MINIREVIEW

BJC

British Journal of Cancer (2016) 114, 485–496 | doi: 10.1038/bjc.2016.19

Keywords: glioblastoma; autophagy; radiotherapy; temozolomide

Therapeutic interactions of autophagy with radiation and temozolomide in glioblastoma: evidence and issues to resolve

Michael I Koukourakis*[,1], Achilleas G Mitrakas[1] and Alexandra Giatromanolaki[2]

[1]Department of Radiotherapy/Oncology, Democritus University of Thrace, PO Box 12, Alexandroupolis 68100, Greece and [2]Department of Pathology, Democritus University of Thrace, Alexandroupolis 68100, Greece

Glioblastoma is a unique model of non-metastasising disease that kills the vast majority of patients through local growth, despite surgery and local irradiation. Glioblastoma cells are resistant to apoptotic stimuli, and their death occurs through autophagy. This review aims to critically present our knowledge regarding the autophagic response of glioblastoma cells to radiation and temozolomide (TMZ) and to delineate eventual research directions to follow, in the quest of improving the curability of this incurable, as yet, disease. Radiation and TMZ interfere with the autophagic machinery, but whether cell response is driven to autophagy flux acceleration or blockage is disputable and may depend on both cell individuality and radiotherapy fractionation or TMZ schedules. Potent agents that block autophagy at an early phase of initiation or at a late phase of autolysosomal fusion are available aside to agents that induce functional autophagy, or even demethylating agents that may unblock the function of autophagy-initiating genes in a subset of tumours. All these create a maze, which if properly investigated can open new insights for the application of novel radio- and chemosensitising policies, exploiting the autophagic pathways that glioblastomas use to escape death.

Glioblastoma is the most common primary brain tumour in adults, and certainly one of the most lethal human tumours (Hayat *et al*, 2007) Its incidence is about 3 new cases per 100 000 population per year, the median age of diagnosis is 64 years and most of the patients have passed away 2 years following diagnosis (Ohgaki *et al*, 2004). Unlike low-grade gliomas, complete surgical removal of glioblastoma is impossible because of the infiltrative nature of the disease and the functional vulnerability of the brain. Post-operative radiotherapy is the standard adjuvant therapy offered worldwide. Following surgery alone, the median survival is 6 months and increases to 12 months with the addition of radiotherapy (Salazar *et al*, 1979). Because of the extent and the location of the disease adjacent to critical brain areas or because of the poor performance status of patients at diagnosis, surgery is often omitted and radiation is offered as the main treatment option.

Attempts to improve the efficacy of radiotherapy with the addition of chemotherapy, mainly nitrosoureas (BCNU, CCNU), go back to the early 1970s. In 1983, Eyre *et al* in a randomised trial failed to show a benefit from the addition of CCNU and procarbazine to radiotherapy. In the same year, an RTOG study provided evidence that radiochemotherapy with BCNU or methyl-CCNU or DTIC was superior to radiotherapy alone (Chang *et al*, 1983). The benefit, however, provided by nitrosoureas was limited and questionable as stressed in subsequent randomised trials (Hatlevoll *et al*, 1985; Deutsch *et al*, 1989; Shapiro *et al*, 1989; Curran *et al*, 1992). The only therapeutic innovation that had an impact in the management of glioblastomas appeared in 2005, when a randomized study showed that temozolomide improved the median survival of patients undergoing radiotherapy by 2.6 months (Stupp *et al*, 2005). In a more recent analysis of the EORTC-NCIC trial on 573 patients, the 2-year survival was 27.2% in the temozolomide group *vs* 10.9% in the radiotherapy-alone group (Stupp, 2009). Nowadays, postoperative radiotherapy with daily administration of TMZ is the gold standard for patients with glioblastomas, although the efficacy of this therapy remains disappointing and attempts to improve the median survival using the combination of TMZ with other drugs, such as motexafin

*Correspondence: Professor MI Koukourakis; E-mail: targ@her.forthnet.gr

Received 21 September 2015; revised 21 December 2015; accepted 31 December 2015; published online 18 February 2016

gadolinium (Brachman *et al*, 2015), has failed, with the 5-year survival remaining $<10\%$.

In the era of targeted therapies, there is an increasing optimism that the identification of key molecular pathways may lead to major improvements in the management of this highly lethal disease. Glioblastomas, being highly angiogenic tumours, were thought to be susceptible to antiangiogenic therapy. Indeed, in 2009, bevacizumab (BVZ), an anti-VEGF humanised monoclonal antibody, received accelerated approval by the FDA for the treatment of recurrent glioblastoma following a randomised phase II study (Kreisl *et al*, 2009). In the most recent randomised RTOG 0825 trial, however, the addition of BVZ ($10\,mg\,kg^{-1}$ every 2 weeks) to standard chemoradiation improved the progression-free interval but had no effect on overall survival (Gilbert *et al*, 2013). Recent studies on newly diagnosed glioblastoma patients showed that the same results and sub-population of patients with better response to BVZ treatment could not be predicted by the molecular data (Poulsen *et al*, 2014). The benefit from BVZ therapy seems to be confined to oedema reduction and a cut down on the usage of corticosteroids (Thomas and Omuro, 2014).

Epidermal growth factor receptor-mediated oncogenic signalling is active in most human carcinomas and has become a target for therapeutic interventions. Indeed, anti-EGFR1 moAb and pharmacological tyrosine kinase inhibitors have shown important clinical activities in the colon, lung and head and neck cancer. About half of glioblastomas bear *EGFR* gene amplification and receptor overexpression (Hatanpaa *et al*, 2010). Mutation of *EGFR* with constitutive activation of the pathway is also quite common. Although the efficacy of such agents as single therapy is limited (van den Bent *et al*, 2009), randomised trials in combination with radiotherapy are missing.

The unique nature of the glioblastoma cells to be resistant to apoptotic stimuli, their death occurring rather through autophagy (Yao *et al*, 2003; Kanzawa *et al*, 2004), suggests that autophagy-interfering agents in combination with radiation and temozolomide may represent a new strategy to test. This hypothesis becomes much more relevant under the new light shed by studies, suggesting that autophagy is the main pathway exploited by cancer stem cells to survive and accelerate their renewal (Jiang *et al*, 2007; Gong *et al*, 2013; Berardi *et al*, 2015). Glioma stem cells is considered the most radioresistant cell population and autophagy targeting may assist to eradicate the disease (Shen *et al*, 2015; Yu *et al*, 2015). This review aims to critically present our knowledge regarding the autophagic response of glioblastoma cells to radiation and TMZ and to delineate eventual research directions to follow in the quest of improving the curability of this incurable, as yet, disease.

AUTOPHAGY AND AUTOPHAGIC DEATH

Macroautophagy (herein stated as autophagy for simplicity) is a major cell pathway dedicated to a continuous renovation of cell constituents through the degradation and recycling of long-lived or damaged proteins and even of entire organelles, such as ribosomes, mitochondria, endoplasmic reticulum and Golgi apparatus (Klionsky and Emr, 2000; Feng *et al*, 2014). During autophagy double-membrane vesicles are formed, which sequester portions of the cytoplasm and fuse with endosomal and lysosomal vesicles (Yorimitsu and Klionsky, 2005). Beclin 1 and ULK (UNC-51-like kinases) proteins are important autophagosome initiation proteins, whereas LC3A, LC3B and LC3C are key structural components of the autophagosomes, which are extensively used for the experimental study of autophagy (Kabeya *et al*, 2000; Wong *et al*, 2013), whereas the difference in expression levels and of subcellular distribution and kinetics of these proteins may be an indication of different roles during autophagy (Giatromanolaki *et al*, 2014; Koukourakis *et al*, 2015).

Autophagy-related protein 8 (Atg8/LC3), an ubiquitin-like protein, has different homologues encoded by different chromosomes in animals, including MAP1LC3A, B and C, as well as the GABARAP proteins, which are the most important constituents of the autophagosomal membranes (Shpilka *et al*, 2011). Most of them are produced as a pre-LC3 25–30 kDa protein that are cleaved by the autophagophagin (Atg4) to the soluble form of LC3, the 18 kDa LC3-I protein. Following an ubiquitination-like reaction, the exposed C terminus conjugates to the head group amine of phosphatidylethanolamine (PE) through an amide bond, becoming the so-called membrane-bound 16 kDa LC3-II form that forms both the outer and inner autophagosome membranes. The p62/sequestrosome protein is the main carrier of the waste material to the LC3-II membranes that engulf the complex within a mature autophagosome (Katsuragi *et al*, 2015). This is followed by fusion with lysosomes and digestion of the autophagolysosomal content, resulting in the disintegration of intracellular waste and at the same time providing energy to the cell. During this process, LC3-II protein can be delipidated by Atg4 proteases and liberated in the cytoplasm. The different forms of LC3 proteins and the kinetics of the p62 protein can be detected by western blot or confocal microscopy methods, providing important tools to study the autolysosomal flux in normal cells and cancer cells under various stresses (Klionsky *et al*, 2012).

Under energy-demanding stress conditions, such as starvation or hypoxia, autophagy is accelerated (Koritzinsky and Wouters, 2013; Bernard *et al*, 2015). On the one hand, excessive intensification of this very process may lead to intolerable cytoplasmic digestion, leading to cell exhaustion and death. On the other hand, accumulation of autophagosomes under stress, which block their fusion with lysosomes (e.g., hydroxychloroquine or 3-methyadenine), may also lead to autophagic death (Kimura *et al*, 2013; Saetre *et al*, 2015). Autophagy is called type II programmed cell death pathway, which is a distinct pathway compared with the apoptotic type I death pathway triggered by DNA or membrane damage followed by release of caspases from the mitochondria. Autophagy is, therefore, a balance that weighs between renovation, energy acquisition, survival and cell death, according to the external or internal stimuli received.

Cancer cells have an increased autophagic activity as shown in a series of clinicopathological studies, where LC3 and autophagy-initiating proteins are overexpressed, a feature linked with aggressive clinical behaviour (Sivridis *et al*, 2010; Giatromanolaki *et al*, 2014; Koukourakis *et al*, 2015). In a recent study, we noted that overexpression of LC3A in the form of stone-like cytoplasmic structures was linked to poor postradiotherapy prognosis in a series of patients with glioblastoma (Mehta *et al*, 2015). Since several decades we know that an inert apoptotic machinery is a very common phenomenon in cancer cells, allowing them to escape death after exposure to cytotoxic chemotherapy and radiotherapy (Hickman, 1996). Evasion from apoptosis is a well-established phenomenon in gliomas and glioblastomas (Krakstad and Chekenya, 2010). Therefore, autophagic death is an alternative pathway to pursue in an attempt to eradicate glioma cells exposed to cytotoxic stress (Zois and Koukourakis, 2009).

