metastasis are associated with altered lysosomal trafficking and increased expression of the lysosomal proteases termed cathepsins. Genetic suppression of cathepsin B and matrix metalloprotease-9 expression significantly reduced tumor cell invasion, tumor growth, and angiogenesis in a mouse glioblastoma model [89]. Further studies are, however, required to determine the function of lysosome in radiation-induced autophagy in malignant glioma cells.

Switch between Apoptosis and Autophagy

There is ample evidence that radiation-induced cell death is affected by various intertwined biochemical processes in the autophagic and apoptotic pathways.

Bax and Bak act as a gateway for caspase-mediated cell death. They play critical roles in mediating the mechanism of cell death following irradiation. The inhibition of apoptosis resulted in an increase in radio-sensitivity of the cancer cells. Irradiation up-regulates autophagic programmed cell death in cells that are unable to undergo Bax/Bak-mediated apoptotic cell death [90].

Activation of PI3K/Akt/mTOR biochemical cascade confers survival advantage in neoplastic cells by both inhibitory effects of mTOR on autophagy and the inhibitory effect of Akt on apoptosis. Some results showed that both apoptosis and autophagy pathways are intertwined through PI3K/Akt/mTOR regulation under irradiation. By blocking apoptosis with the pan-caspase inhibitor zVAD, autophagy was effectively increased in both the PC-3 and DU145 prostate cancer cell lines. Furthermore, both of the cell lines exhibited overall decreased cell survival when zVAD was combined with RAD001. The zVAD-induced inhibition of apoptosis or the RAD001-induced autophagy resulted in increased radio-sensitivity when employed singularly, while combination of zVAD and RAD001 led to additive, rather than synergistic, effects on cell death [65].

The cytotoxicity of radiation is increased in the situations of autophagy upregulation, possibly because of the synergistic and redundant mechanisms that can amplify the death trigger signaling through the endoplasmic reticulum (ER) stress. ER is an organelle present in eukaryotic cells for key functions, such as calcium sequestration, protein translation and folding, and maturation. Although ER stress has primarily been associated with cell survival under cellular stress, insurmountable cell stress triggers programmed death pathway, usually via apoptosis. However, it has been shown recently that ER stress can also induce cell death through activation of alternative pathway in autophagy [91].

Whether autophagy observed in treated cancer cells represents a mechanism that contributes to tumor cell resistance to therapy-induced apoptosis or a mechanism for initiating a non-apoptotic form of programmed cell death remains controversial. The ability of radiation or chemotherapy to induce cell death in cancer cell lines that display resistance to apoptosis depends on type II programmed cell death executed by autophagy [92]. Some data demonstrate that inhibitors of autophagy enhance the efficacy of therapeutic strategies designed to induce tumor cell apoptosis [93]. Another report provides evidences that besides apoptosis induction 4-HPR can also induce autophagy in glioma cell. 4-HPR-induced autophagy may provide survival advantage and inhibition of autophagy may enhance the cytotoxicity to 4-HPR [94]. There are many important questions to be addressed in future investigations in trying to determine the relative influences on apoptosis and autophagy in glioma cells. It will be important to identify the presence of biochemical switches that direct glioma cells towards apoptosis or autophagy.

Glioma Stem Cells in Radiation Resistance

The brain tumor stem cell hypothesis proposes the existence of original multipotent glioma cells that are characterized by the expression of stem cell markers and by the capacity for self-renewal, multi-lineage differentiation, and re-establishment of tumors after transplantation [21–25]. An implication of the brain tumor stem cell model is that brain tumor stem cells are resistant to radiation and chemotherapy and may therefore be responsible for tumor recurrence [95,96]. The molecular signatures underline the need for development of multimodality treatments targeting not only the tumor cells, but also including strategies aimed at the glioma stem-like cell compartment, and interfering with tumor–host interaction that provides the specialized microenvironment relevant for the maintenance of tumor stem-like cells (the stem cell niche), angiogenesis, and immune response [97]. Targeting L1CAM, a neuronal cell adhesion molecule, using lentiviral-mediated short hairpin RNA (shRNA) interference in $CD133^{+}$ glioma cells potently disrupted neurosphere formation, induced apoptosis, and inhibited growth specifically in