RADIOTHERAPY AND AUTOPHAGY IN GLIOMAS/GLIOBLASTOMAS

Several experimental studies have confirmed that glioma and glioblastoma cells exhibit important resistance to apoptosis after exposure to ionising radiation. Expression status of apoptosis-related genes such as *PTEN* may be involved in the phenomenon (Lee *et al*, 2011), and interference with various signaling pathways

such as STAT3 and EGFR may restore the ability for apoptotic death (Gao *et al*, 2010).

The study by Yao *et al* (2003) is probably the first to show that irradiation of human glioblastoma cells results in enhanced autophagy, whereas no apoptosis was evident in radiosensitive or radioresistant cell lines. Ito *et al* (2005) subsequently confirmed that ionising radiation induces cell cycle arrest and autophagic death, but not apoptosis, in glioma cell lines. Moreover, Jo *et al* (2014) suggested that autophagy is a pathway to cell death after glioma irradiation. In 2005, a study from the MD Anderson Cancer Center showed that DNA-protein kinase (DNA-PK)- (enzyme involved in the repair of DNA double-strand breaks) deficient glioma cells suffered massive autophagic death even after low doses of radiation (Daido *et al*, 2005). Intact DNA-PK pathway prevented autophagic death, but cells still exhibited a low apoptotic tendency. Of interest, induction of autophagy using siRNA against DNA-PK sensitised glioblastoma cell lines (Zhuang *et al*, 2011a, b). An additional study using particle radiation, which mainly kills through DNA double-strand damage, supports the suggestion that DNA repair disabling is a potent stimulus not only for apoptotic death but also for autophagic death in gliomas (Jinno-Oue *et al*, 2010).

The Akt/m-TOR is recognised as a major pathway regulating autophagy. Inhibitors of Akt/m-TOR activity, such as rapamycin analogues, intensify the autophagic process (Martelli *et al*, 2011). This pathway is often upregulated in tumours, including glioma (Akhavan *et al*, 2010). Akt inhibitors induce autophagic death, but not apoptotic death, in both radioresistant and radiosensitive U87 glioma cell lines and enhance sensitivity to radiation (Fujiwara *et al*, 2007). In the same cell line, Benzina *et al* (2008) found that high LET radiation kills cells through autophagy. Mehta *et al* (2015) further confirmed that Akt inhibition radiosensitises primary human glioblastoma stem-like cells. Moreover, silencing of *EGFR*, an activator of PI3K/Akt/mTOR pathway, with simultaneous induction of autophagy led to better response to IR and suppressed migration in the T98G cell line (Palumbo *et al*, 2014). These results are further supported by Gini *et al* (2013), who showed that the growth of EGFRvIII-activated glioblastoma was blocked after treatment with CC214-1 and CC214-2, which are inhibitors of mTORC1 and mTORC2. The study by Chiu *et al* (2009) suggested that arsenic trioxide also enhances the activity of radiation in glioma cell lines by augmenting the autophagic cell death, which is also supported by Carmignani *et al* (2014), who showed that glioblastoma stem cells differentiated into non-tumourigenic cells as a result of autophagy induction, after inhibition of PI3K/Akt and stimulation of mitogen-activated protein kinase pathway using arsenic trioxide and metformin, respectively. Rapamycin has also been shown to induce differentiation of glioma-initiating cells and increase their radiosensitivity by activating autophagy (Zhuang *et al*, 2011a, b).

Furthermore, research related to NF-κB, a factor that suppresses autophagy in response to TNFa (Djavaheri-Mergny *et al*, 2007), showed that treatment of glioma cells with pitavastatin (inhibitor of NF-kB) resulted in autophagic death but not apoptotic death, and empowered the activity of radiation (Tsuboi *et al*, 2009). Nuclear factor-κB is a transcription factor negatively regulating apoptotic death. Early phase blockers of autophagy, such as 3-methyladenine (3-MA), counteracted the cytotoxic effect of radiation and of the combination with pitavastatin.

A variety of researches focused on glioma radiosensitisation via autophagy suppression. Yuan *et al* (2015) exhibited that suppression of *ATG5* using siRNA or suppression of autophagy using 3-methyladenine increased the radiosensitisation effect of gliomas after *STAT3* inhibition. Moreover, according to Ye *et al* (2013), the resistant clones of glioma stem cells bear high expression levels of early growth response 1 that induce autophagy. Additionally, mitochondrial isoenzyme of $NADP^+$-dependent isocitrate dehydrogenase siRNA-transfected A172 glioma cells were sensitised after inhibition of autophagy (Kim *et al*, 2013).

There is, therefore, important evidence that in glioma cells autophagic death is the most common pathway exploited by radiation compared with apoptosis. Although reactive autophagy may occur in the context of a cell survival response to radiation, which may represent a radioresistance pathway, augmenting the autophagic response through combination with Akt/mTOR inhibitors would shift the balance to death. DNA repair enzyme inhibitors seem also to facilitate radiation-induced autophagic death, and at the same time restoring to a certain extent the apoptotic pathway. The fact that early phase autophagy inhibitors also sensitise glioma cells to radiation (Lamonaco *et al*, 2009) is not contradictory to the whole concept, as abrogating low-level autophagy that keeps the balance to the survival side is expected to increase vulnerability to radiation. Late phase autophagy inhibitors that block autophagolysosomal formation are also expected to be radiosensitisers as they abrogate cytoprotective autophagy and load the cell with waste material, resulting in autophagy-mediated death.

TEMOZOLOMIDE AND AUTOPHAGY IN GLIOMAS/ GLIOBLASTOMAS

One of the first studies regarding the role of autophagy in malignant gliomas showed that TMZ at clinically relevant concentration of 100 μM induces autophagy rather than apoptosis in malignant glioma cells, as shown by the accumulation of the LC3 protein on autophagosomal membranes. Lee *et al* (2015) suggest that TMZ in combination with chloroquine could inhibit the growth and apoptosis of glioblastomas, whereas autophagy suppression leads to the abolishment of the combination effect of TMZ and chloroquine (Lee *et al*, 2015). Moreover, blockage of autophagy at an early step by 3-MA prevented the accumulation of autophagosomes and suppressed the cytotoxic effect of TMZ. Zanotto-Filho *et al* (2015), however, suggested that autophagy has a protective role in gliomas as TMZ/curcumin treatment in combination with 3-MA leads to a reduction of cell viability. Nevertheless, bafilomycin that blocks autophagy at a late step, by preventing the fusion of mature autophagosomes with lysosomes, sensitised glioma cells to TMZ by inducing apoptotic rather than autophagic death.

These studies suggest that autophagy and drug interaction is a very complex process. Blockage of autophagosome formation abrogates the autophagic death induced by TMZ. On the contrary, non-functional accumulation of autophagosomes (induced by TMZ and blocked by late phase autophagy drugs) releases an apparent pre-existing obstacle in the apoptotic pathway, driving cells to apoptotic death. Lefranc and Kiss (2006) suggested that as glioblastoma cells are resistant to apoptosis, blocking targets that keep autophagy suppressed, such as mTOR, may enhance the activity of TMZ-induced autophagic death (Zhivotovsky *et al*, 1999).

An important link between TMZ-induced autophagic death and cell metabolism has been subsequently brought forward by Katayama *et al* (2007). In experiments in multiple glioma cell lines, TMZ consistently induced autophagy in parallel with an increase of ATP production. This ATP surge could not be blocked by glucose starvation but was blocked by agents blocking early steps of autophagy (methyladenine and *beclin 1* siRNA). As this inhibition resulted in micronucleation, it was suggested that autophagy-induced ATP surge counteracts the TMZ-induced autophagic death. Indeed, administration of pyruvate abrogated the activity of early phase autophagy inhibitors and enhanced glioma cell survival. Small molecules, such as dasatinib, which antagonise the ATP binding pocket of abl, kit or EGFR proteins,

induce autophagic cell death in glioblastoma cells and have synergistic effect with TMZ (Milano *et al*, 2009). Moreover, the association between metabolism and mitochondria with the efficacy of TMZ is supported by a several recent studies. You *et al* (2013) showed that the expression of ATAD3A, a mitochondrial protein ATPase (ATAD3A), is a prognostic factor for glioblastomas and a predictor of TMZ or radiation resistance (You *et al*, 2013). Another study that supports the hypothesis of association between the function of mitochondria and TMZ showed that metformin, which inhibits mitochondrial electron transport chain complex I, enhances the cell cytotoxicity of TMZ (Sesen *et al*, 2015).

It seems, therefore, that although exacerbation of autophagy is a death pathway exploited by TMZ, the energy released by this very process counteracts TMZ efficacy. Overactivity of the autophagic pathway in the context of a cell-cytoprotective mechanism against TMZ can be at the same time the cause of temozolomide-induced cell death. Abrogation of the end point of such an autophagic-protective mechanism, which is energy production, inevitably shifts the balance to increased autophagic death. In pathological studies, the majority of glioblastoma cases examined had an upregulated autophagic pathway at diagnosis (Aoki *et al*, 2008; Pirtoli *et al*, 2009), with overexpression of LC3, beclin 1 and ULK proteins (Giatromanolaki *et al*, 2014), presumably as an indispensable pathway for energy acquisition under the stressful metabolic demands of an accelerated growth. Any stressor, like TMZ, that further accelerates autophagy may result in autophagic death by disrupting a delicate balance between energy replenishment and suicide autophagy. The synergism found between TMZ-induced autophagy and autophagy-mediated killing effect of oncolytic adenoviruses (Yokoyama *et al*, 2008; Ulasov *et al*, 2009) may also be explained under this point of view.

An interesting observation from the clinicopathological studies is that a glioblastoma group with low levels of LC3 and beclin 1 has a poorer prognosis and may be more resistant to TMZ, although this hypothesis demands further confirmation in larger studies. A study by Fu *et al* (2009) provides important insights into this phenomenon. Following separation of CD133-positive and -negative glioblastoma cells from a freshly resected tumour, positive cells (representing glioblastoma stem cells) had lower LC3-II and beclin 1 levels compared with negative ones and were more resistant to TMZ (Fu *et al*, 2009). Both cells, however, were resistant to caspase-3 induction following incubation with TMZ. An important hypothesis that emerges from these studies is that, although autophagic death is a main pathway of glioma cell death after exposure to TMZ, glioma stem cells may have an autophagic machinery refractory to the activity of the drug and, therefore, more easily survive under exposure to TMZ, leading subsequently to tumour repopulation and growth.

The above data contrast the study by Lomonaco *et al* (2009) where CD133-positive cells seem to upregulate autophagy more potently than negative cells after exposure to radiation and to the finding that autophagy inhibition sensitises the stem cell sub-population to radiation. Similar results have been reported by Winardi *et al* (2014), who supports that high level of CD133 population and high rate of autophagy lead to a poor prognosis in astrocytomas (Winardi *et al*, 2014). Although further studies are demanded to elucidate this discrepancy that may be cancer cell line-dependent, differences in the mechanism of autophagy induction between radiation and TMZ can also underlie. In addition, it is stressed that for currently applied experimental techniques, it is difficult to distinguish between autophagosomal or autolysosomal non-functional accumulation *vs* intensified autophagy. Moreover, as LC3A and LC3B are distinct proteins with different response at various stimuli, studying the autophagic response with nonspecific LC3 subtype markers may provide confusing results and mask specific response patterns that may be important to TMZ activity.

In any case, restoration or protection of the autophagic flux may be important in sensitising cells to TMZ. Methylation of the promoter of *Beclin 1* gene has been reported to be a common event in breast cancer (Li *et al*, 2010). Whether hypomethylating agents can be of value to restore autophagic responsiveness in glioma stem cells and sensitivity to TMZ is a sound hypothesis. 5-Aza-20-deoxycytidine, a potent demethylating agent, is an autophagy promoter (Chen *et al*, 2011). Although it is unknown whether demethylating agents can restore autophagy in glioblastomas, we know that they restore the expression of genes involved in apoptosis (Eramo *et al*, 2005) like caspase-8, which is hypermethylated in stem cell-like glioma cells (Capper *et al*, 2009). However, differentiation-inducing agent inhibitors of histone deacetylase, such as valproic acid (Park *et al*, 2011), are potent inducers of autophagy in glioma cells, enhancing autophagic cell death but not apoptosis (Fu *et al*, 2010). Whether such agents can target TMZ-resistant glioma stem cells is another hypothesis to test. Carbamazepine is also another drug that increases the autophagic flux and protects normal tissues against radiation (Kim *et al*, 2011), presumably due to restoration of the radiation-induced autophagy blockage (Zois *et al*, 2011).

RESTORING APOPTOSIS

Another important approach aside to autophagy death pathway manipulation is to interfere with the molecular pathways aiming to overcome the relative resistance of glioma cells to apoptotic death. Both radiotherapy and TMZ are DNA-damaging agents. Radiotherapy induces both DNA single- and double-strand breaks, the kinetics and fidelity of repair of which define cell survival and clonogenic ability (Mirzayans *et al*, 2013; Morgan and Lawrence, 2015). Whether apoptosis, mitotic catastrophe or senescence follows defective DNA repair is an outcome defined, at least partially, by the oncogene and apoptosis-related gene activity in cancer cells (Vakifahmetoglu *et al*, 2008; Mirzayans *et al*, 2013). Gene therapy or small molecules restoring p53 function increase the radiation-induced apoptosis in cancer cells, as well as in glioblastomas (Villalonga-Planells *et al*, 2011; Pflaum *et al*, 2014). Temozolomide is also a DNA-damaging agent. Following its conversion to 5-aminoimidazole-4-carboxamide, it delivers methyl groups to DNA, resulting in the formation of O^6-methylguanine, which mispairs erroneously with thymine (instead of cytosine) during DNA replication, resulting in DNA double-strand break formation (Fukushima *et al*, 2009). The death pathway that follows depends on the gene expression profile of the cancer cell, the p53, PUMA and bcl-2 family of protein expression status being decisive for the activation of the apoptosis pathway under exposure to temozlomide, even of glioma stem cells (Gratas *et al*, 2014; Miao *et al*, 2015).

Gene therapy or novel therapeutic agents that restore the apoptotic machinery in gliomas may prove to be of importance in reactivating apoptotic death induced by both ionising radiation and TMZ. Inhibitors of the PDGFR seem to initiate the apoptotic pathway in apoptosis-resistant glioblastoma cells (Ziegler *et al*, 2008). Anti-EGFR therapy has been reported to sensitise CD133-positive glioma cells to radiation, presumably by restoring their apoptotic ability (Diaz Miqueli *et al*, 2009). Specific inhibitors of the checkpoint kinases Chk1 and Chk2 reverse the radioresistane of these cells (Bao *et al*, 2006). Drugs targeting the mitochondrial pore, such as lonidamine, have been shown to induce apoptotic death in TMZ-resistant glioma cells (Lena *et al*, 2009). Chang *et al* (2009) found that *Sirtuin 1* gene is exclusively expressed in CD133-positive radioresistant stem cells and that silencing this gene improves curability in experimental models. Agonist antibodies of the TRAIL death receptor 5 that induce apoptosis are

also shown to improve the efficacy of radiotherapy and TMZ (Fiveash *et al*, 2008). Gene therapy approaches, such as introduction of the caspase-8 gene or IL-24 gene, may be also useful in restoring apoptosis of glioma cells (Tsurushima *et al*, 2008; Yacoub *et al*, 2008).

WHICH AUTOPHAGIC DEATH?

The above data suggest that glioma and glioblastoma cells exhibit an autophagic response after exposure to ionising radiation and TMZ. This assumption is based on the increased accumulation of LC3-positive autophagosomes. Such a finding, however, does not necessarily mean an intensified 'functional autophagy'. In fact, disruption of the lysosomal and autophagosomal fusion may also result in LC3-positive autophagosomal accumulation, without intensification of the production of new autophagosomes. This is an important point we need to clarify regarding the actual effect of ionising radiation on the autophagic machinery. Autophagic flux studies in cancer cells are demanded to elucidate the phenomenon, and such studies are absent in the literature. Our studies in normal fibroblasts and endothelial cells suggest that radiation results in an early blockage of the autophagic flux within the first days of irradiation (Kalamida *et al*, 2015). The increase of LC3-II membrane-bound form in the soluble fraction of cells instead of the pellet fraction and the sharp increase of p62 protein (that normally disintegrates in the autophagolysosomal environment once incorporated) strongly suggest that radiation induces a non-functional accumulation of autophagosomes in cells, inducing autophagic death in a similar way that late phase autophagy blockers (such as chloroquine and bafilomycin) do. In fact, restoration of autopagic flux protects normal cells from radiation death.

Whether this phenomenon also applies in cancer cells or whether these sustain a resistant attitude against radiation-induced autophagy flux blockage demands thorough investigation. Preliminary experiments show that the response of cancer cells seems to be cell line- and radiation dose-specific (unpublished data). A biphasic relation linking radiation dose levels and the autophagic survival/death balance in cancer cells cannot be excluded. Lower doses, especially in radioresistant cells, could trigger a functional autophagic response with a rather cytoprotective role providing energy to the cells. In contrast, higher doses may disrupt the autophagic process by downregulating autophagy gene expression and/or by blocking autophagosome/lysosomal interaction shifting the balance to an autophagic death because of an accumulation of waste. This, however, is nothing more than a hypothesis that should be thoroughly examined, as different fractionation of radiotherapy may have a different effect on autophagy and may synergise better with different autophagy-interfering agents. Nevertheless, in a recent study in prostate cancer we provided a strong evidence that such a hypothesis is valid, as a radioresistant cell line intensified its autophagic flux after low-dose radiation, whereas this was blocked in a radiosensitive one using the same radiation dose (Koukourakis *et al*, 2015). It may be, therefore, that radioresistant tumours continue or accelerate their autophagic flux when exposed to low dose per fraction (i.e. within the range of standard 2 Gy per day fractionation), whereas larger fractions (hypofractionation) may block the autophagic flux and at least partially overcome radioresistance. Silencing of the LC3A gene results in important radiosensitisation, suggesting that autophagy is eventually a pathway of survival following irradiation of radioresistant cells and tumours (Koukourakis *et al*, 2015).

Nowadays, it is more than clear that autophagy is overactive in a large fraction of tumours, including gliomas (Capper *et al*, 2009), and cell death features representative of an autophagic exhaustion of the cytoplasmic material are commonly evident in the form of stone-like cytoplasmic structures. Indeed, we identified the formation of the so-called LC3A-positive 'stone-like structures' in various cancer tissues, showing a complete elimination of the cytoplasm of cells and its substitution by one or more dense structures of amorphous undigested LC3-positive material (Sivridis *et al*, 2010). This feature, also evident in human glioblastomas, was inducible under acidic conditions and glucose antagonism, showing the existence of this type of autophagic cell death in glioblastomas (Capper *et al*, 2009). This autophagic death path presumably demands functional and overactivated autophagy to uncontrollably consume the cytoplasmic content, shifting the balance from autophagic cytoprotection to autophagic death. Thus, as cancer cells use functional upregulated autophagy to survive the hypoxic and overall unfavorable tumour environment, further exogenous induction of functional autophagy, for example, through Akt/mTOR inhibitors, may push the balance towards cytoplasmic exhaustion and autophagic death.

It can be, therefore, suggested that autophagic death is not a single phenomenon but is rather characterised by at least two distinct paths: (i) the excessive autophagic activity resulting in stone-like structure formation (functional autophagic death) by exhaustion of the lysosomal potential and; (ii) the intolerable accumulation of waste non-functional autophagosomes (non-functional autophagic death) by abrogation of lysosomal fusion. Whether this latter path represents a link of autophagy with apoptosis, necrosis or mitotic catastrophe induction or it represents an entirely distinct death pathway is unknown.

THE PARADOXICAL SENSITISATION BY BOTH INDUCERS AND BLOCKERS

Nevertheless, both 'functional' and 'non-functional' autophagic death pathways triggered by radiation and TMZ can be boosted by early or late phase autophagy blockers and by autophagy inducers. This paradox can be explained as follows:

(1) Early phase autophagy blockers prevent the formation of autophagosomes, blocking therefore an important source of energy and the cytoprotective effect of a controllable functional autophagy. This, on the one hand, leads *per se* to the death of a varying fraction of tumour cells, the survival of which depended on functional autophagy. On the other hand, by blocking autophagosome formation tumour cells enter a state of energy deficit and excessive loading with damaged proteins and organelles that radiation or TMZ produces, facilitating the apoptotic death effect of these agents. Thus, early autophagy blockers may activate a link with apoptosis-like pathways. For such therapeutic approaches, apoptosis restoration policies may secure a superadditive effect.
(2) Late phase autophagy blockers, for example, those that allow the formation of autophagosomes but block their fusion with lysosomes, repress by one hand the acquisition of energy through autophagy and on the other hand load the cell with waste non-degradable autophagic vesicles. Thus, similarly to early phase blockers, they kill a varying percentage of tumour cells through energy deprivation and waste overloading. If a radiotherapy schedule and/or TMZ also induces non-functional autophagosome formation, the synergistic effect with the energy austerity effect or with the autophagosome accumulation is antagonistic or at least additive. However, if a radiotherapy schedule is given in those small fractions to induce functional autophagy, the intensified accumulation of autophagosomes using late blockers would result in superadditive effect. In fact, a combination of an early phase autophagy inducer with a late phase blocker seem an appealing therapeutic proposal.