glioma stem cells [98]. Malignant glioma cells expressing CD133, which was recently identified as a potential brain tumor stem cell marker in brain cancer [21] and in other solid tumors [99,100], are resistant to IR because they are more efficient at inducing the repair of damaged DNA than is the bulk of the tumor cells. Radiation treatment fails in the long run because it cannot kill the subpopulation of $CD133^+$ tumor-initiating cells [96]. Cancer stem cells (CSCs) contribute to glioma radioresistance through preferential activation of the DNA damage checkpoint response and an increase in DNA repair capacity [96,101]. Although IR damages tumor cells through several mechanisms, IR kills cancer cells primarily through DNA damage. The ability to repair DNA damage is essential to cellular survival because maintaining DNA breaks induces apoptosis, senescence or autophagy [16,102–104]. Delta-24-RGD, an oncolytic adenovirus with enhanced tropism to glioma cells and selective replication in cancer cells with an abnormal Rb pathway [51,105], efficiently eliminates glioma CSCs. Glioma CSCs are susceptible to adenovirus-mediated cell death via autophaghy *in vitro* and *in vivo* [106]. These results imply that therapeutically targeting the glioma stem cells may yield significant benefits for glioma patients.

However, Kelly *et al.* [107] questioned xenotransplant experiments supporting the CSC hypothesis because they found a high frequency of leukemia-initiating cells (L-IC) in some transgenic mouse models. Questions remain as to whether the currently identified glioma stem cells are the cell-of-origin for glioma initiation and progression, or the results of such processes. $CD133^+$ CSC maintain only a subset of primary glioblastomas. The remainder stems from previously unknown $CD133^-$ tumor cells with apparent stem cell-like properties but distinct molecular profiles and growth characteristics *in vitro* and *in vivo* [108]. Ogden *et al.* [109] characterize the expression of a putative CSC marker (CD133) and a glial progenitor marker (A2B5) in a diverse set of human gliomas. They tested the tumorigenic potential of three different populations of glioma cells ($A2B5^+CD133^+$, $A2B5^+CD133^-$, and $A2B5^-CD133^-$) from six different tumors and found that in some cases (four of six), $CD133^-$ cells could give rise to tumors. The implication of such findings in cancer development is so far unclear.

Taken together, determining whether the growth of gliomas is sustained by most of the tumor cells or by a rare subpopulation has important ramifications for the design of novel therapies. Therefore, the brain tumor stem cell hypothesis merits more rigorous tests.

Summary

New therapies that provide highly specific tumor cell killing and complete eradication of cancer cells are urgently needed. Pretreatment of glioma cells with locked nucleic acid (LNA)-antimiR-21 oligonucleotides leads to synergistic anti-tumor efficacy both *in vitro* and *in vivo* [110]. The induced cytotoxic T lymphocyte (CTL) specific for interleukin-13 receptor alpha2 (IL-13Ralpha2) peptide could be a potential target of specific immunotherapy for human leukocyte antigen (HLA)-A2 patients with malignant glioma [111].

Given that various malignant glioma cells undergo autophagy after radiation, we propose to use autophagy to our benefit to kill malignant glioma cells. Enhancement of autophagy may promote radiation-induced autophagic cell death, and its inhibition may lead to apoptosis, thus resulting in a greater degree of malignant glioma cell death than currently available therapies do. In recent studies, we found out that malignant glioma U251 cells undergo autophagy instead of apoptosis when irradiated. We detected increases in the mRNA and protein levels of LC3-II, the protein levels of cathepsin L and the formation of autophagosomes (data not shown). By manipulating the pathways of autophagy, we may be able to develop more effective adjunctive treatment strategies to increase the sensitivity of malignant glioma to radiation. However, the role of autophagy in radiation sensitivity has not previously been established. More studies will be necessary to clarify how to best manipulate these pathways before such new therapies can be developed.

Funding

This work was supported by grants from the Natural Science Foundation of Jiangsu Province (BK20066051).

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