(3) Autophagy inducers could not only target tumour cells with increased autophagy but also tumour cells with reduced autophagy. In tumour cells with active autophagy, augmentation of autophagy may shift the balance to functional autophagic death through lysosomal exhaustion and cytoplasmic degradation (stone-like death). If radiation and TMZ is scheduled to induce functional autophagy, autophagy inducers administered concurrently with radiation will accelerate functional autophagic death. If radiation and TMZ are scheduled to induce non-functional autophagosome accumulation, administration of autophagy inducers immediately before radiochemotherapy may enhance the non-functional autophagic death.

CLINICAL EXPERIENCE

Clinical experience with autophagy-manipulating agents in the treatment of glioblastoma is limited. Chloroquine (Kimura *et al*, 2013), presumably because it is already availabile in the clinical practice as an antimalaria agent (Hall, 1976), is the only autophagy inhibitor studied for the treatment of glioblastoma patients. In a phase II clinical trial, the administration of hydroxychloroquine to patients with glioblastoma undergoing radiotherapy with temozolomide confirmed an increase of autophagic vacuoles and of the LC3-II form in the peripheral blood cells, supportive of an antiautophagic activity, but the haematological toxicity of the regimen was unacceptable and the benefit in terms of survival was not evident (Rosenfeld *et al*, 2014). A small pilot study on the combination of chloroquine with radiation for the treatment of recurrent glioblastoma confirmed the feasibility of the regimen and the authors claimed encouraging treatment outcomes (Bilger *et al*, 2014). The recent observation that the acidic extracellular pH neutralises the autophagy-inhibiting activity of chloroquine stresses the importance of the development of potent autophagy inhibitors, the activity of which is independent or even better take advantage of the intratumoral hypoxic and acidic conditions (Pellegrini *et al*, 2014).

Mammalian target of rapamycin inhibitors have multiple biological activities, including the induction of autophagy (Huang and Fingar, 2014). Such agents have been introduced in the clinical practice for the treatment of renal carcinoma (Amato, 2011), and several clinical trials have investigated the activity mTOR inhibitors in patients with glioblastoma. Temsirolimus, for example, showed clinical efficacy in one-third of patients treated for recurrent glioblastoma (Galanis *et al*, 2005). Sarkaria *et al* (2011) combined everolimus in combination with radiotherapy and TMZ in 18 patients, showing a metabolic effect of everolimus in most patients, as detected with fluorodeoxyglucose PET scan. More recently, the authors reported a phase II study on 100 patients, which, however, did not detect any beneficial effect in terms of survival (Ma *et al*, 2015). An interesting phase II study by Hainsworth *et al* (2012) administered BVZ with everolimus after radiochemotherapy for 68 glioblastoma patients (Hainsworth *et al*, 2012). The progression-free survival interval compared favorably with the authors' previous experience.

IMPORTANT ISSUES TO RESOLVE

It is evident that although we know that radiation and TMZ kill cells through autophagy, it is unclear which of the functional or non-functional autophagic death pathway is followed. It is very important to investigate the role of radiotherapy fractionation on the autophagic pathways that this activates. Several tumours, such as gliomas, melanomas or even prostate cancer, exhibit a higher sensitivity to large radiotherapy fractions. It may be that small radiotherapy fractions (around 2 Gy) can simply stimulate functional autophagy in such cells, triggering therefore a cytoprotective pathway during the fractionated course of radiotherapy. Larger fractions can block autophagic flux leading to non-functional accumulation of autophagosomes, so that in such radiation scheme self-sensitises cells to its effect.

If such a differential effect on autophagy exists, then the choice and sequence of autophagy-interfering agent for combined administration should be made according to the fractionation of radiotherapy we wish to apply. The same principle can be also applicable for TMZ as the short schedule of 200–250 mg $m^{-2} \times 5$ days every month or the prolonged schedule of 70 mg m^{-2} per day continuously used in the clinical practice may have a different effect on the autophagic flux of gliomas and may demand different autophagic interference to achieve radio/chemosensitisation.

It seems that the interfering autophagy may indeed help us make a difference in the treatment of gliomas and glioblastomas. A hypothetical scheme for intervention targeting autophagy aiming to radiosensitisation is shown in Figure 1. Inducers of autophagy, like mTOR inhibitors available in the clinical practice (Houghton, 2010), can lead to a functional autophagic death when combined with standard radiotherapy and a schedule of continuous low daily dose of TMZ. Pretreatment of tumours with autophagy inducers, followed by hypofractionated radiotherapy and or high daily dose schedules of TMZ, in combination with late phase autophagy blockers, could enhance antineoplastic efficacy by leading to non-functional autophagic death. Whether early phase autophagy blockers could be of value in the combination of agents that restore apoptotic death pathways with radiotherapy or TMZ is also a hypothesis to study. Important issues remain, however, unresolved. One key question that has to be answered is whether radiotherapy and TMZ trigger a functional autophagic response or they just deregulate autophagy resulting in non-degradable autophagosome accumulation. This demands studies of monitoring the autophagic flux and lysosomal kinetics in cell lines after escalated doses of radiotherapy or TMZ, as dose schedule may be a principal factor defining the type of autophagic response.

Another important issue to resolve is the characterisation of the autophagic activity and response tendency of the tumour itself. Not all gliomas or glioblastomas are the same as, indeed, some of them are more radiosensitive than others. Assessment of the expression status of autophagy-blocking pathways such as Akt/mTOR may help to identify tumours of defective autophagy, the radiosensitivity of which may increase by specific Akt/mTOR inhibitors. As beclin 1 protein is downregulated in a large percentage of gliomas, detection of hypermethylated *Beclin 1* gene or other autophagy-initiating proteins may reveal targets for demethylating agent administration so that subsequent interference with autophagy inducers may become beneficial. Extensive expression of LC3A-positive stone-like structures may characterise tumours with functional autophagy that may benefit from concurrent radiotherapy with either early autophagy and DNA-repair blockers or autophagy induction therapy with mTOR inhibitors (depending upon radiotherapy fractionation).

Assessment of the content of CD133-positive compartment can be easily performed in immunohistochemistry of biopsies. Pretreatment of these patients with differentiation-inducing agents such as histone deacetylase inhibitors may prove a useful preradiotherapy policy. Silencing of stem-cell-specific genes, such as *Sirtuin 1*, or apoptosis restoration treatments, such as caspase or IL-24 adenoviruses, may also be of value in these cases.

CONCLUSION

Malignant glioma is probably a unique model of non-metastasising disease that kills the majority of patients through local growth.

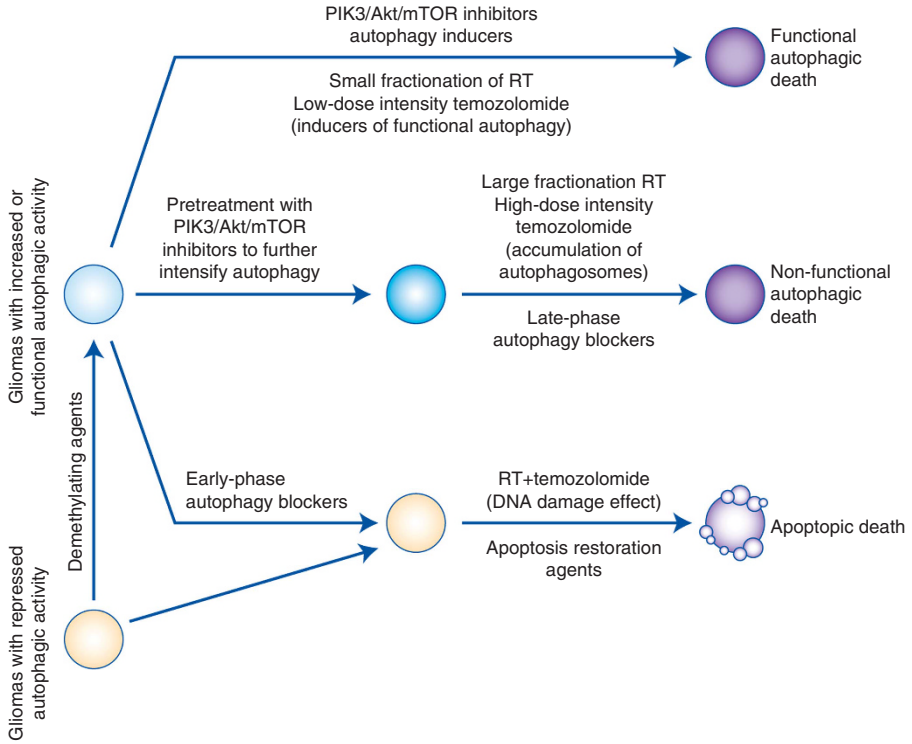

Figure 1. A hypothetical scheme targeting autophagy for glioblastoma sensitisation during radio-chemotherapy with TMZ.

It consists, therefore, of one of the biggest challenges in radiation therapy, especially nowadays when novel radiotherapy techniques allow the delivery of high dose per fraction to the tumour at the same time reducing the dose to the surrounding normal brain tissue. Whether hypofractionation that would block the autophagy flux, even in radioresistant cell lines, can be effectively combined with late autophagy blockers to exploit a non-functional autophagic death is a question that deserves investigation. Temozolomide is an important drug when combined with radiotherapy, or even for relapsed tumours after radiotherapy, and just like radiotherapy kills glioma cells through autophagic death. The combination of mTOR inhibitors (intensifiers or autophagy) with low daily dose of TMZ during standard radiotherapy or after failure of radiotherapy, aiming to trigger functional autophagic death, may also be a promising approach. Modulation of radiation or of TMZ autophagic response appears one of the most promising approaches to prolong survival and to better understand the glioblastoma therapy riddle, but important insights demanded to create a reliable concept are still missing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akhavan D, Cloughesy TF, Mischel PS (2010) mTOR signaling in glioblastoma: lessons learned from bench to bedside. *Neuro Oncol* **12**: 882–889.

Amato R (2011) Everolimus for the treatment of advanced renal cell carcinoma. *Expert Opin Pharmacother* **12**: 1143–1155.

Aoki H, Kondo Y, Aldape K, Yamamoto A, Iwado E, Yokoyama T, Hollingsworth EF, Kobayashi R, Hess K, Shinojima N, Shingu T, Tamada Y, Zhang L, Conrad C, Bogler O, Mills G, Sawaya R, Kondo S (2008) Monitoring autophagy in glioblastoma with antibody against isoform B of human microtubule-associated protein 1 light chain 3. *Autophagy* **4**: 467–475.

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **7**: 756–760.

Benzina S, Altmeyer A, Malek F, Dufour P, Denis JM, Gueulette J, Bischoff P (2008) High-LET radiation combined with oxaliplatin induce autophagy in U-87 glioblastoma cells. *Cancer Lett.* **8**: 63–70.

Berardi DE, Flumian C, Rodriguez CE, Díaz Bessone MI, Cirigliano SM, Bal de Kier Joffé ED, Fiszman GL, Urtreger AJ, Todaro LB (2015) PKCδ inhibition impairs mammary cancer proliferative capacity but selects cancer stem cells, involving autophagy. *J Cell Biochem* **9999**: 1–11.

Bernard A, Jin M, Xu Z, Klionsky DJ (2015) A large-scale analysis of autophagy-related gene expression identifies new regulators of autophagy. *Autophagy* **11**: 2114–2122.

Bilger A, Bittner MI, Grosu AL, Wiedenmann N, Meyer PT, Firat E, Niedermann G, Weber WA, Milanović D (2014) FET-PET-based reirradiation and chloroquine in patients with recurrent glioblastoma: first tolerability and feasibility results. *Strahlenther Onkol* **190**: 957–961.

Brachman DG, Pugh SL, Ashby LS, Thomas TA, Dunbar EM, Narayan S, Robins HI, Bovi JA, Rockhill JK, Won M, Curran WP (2015) Phase 1/2 trials of temozolomide, motexafin gadolinium, and 60-Gy fractionated radiation for newly diagnosed supratentorial glioblastoma multiforme: final results of RTOG 0513. *Int J Radiat Oncol Biol Phys.* **1**: 961–967.

Capper D, Gaiser T, Hartmann C, Habel A, Mueller W, Herold-Mende C, von Deimling A, Siegelin MD (2009) Stem-cell-like glioma cells are resistant to TRAIL/Apo2L and exhibit down-regulation of caspase-8 by promoter methylation. *Acta Neuropathol* **117**: 445–456.

Carmignani M, Volpe AR, Aldea M, Soritau O, Irimie A, Florian IS, Tomuleasa C, Baritchii A, Petrushev B, Crisan G, Valle G (2014) Glioblastoma stem cells: a new target for metformin and arsenic trioxide. *J Biol Regul Homeost Agents* **28**: 1–15.

Chang CH, Horton J, Schoenfeld D (1983) Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group study. *Cancer* **52**: 997–1007.

Chang CJ, Hsu CC, Yung MC, Chen KY, Tzao C, Wu WF, Chou HY, Lee YY, Lu KH, Chiou SH, Ma HI (2009) Enhanced radiosensitivity and radiation-induced apoptosis in glioma CD133-positive cells by knockdown of SirT1 expression. *Biochem Biophys Res Commun* **6**: 236–242.

Chen MY, Liao WS, Lu Z, Bornmann WG, Hennessey V, Washington MN, Rosner GL, Yu Y, Ahmed AA, Bast RC Jr (2011) Decitabine and suberoylanilide hydroxamic acid (SAHA) inhibit growth of ovarian cancer cell lines and xenografts while inducing expression of imprinted tumour suppressor genes, apoptosis, G2/M arrest, and autophagy. *Cancer* **117**: 4424–4438.

Chiu HW, Ho SY, Guo HR, Wang YJ (2009) Combination treatment with arsenic trioxide and irradiation enhances autophagic effects in U118-MG cells through increased mitotic arrest and regulation of PI3K/Akt and ERK1/2 signaling pathways. *Autophagy* **5**: 472–483.

Curran Jr WJ, Scott CB, Nelson JS (1992) A randomized trial of accelerated hyperfractionated radiation therapy and bis-chloroethyl nitrosourea for malignant glioma. A preliminary report of Radiation Therapy Oncology Group 83-02. *Cancer* **70**: 2909–2917.

Daido S, Yamamoto A, Fujiwara K, Sawaya R, Kondo S, Kondo Y (2005) Inhibition of the DNA-dependent protein kinase catalytic subunit radiosensitizes malignant glioma cells by inducing autophagy. *Cancer Res* **65**: 4368–4375.

Deutsch M, Green SB, Strike TA (1989) Results of a randomized trial comparing BCNU plus radiotherapy, streptozotocin plus radiotherapy, BCNU plus hyperfractionated radiotherapy, and BCNU following misonidazole plus radiotherapy in the postoperative treatment of malignant glioma. *Int J Radiat Oncol Biol Phys* **16**: 1389–1396.

Diaz Miqueli A, Rolff J, Lemm M, Fichtner I, Perez R, Montero E (2009) Radiosensitisation of U87MG brain tumours by anti-epidermal growth factor receptor monoclonal antibodies. *Br J Cancer* **100**: 950–958.

Djavaheri-Mergny M, Amelotti M, Mathieu J, Besançon F, Bauvy C, Codogno P (2007) Regulation of autophagy by NFkappaB transcription factor and reactives oxygen species. *Autophagy* **3**: 390–392.

Eramo A, Pallini R, Lotti F, Sette G, Patti M, Bartucci M, Ricci-Vitiani L, Signore M, Stassi G, Larocca LM, Crinò L, Peschle C, De Maria R (2005) Inhibition of DNA methylation sensitizes glioblastoma for tumour necrosis factor-related apoptosis-inducing ligand-mediated destruction. *Cancer Res* **65**: 11469–11477.

Eyre HJ, Quagliana JM, Eltringham JR (1983) Randomized comparisons of radiotherapy and CCNU versus radiotherapy, CCNU plus procarbazine for the treatment of malignant gliomas following surgery. A Southwest Oncology Group Report. *J Neurooncol* **1**: 171–177.

Feng Y, He D, Yao Z, Klionsky DJ (2014) The machinery of macroautophagy. *Cell Res* **24**: 24–41.

Fiveash JB, Gillespie GY, Oliver PG, Zhou T, Belenky ML, Buchsbaum DJ (2008) Enhancement of glioma radiotherapy and chemotherapy response with targeted antibody therapy against death receptor 5. *Int J Radiat Oncol Biol Phys.* **71**: 507–516.

Fu J, Liu ZG, Liu XM, Chen FR, Shi HL, Pangjesse CS, Ng HK, Chen ZP (2009) Glioblastoma stem cells resistant to temozolomide-induced autophagy. *Chin Med J (Engl)* **122**: 1255–1259.

Fu J, Shao CJ, Chen FR, Ng HK, Chen ZP (2010) Autophagy induced by valproic acid is associated with oxidative stress in glioma cell lines. *Neuro-Oncology* **12**: 328–340.

Fujiwara K, Iwado E, Mills GB, Sawaya R, Kondo S, Kondo Y (2007) Akt inhibitor shows anticancer and radiosensitizing effects in malignant glioma cells by inducing autophagy. *Int J Oncol* **31**: 753–760.

Fukushima T, Takeshima H, Kataoka H (2009) Anti-glioma therapy with temozolomide and status of the DNA-repair gene MGMT. *Anticancer Res* **29**: 4845–4854.

Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, Peralba JM, Jenkins RB, Dakhil SR, Morton RF, Jaeckle KA, Scheithauer BW, Dancey J, Hidalgo M, Walsh DJ. North Central Cancer Treatment Group (2005) Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* **23**: 5294–5304.

Gao L, Li F, Dong B, Zhang J, Rao Y, Cong Y, Mao B, Chen X (2010) Inhibition of STAT3 and ErbB2 suppresses tumour growth, enhances radiosensitivity, and induces mitochondria-dependent apoptosis in glioma cells. *Int J Radiat Oncol Biol Phys* **77**: 1223–1231.

Giatromanolaki A, Sivridis E, Mitrakas A, Kalamida D, Zois CE, Haider S, Piperidou C, Pappa A, Gatter KC, Harris AL, Koukourakis MI (2014) Autophagy and lysosomal related protein expression patterns in human glioblastoma. *Cancer Biol Ther* **15**: 1468–1478.

Gilbert MR, Dignam J, Won M, Blumenthal DT, Vogelbaum MA, Aldape KD, Colman H, Chakravarti A, Jeraj R, Armstrong TS, Wefel JS, Brown PD, Jaeckle KA, Schiff D, Atkins JN, Brachman D, Werner-Wasik M, Komaki R, Sulman EP, Mehta MP (2013) A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med* **370**: 699–708.

Gini B, Zanca C, Guo D, Matsutani T, Masui K, Ikegami S, Yang H, Nathanson D, Villa GR, Shackelford D, Zhu S, Tanaka K, Babic I, Akhavan D, Lin K, Assuncao A, Gu Y, Bonetti B, Mortensen DS, Xu S, Raymon HK, Cavenee WK, Furnari FB, James CD, Kroemer G, Heath JR, Hege K, Chopra R, Cloughesy TF, Mischel PS (2013) The mTOR kinase inhibitors, CC214-1 and CC214-2, preferentially block the growth of EGFRvIII-activated glioblastomas. *Clin Cancer Res* **19**: 5722–5732.

Gong C, Bauvy C, Tonelli G, Yue W, Deloménie C, Nicolas V, Zhu Y, Domergue V, Marin-Esteban V, Tharinger H, Delbos L, Gary-Gouy H, Morel AP, Ghavami S, Song E, Codogno P, Mehrpour M (2013) Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* **32**: 2261–2272.

Gratas C, Séry Q, Rabé M, Oliver L, Vallette FM (2014) Bak and Mcl-1 are essential for Temozolomide induced cell death in human glioma. *Oncotarget* **5**: 2428–2435.

Hainsworth JD, Shih KC, Shepard GC, Tillinghast GW, Brinker BT, Spigel DR (2012) Phase II study of concurrent radiation therapy, temozolomide, and bevacizumab followed by bevacizumab/everolimus as first-line treatment for patients with glioblastoma. *Clin Adv Hematol Oncol* **10**: 240–246.

Hall AP (1976) The treatment of malaria. *BMJ* **1**: 323–328.

Hatanpaa KJ, Burma S, Zhao D, Habib AA (2010) Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia* **12**: 675–684.

Hatlevoll R, Lindegaard KF, Hagen S (1985) Combined modality treatment of operated astrocytomas grade 3 and 4. A prospective and randomized study of misonidazole and radiotherapy with two different radiation schedules and subsequent CCNU chemotherapy. Stage II of a prospective multicenter trial of the Scandinavian Glioblastoma Study Group. *Cancer* **56**: 41–47.

Hayat MJ, Howlader N, Reichman ME, Edwards BK (2007) Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. *Oncologist* **12**: 20–37.

Hickman JA (1996) Apoptosis and chemotherapy resistance. *Eur J Cancer* **32A**: 921–926.

Houghton PJ (2010) Everolimus. *Clin Cancer Res* **16**(5): 1368–1372.

Huang K, Fingar DC (2014) Growing knowledge of the mTOR signaling network. *Semin Cell Dev Biol* **36**: 79–90.

Ito H, Daido S, Kanzawa T, Kondo S, Kondo Y (2005) Radiation-induced autophagy is associated with LC3 and its inhibition sensitizes malignant glioma cells. *Int J Oncol* **26**: 1401–1410.

Jiang H, Gomez-Manzano C, Aoki H, Alonso MM, Kondo S, McCormick F, Xu J, Kondo Y, Bekele BN, Colman H, Lang FF, Fueyo J (2007) Examination of the therapeutic potential of Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. *J Natl Cancer Inst* **99**: 1410–1414.

Jinno-Oue A, Shimizu N, Hamada N, Wada S, Tanaka A, Shinagawa M, Ohtsuki T, Mori T, Saha MN, Hoque AS, Islam S, Kogure K, Funayama T, Kobayashi Y, Hoshino H (2010) Irradiation with carbon ion beams induces apoptosis, autophagy, and cellular senescence in a human glioma-derived cell line. *Int J Radiat Oncol Biol Phys* **76**: 229–241.

Jo GH, Bögler O, Chwae YJ, Yoo H, Lee SH, Park JB, Kim YJ, Kim JH, Gwak HS (2014) Radiation-induced autophagy contributes to cell death and induces apoptosis partly in malignant glioma cells. *Cancer Res* **47**: 221–241.

Kabeya Y, Mizushima N, Ueno T (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* **19**: 5720–5728.

Kalamida D, Karagounis IV, Mitrakas A, Kalamida S, Giatromanolaki A, Koukourakis MI (2015) Fever-range hyperthermia vs hypothermia effect on cancer cell viability, proliferation and HSP90 expression. *PLoS One* **10**: e0116021.

Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, Kondo S (2004) Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. *Cell Death Differ* **11**: 448–457.

Katayama M, Kawaguchi T, Berger MS, Pieper RO (2007) DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. *Cell Death Differ* **14**: 548–558.

Katsuragi Y, Ichimura Y, Komatsu M (2015) p62/SQSTM1 functions as a signaling hub and an autophagy adaptor. *FEBS J* **282**: 4672–4678.

Kim H, Bernard ME, Flickinger J, Epperly MW, Wang H, Dixon TM, Shields D, Houghton F, Zhang X, Greenberger JS (2011) The autophagy-inducing drug carbamazepine is a radiation protector and mitigator. *Int J Radiat Biol* **87**: 1052–1060.

Kim SY, Yoo YH, Park JW (2013) Silencing of mitochondrial NADP(+)-dependent isocitrate dehydrogenase gene glioma radiosensitivity. *Biochem Biophys Res Commun* **433**: 260–265.

Kimura T, Takabatake Y, Takahashi A, Isaka Y (2013) Chloroquine in cancer therapy: adouble-edged sword of autophagy. *Cancer Res* **73**: 3–7.

Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, Ahn HJ, Ait-Mohamed O, Ait-Si-Ali S, Akematsu T, Akira S, Al-Younes HM, Al-Zeer MA, Albert ML, Albin RL, Alegre-Abarrategui J, Aleo MF, Alirezaei M, Almasan A, Almonte-Becerril M, Amano A, Amaravadi R, Amarnath S, Amer AO, Andrieu-Abadie N, Anantharam V, Ann DK, Anoopkumar-Dukie S, Aoki H, Apostolova N, Arancia G, Aris JP, Asanuma K, Asare NY, Ashida H, Askanas V, Askew DS, Auberger P, Baba M, Backues SK, Baehrecke EH, Bahr BA, Bai XY, Bailly Y, Baiocchi R, Baldini G, Balduini W, Ballabio A, Bamber BA, Bampton ET, Bánhegyi G, Bartholomew CR, Bassham DC, Bast Jr RC, Batoko H, Bay BH, Beau I, Béchet DM, Begley TJ, Behl C, Behrends C, Bekri S, Bellaire B, Bendall LJ, Benetti L, Berliocchi L, Bernardi H, Bernassola F, Besteiro S, Bhatia-Kissova I, Bi X, Biard-Piechaczyk M, Blum JS, Boise LH, Bonaldo P, Boone DL, Bornhauser BC, Bortoluci KR, Bossis I, Bost F, Bourquin JP, Boya P, Boyer-Guittaut M, Bozhkov PV, Brady NR, Brancolini C, Brech A, Brenman JE, Brennand A, Bresnick EH, Brest P, Bridges D, Bristol ML, Brookes PS, Brown EJ, Brumell JH, Brunetti-Pierri N, Brunk UT, Bulman DE, Bultman SJ, Bultynck G, Burbulla LF, Bursch W, Butchar JP, Buzgariu W, Bydlowski SP, Cadwell K, Cahová M, Cai D, Cai J, Cai Q, Calabretta B, Calvo-Garrido J, Camougrand N, Campanella M, Campos-Salinas J, Candi E, Cao L, Caplan AB, Carding SR, Cardoso SM, Carew JS, Carlin CR, Carmignac V, Carneiro LA, Carra S, Caruso RA, Casari G, Casas C, Castino R, Cebollero E, Cecconi F, Celli J, Chaachouay H, Chae HJ, Chai CY, Chan DC, Chan EY, Chang RC, Che CM, Chen CC, Chen GC, Chen GQ, Chen M, Chen Q, Chen SS, Chen W, Chen X, Chen X, Chen X, Chen YG, Chen Y, Chen Y, Chen YJ, Chen Z, Cheng A, Cheng CH, Cheng Y, Cheong H, Cheong JH, Cherry S, Chess-Williams R, Cheung ZH, Chevet E, Chiang HL, Chiarelli R, Chiba T, Chin LS, Chiou SH, Chisari FV, Cho CH, Cho DH, Choi AM, Choi D, Choi KS, Choi ME, Chouaib S, Choubey D, Choubey V, Chu CT, Chuang TH, Chueh SH, Chun T, Chwae YJ, Chye ML, Ciarcia R, Ciriolo MR, Clague MJ, Clark RS, Clarke PG, Clarke R, Codogno P, Coller HA, Colombo MI, Comincini S, Condello M, Condorelli F, Cookson MR, Coombs GH, Coppens I, Corbalan R, Cossart P, Costelli P, Costes S, Coto-Montes A, Couve E, Coxon FP, Cregg JM, Crespo JL, Cronjé MJ, Cuervo AM, Cullen JJ, Czaja MJ, D'Amelio M, Darfeuille-Michaud A, Davids LM, Davies FE, De Felici M, de Groot JF, de Haan CA, De Martino L, De Milito A, De Tata V, Debnath J, Degterev A, Dehay B, Delbridge LM, Demarchi F, Deng YZ, Dengjel J, Dent P, Denton D, Deretic V, Desai SD, Devenish RJ, Di Gioacchino M, Di Paolo G, Di Pietro C, Díaz-Araya G, Díaz-Laviada I, Diaz-Meco MT, Diaz-Nido J, Dikic I, Dinesh-Kumar SP, Ding WX, Distelhorst CW, Diwan A, Djavaheri-Mergny M, Dokudovskaya S, Dong Z, Dorsey FC, Dosenko V, Dowling JJ, Doxsey S, Dreux M, Drew ME, Duan Q, Duchosal MA, Duff K, Dugail I, Durbeej M, Duszenko M, Edelstein CL, Edinger AL, Egea G, Eichinger L, Eissa NT, Ekmekcioglu S, El-Deiry WS, Elazar Z, Elgendy M, Ellerby LM, Eng KE, Engelbrecht AM, Engelender S, Erenpreisa J, Escalante R, Esclatine A, Eskelinen EL, Espert L, Espina V, Fan H, Fan J, Fan QW, Fan Z, Fang S, Fang Y, Fanto M, Fanzani A, Farkas T, Farré JC, Faure M, Fechheimer M, Feng CG, Feng J, Feng Q, Feng Y, Fésüs L, Feuer R, Figueiredo-Pereira ME, Fimia GM, Fingar DC, Finkbeiner S, Finkel T, Finley KD, Fiorito F, Fisher EA, Fisher PB, Flajolet M, Florez-McClure ML, Florio S, Fon EA, Fornai F, Fortunato F, Fotedar R, Fowler DH, Fox HS, Franco R, Frankel LB, Fransen M, Fuentes JM, Fueyo J, Fujii J, Fujisaki K, Fujita E, Fukuda M, Furukawa RH, Gaestel M, Gailly P, Gajewska M, Galliot B, Galy V, Ganesh S, Ganetzky B, Ganley IG, Gao FB, Gao GF, Gao J, Garcia L, Garcia-Manero G, Garcia-Marcos M, Garmyn M, Gartel AL, Gatti E, Gautel M, Gawriluk TR, Gegg ME, Geng J, Germain M, Gestwicki JE, Gewirtz DA, Ghavami S, Ghosh P, Giammarioli AM, Giatromanolaki AN, Gibson SB, Gilkerson RW, Ginger ML, Ginsberg HN, Golab J, Goligorsky MS, Golstein P, Gomez-Manzano C, Goncu E, Gongora C, Gonzalez CD, Gonzalez R, González-Estévez C, González-Polo RA, Gonzalez-Rey E, Gorbunov NV, Gorski S, Goruppi S, Gottlieb RA, Gozuacik D, Granato GE, Grant GD, Green KN, Gregorc A, Gros F, Grose C, Grunt TW, Gual P, Guan JL, Guan KL, Guichard SM, Gukovskaya AS, Gukovsky I, Gunst J, Gustafsson AB, Halayko AJ, Hale AN, Halonen SK, Hamasaki M, Han F, Han T, Hancock MK, Hansen M, Harada H, Harada M, Hardt SE, Harper JW, Harris AL, Harris J, Harris SD, Hashimoto M, Haspel JA, Hayashi S, Hazelhurst LA, He C, He YW, Hébert MJ, Heidenreich KA, Helfrich MH, Helgason GV, Henske EP, Herman B, Herman PK, Hetz C, Hilfiker S, Hill JA, Hocking LJ, Hofman P, Hofmann TG, Höhfeld J, Holyoake TL, Hong MH, Hood DA, Hotamisligil GS, Houwerzijl EJ, Høyer-Hansen M, Hu B, Hu CA, Hu HM, Hua Y, Huang C, Huang J, Huang S, Huang WP, Huber TB, Huh WK, Hung TH, Hupp TR, Hur GM, Hurley JB, Hussain SN, Hussey PJ, Hwang JJ, Hwang S, Ichihara A, Ilkhanizadeh S, Inoki K, Into T, Iovane V, Iovanna JL, Ip NY, Isaka Y, Ishida H, Isidoro C, Isobe K, Iwasaki A, Izquierdo M, Izumi Y, Jaakkola PM, Jäättelä M, Jackson GR, Jackson WT, Janji B, Jendrach M, Jeon JH, Jeung EB, Jiang H, Jiang H, Jiang JX, Jiang M, Jiang Q, Jiang X, Jiang X, Jiménez A, Jin M, Jin S, Joe CO, Johansen T, Johnson DE, Johnson GV, Jones NL, Joseph B, Joseph SK, Joubert AM, Juhász G, Juillerat-Jeanneret L, Jung CH, Jung YK, Kaarniranta K, Kaasik A, Kabuta T, Kadowaki M, Kagedal K, Kamada Y, Kaminskyy VO, Kampinga HH, Kanamori H, Kang C, Kang KB, Kang KI, Kang R, Kang YA, Kanki T, Kanneganti TD, Kanno H, Kanthasamy AG, Kanthasamy A, Karantza V, Kaushal GP, Kaushik S, Kawazoe Y, Ke PY, Kehrl JH, Kelekar A, Kerkhoff C, Kessel DH, Khalil H, Kiel JA, Kiger AA, Kihara A, Kim DR, Kim DH, Kim DH, Kim EK, Kim HR, Kim JS, Kim JH, Kim JC, Kim JK, Kim PK, Kim SW, Kim YS, Kim Y, Kimchi A, Kimmelman AC, King JS, Kinsella TJ, Kirkin V, Kirshenbaum LA, Kitamoto K, Kitazato K, Klein L, Klimecki WT, Klucken J, Knecht E, Ko BC, Koch JC, Koga H, Koh JY, Koh YH, Koike M, Komatsu M, Kominami E, Kong HJ, Kong WJ, Korolchuk VI, Kotake Y, Koukourakis MI, Kouri Flores JB, Kovács AL, Kraft C, Krainc D, Krämer H, Kretz-Remy C, Krichevsky AM, Kroemer G, Krüger R, Krut O, Ktistakis NT, Kuan CY, Kucharczyk R, Kumar A, Kumar R, Kumar S, Kundu M, Kung HJ, Kurz T, Kwon HJ, La Spada AR, Lafont F, Lamark T, Landry J, Lane JD, Lapaquette P, Laporte JF, László L, Lavandero S, Lavoie JN, Layfield R, Lazo PA, Le W, Le Cam L, Ledbetter DJ, Lee AJ, Lee BW, Lee GM, Lee J, Lee JH, Lee M, Lee MS, Lee SH, Leeuwenburgh C, Legembre P, Legouis R, Lehmann M, Lei HY, Lei QY, Leib DA, Leiro J, Lemasters JJ, Lemoine A, Lesniak MS, Lev D, Levenson VV, Levine B, Levy E, Li F, Li JL, Li L, Li S, Li W, Li XJ, Li YB, Li YP, Liang C, Liang Q, Liao YF, Liberski PP, Lieberman A, Lim HJ, Lim KL, Lim K, Lin CF, Lin FC, Lin J, Lin JD, Lin K, Lin WW, Lin WC, Lin YL, Linden R, Lingor P, Lippincott-Schwartz J, Lisanti MP, Liton PB, Liu B, Liu CF, Liu K, Liu L, Liu QA, Liu W, Liu YC, Liu Y, Lockshin RA, Lok CN, Lonial S, Loos B, Lopez-Berestein G, López-Otín C, Lossi L, Lotze MT, Lőw P, Lu B, Lu B, Lu B, Lu Z, Luciano F, Lukacs NW, Lund AH, Lynch-Day MA, Ma Y, Macian F, MacKeigan JP, Macleod KF, Madeo F, Maiuri L, Maiuri MC, Malagoli D, Malicdan MC, Malorni W, Man N, Mandelkow EM, Manon S, Manov I, Mao K, Mao X, Mao Z, Marambaud P, Marazziti D, Marcel YL, Marchbank K, Marchetti P, Marciniak SJ, Marcondes M, Mardi M, Marfe G, Mariño G, Markaki M, Marten MR, Martin SJ, Martinand-Mari C, Martinet W, Martinez-Vicente M, Masini M, Matarrese P, Matsuo S, Matteoni R, Mayer A, Mazure NM, McConkey DJ, McConnell MJ, McDermott C, McDonald C, McInerney GM, McKenna SL, McLaughlin B, McLean PJ, McMaster CR, McQuibban GA, Meijer AJ, Meisler MH, Meléndez A, Melia TJ, Melino G, Mena MA, Menendez JA, Menna-Barreto RF, Menon MB, Menzies FM, Mercer CA, Merighi A, Merry DE, Meschini S, Meyer CG, Meyer TF, Miao CY, Miao JY, Michels PA, Michiels C, Mijaljica D, Milojkovic A, Minucci S, Miracco C, Miranti CK, Mitroulis I, Miyazawa K, Mizushima N, Mograbi B, Mohseni S, Molero X, Mollereau B, Mollinedo F, Momoi T, Monastyrska I, Monick MM, Monteiro MJ, Moore MN, Mora R, Moreau K, Moreira PI, Moriyasu Y, Moscat J, Mostowy S, Mottram JC,

Motyl T, Moussa CE, Müller S, Muller S, Münger K, Münz C, Murphy LO, Murphy ME, Musarò A, Mysorekar I, Nagata E, Nagata K, Nahimana A, Nair U, Nakagawa T, Nakahira K, Nakano H, Nakatogawa H, Nanjundan M, Naqvi NI, Narendra DP, Narita M, Navarro M, Nawrocki ST, Nazarko TY, Nemchenko A, Netea MG, Neufeld TP, Ney PA, Nezis IP, Nguyen HP, Nie D, Nishino I, Nislow C, Nixon RA, Noda T, Noegel AA, Nogalska A, Noguchi S, Notterpek L, Novak I, Nozaki T, Nukina N, Nürnberger T, Nyfeler B, Obara K, Oberley TD, Oddo S, Ogawa M, Ohashi T, Okamoto K, Oleinick NL, Oliver FJ, Olsen LJ, Olsson S, Opota O, Osborne TF, Ostrander GK, Otsu K, Ou JH, Ouimet M, Overholtzer M, Ozpolat B, Paganetti P, Pagnini U, Pallet N, Palmer GE, Palumbo C, Pan T, Panaretakis T, Pandey UB, Papackova Z, Papassideri I, Paris I, Park J, Park OK, Parys JB, Parzych KR, Patschan S, Patterson C, Pattingre S, Pawelek JM, Peng J, Perlmutter DH, Perrotta I, Perry G, Pervaiz S, Peter M, Peters GJ, Petersen M, Petrovski G, Phang JM, Piacentini M, Pierre P, Pierrefite-Carle V, Pierron G, Pinkas-Kramarski R, Piras A, Piri N, Platanias LC, Pöggeler S, Poirot M, Poletti A, Poüs C, Pozuelo-Rubio M, Prætorius-Ibba M, Prasad A, Prescott M, Priault M, Produit-Zengaffinen N, Progulske-Fox A, Proikas-Cezanne T, Przedborski S, Przyklenk K, Puertollano R, Puyal J, Qian SB, Qin L, Qin ZH, Quaggin SE, Raben N, Rabinowich H, Rabkin SW, Rahman I, Rami A, Ramm G, Randall G, Randow F, Rao VA, Rathmell JC, Ravikumar B, Ray SK, Reed BH, Reed JC, Reggiori F, Régnier-Vigouroux A, Reichert AS, Reiners Jr JJ, Reiter RJ, Ren J, Revuelta JL, Rhodes CJ, Ritis K, Rizzo E, Robbins J, Roberge M, Roca H, Roccheri MC, Rocchi S, Rodemann HP, Rodríguez de Córdoba S, Rohrer B, Roninson IB, Rosen K, Rost-Roszkowska MM, Rouis M, Rouschop KM, Rovetta F, Rubin BP, Rubinsztein DC, Ruckdeschel K, Rucker III EB, Rudich A, Rudolf E, Ruiz-Opazo N, Russo R, Rusten TE, Ryan KM, Ryter SW, Sabatini DM, Sadoshima J, Saha T, Saitoh T, Sakagami H, Sakai Y, Salekdeh GH, Salomoni P, Salvaterra PM, Salvesen G, Salvioli R, Sanchez AM, Sánchez-Alcázar JA, Sánchez-Prieto R, Sandri M, Sankar U, Sansanwal P, Santambrogio L, Saran S, Sarkar S, Sarwal M, Sasakawa C, Sasnauskiene A, Sass M, Sato K, Sato M, Schapira AH, Scharl M, Schätzl HM, Scheper W, Schiaffino S, Schneider C, Schneider ME, Schneider-Stock R, Schoenlein PV, Schorderet DF, Schüller C, Schwartz GK, Scorrano L, Sealy L, Seglen PO, Segura-Aguilar J, Seiliez I, Seleverstov O, Sell C, Seo JB, Separovic D, Setaluri V, Setoguchi T, Settembre C, Shacka JJ, Shanmugam M, Shapiro IM, Shaulian E, Shaw RJ, Shelhamer JH, Shen HM, Shen WC, Sheng ZH, Shi Y, Shibuya K, Shidoji Y, Shieh JJ, Shih CM, Shimada Y, Shimizu S, Shintani T, Shirihai OS, Shore GC, Sibirny AA, Sidhu SB, Sikorska B, Silva-Zacarin EC, Simmons A, Simon AK, Simon HU, Simone C, Simonsen A, Sinclair DA, Singh R, Sinha D, Sinicrope FA, Sirko A, Siu PM, Sivridis E, Skop V, Skulachev VP, Slack RS, Smaili SS, Smith DR, Soengas MS, Soldati T, Song X, Sood AK, Soong TW, Sotgia F, Spector SA, Spies CD, Springer W, Srinivasula SM, Stefanis L, Steffan JS, Stendel R, Stenmark H, Stephanou A, Stern ST, Sternberg C, Stork B, Strålfors P, Subauste CS, Sui X, Sulzer D, Sun J, Sun SY, Sun ZJ, Sung JJ, Suzuki K, Suzuki T, Swanson MS, Swanton C, Sweeney ST, Sy LK, Szabadkai G, Tabas I, Taegtmeyer H, Tafani M, Takács-Vellai K, Takano Y, Takegawa K, Takemura G, Takeshita F, Talbot NJ, Tan KS, Tanaka K, Tanaka K, Tang D, Tang D, Tanida I, Tannous BA, Tavernarakis N, Taylor GS, Taylor GA, Taylor JP, Terada LS, Terman A, Tettamanti G, Thevissen K, Thompson CB, Thorburn A, Thumm M, Tian F, Tian Y, Tocchini-Valentini G, Tolkovsky AM, Tomino Y, Tönges L, Tooze SA, Tournier C, Tower J, Towns R, Trajkovic V, Travassos LH, Tsai TF, Tschan MP, Tsubata T, Tsung A, Turk B, Turner LS, Tyagi SC, Uchiyama Y, Ueno T, Umekawa M, Umemiya-Shirafuji R, Unni VK, Vaccaro MI, Valente EM, Van den Berghe G, van der Klei IJ, van Doorn W, van Dyk LF, van Egmond M, van Grunsven LA, Vandenabeele P, Vandenberghe WP, Vanhorebeek I, Vaquero EC, Velasco G, Vellai T, Vicencio JM, Vierstra RD, Vila M, Vindis C, Viola G, Viscomi MT, Voitsekhovskaja OV, von Haefen C, Votruba M, Wada K, Wade-Martins R, Walker CL, Walsh CM, Walter J, Wan XB, Wang A, Wang C, Wang D, Wang F, Wang F, Wang G, Wang H, Wang HG, Wang HD, Wang J, Wang K, Wang M, Wang RC, Wang X, Wang X, Wang YJ, Wang Y, Wang Z, Wang ZC, Wang Z, Wansink DG, Ward DM, Watada H, Waters SL, Webster P, Wei L, Weihl CC, Weiss WA, Welford SM, Wen LP, Whitehouse CA, Whitton JL, Whitworth AJ, Wileman T, Wiley JW, Wilkinson S, Willbold D, Williams RL, Williamson PR, Wouters BG, Wu C, Wu DC, Wu WK, Wyttenbach A, Xavier RJ, Xi Z, Xia P, Xiao G, Xie Z, Xie Z, Xu DZ, Xu J, Xu L, Xu X, Yamamoto A, Yamamoto A, Yamashina S, Yamashita M, Yan X, Yanagida M, Yang DS, Yang E, Yang JM, Yang SY, Yang W, Yang WY, Yang Z, Yao MC, Yao TP, Yeganeh B, Yen WL, Yin JJ, Yin XM, Yoo OJ, Yoon G, Yoon SY, Yorimitsu T, Yoshikawa Y, Yoshimori T, Yoshimoto K, You HJ, Youle RJ, Younes A, Yu L, Yu L, Yu SW, Yu WH, Yuan ZM, Yue Z, Yun CH, Yuzaki M, Zabirnyk O, Silva-Zacarin E, Zacks D, Zacksenhaus E, Zaffaroni N, Zakeri Z, Zeh III HJ, Zeitlin SO, Zhang H, Zhang HL, Zhang J, Zhang JP, Zhang L, Zhang L, Zhang MY, Zhang XD, Zhao M, Zhao YF, Zhao Y, Zhao ZJ, Zheng X, Zhivotovsky B, Zhong Q, Zhou CZ, Zhu C, Zhu WG, Zhu XF, Zhu X, Zhu Y, Zoladek T, Zong WX, Zorzano A, Zschocke J, Zuckerbraun B (2012) Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* **8**: 445–544.

Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. *Science* **290**: 1717–1721.

Koritzinsky M, Wouters BG (2013) The roles of reactive oxygen species and autophagy in mediating the tolerance of tumor cells to cycling hypoxia. *Semin Radiat Oncol* **23**: 252–261.

Koukourakis MI, Kalamida D, Giatromanolaki A, Zois C, Sivridis E, Pouliliou S, Mitrakas A, Gatter GC, Harris AL (2015) Autophagosome proteins LC3A, LC3B and LC3C have distinct subcellular distribution kinetics and expression in cancer cell lines. *PLoS One* **10**(9): e0137675.

Koukourakis MI, Kalamida D, Mitrakas A, Pouliliou S, Kalamida S, Sivridis E, Giatromanolaki A (2015) Intensified autophagy compromises the efficacy of radiotherapy against prostate cancer. *Biochem Biophys Res Commun* **461**: 268–274.

Krakstad C, Chekenya M (2010) Survival signalling and apoptosis resistance in glioblastomas: opportunities for targeted therapeutics. *Mol Cancer* **9**: 135.

Kreisl TN, Kim L, Moore K (2009) Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumour progression in recurrent glioblastoma. *J Clin Oncol* **27**: 740–745.

Lee JJ, Kim BC, Park MJ, Lee YS, Kim YN, Lee BL, Lee JS (2011) PTEN status switches cell fate between premature senescence and apoptosis in glioma exposed to ionizing radiation. *Cell Death Differ* **18**: 666–677.

Lee SW, Kim HK, Lee NH, Yi HY, Kim HS, Hong SH, Hong YK, Joe YA (2015) The synergistic effect of combination temozolomide and chloroquine treatment is dependent on autophagy formation and p53 status in glioma cells. *Cancer Lett* **360**: 195–204.

Lefranc F, Kiss R (2006) Autophagy, the Trojan horse to combat glioblastomas. *Neurosurg Focus* **20**: E7.

Lena A, Rechichi M, Salvetti A, Bartoli B, Vecchio D, Scarcelli V, Amoroso R, Benvenuti L, Gagliardi R, Gremigni V, Rossi L (2009) Drugs targeting the mitochondrial pore act as cytotoxic and cytostatic agents in temozolomide-resistant glioma cells. *J Transl Med* **7**: 13.

Li Z, Chen B, Wu Y, Jin F, Xia Y, Liu X (2010) Genetic and epigenetic silencing of the beclin 1 gene in sporadic breast tumours. *BMC Cancer* **10**: 98.

Lomonaco SL, Finniss S, Xiang C, Decarvalho A, Umansky F, Kalkanis SN, Mikkelsen T, Brodie C (2009) The induction of autophagy by gamma-radiation contributes to the radioresistance of glioma stem cells. *Int J Cancer* **125**: 717–722.

Ma DJ, Galanis E, Anderson SK, Schiff D, Kaufmann TJ, Peller PJ, Giannini C, Brown PD, Uhm JH, McGraw S, Jaeckle KA, Flynn PJ, Ligon KL, Buckner JC, Sarkaria JN (2015) A phase II trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed glioblastoma: NCCTG N057K. *Neuro-Oncology* **17**: 1261–1269.

Martelli AM, Evangelisti C, Follo MY, Ramazzotti G, Fini M, Giardino R, Manzoli L, McCubrey JA, Cocco L (2011) Targeting the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in cancer stem cells. *Curr Med Chem* **18**: 2715–2726.

Mehta M, Khan A, Danish S, Haffty BG, Sabaawy HE (2015) Radiosensitization of primary human glioblastoma stem-like cells with low-dose AKT inhibition. *Mol Cancer Ther* **14**: 1171–1180.

Miao W, Liu X, Wang H, Fan Y, Lian S, Yang X, Wang X, Guo G, Li Q, Wang S (2015) p53 upregulated modulator of apoptosis sensitizes drug-resistant U251 glioblastoma stem cells to temozolomide through enhanced apoptosis. *Mol Med Rep* **11**: 4165–4173.

Milano V, Piao Y, LaFortune T, de Groot J (2009) Dasatinib-induced autophagy is enhanced in combination with temozolomide in glioma. *Mol Cancer Ther* **8**: 394–406.

Mirzayans R, Andrais B, Scott A, Wang YW, Murray D (2013) Ionizing radiation-induced responses in human cells with differing TP53 status. *Int J Mol Sci* **14**: 22409–22435.

Morgan MA, Lawrence TS (2015) Molecular pathways: overcoming radiation resistance by targeting DNA damage response pathways. *Clin Cancer Res* **21**: 2898–28904.

Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schüler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lütolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. *Cancer Res* **64**: 6892–6899.

Palumbo S, Tini P, Toscano M, Allavena G, Angeletti F, Manai F, Miracco C, Comincini S, Pirtoli L (2014) Combined EGFR and autophagy modulation impairs cell migration and enhances radiosensitivity in human glioblastoma cells. *J Cell Physiol* **229**: 1863–1873.

Park JA, Kim YE, Seok HJ, Park WY, Kwon HJ, Lee Y (2011) Differentiation and upregulation of heat shock protein 70 induced by a subset of histone deacetylase inhibitors in mouse and human embryonic stem cells. *BMB Rep* **44**: 176–181.

Pellegrini P, Strambi A, Zipoli C, Hägg-Olofsson M, Buoncervello M, Linder S, De Milito A (2014) Acidic extracellular pH neutralizes the autophagy-inhibiting activity of chloroquine: implications for cancer therapies. *Autophagy* **10**: 562–571.

Pflaum J, Schlosser S, Müller M (2014) p53 Family and cellular stress responses in cancer. *Front Oncol* **4**: 285.

Pirtoli L, Cevenini G, Tini P, Vannini M, Oliveri G, Marsili S, Mourmouras V, Rubino G, Miracco C (2009) The prognostic role of Beclin 1 protein expression in high-grade gliomas. *Autophagy* **5**: 930–936.

Poulsen HS, Urup T, Michaelsen SR, Staberg M, Villingshøj M, Lassen U (2014) The impact of bevacizumab treatment on survival and quality of life in newly diagnosed glioblastoma patients. *Cancer Manag Res* **6**: 373–387.

Rosenfeld MR, Ye X, Supko JG, Desideri S, Grossman SA, Brem S, Mikkelson T, Wang D, Chang YC, Hu J, McAfee Q, Fisher J, Troxel AB, Piao S, Heitjan DF, Tan KS, Pontiggia L, O'Dwyer PJ, Davis LE, Amaravadi RK (2014) 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* **10**: 1359–1368.

Saetre F, Hagen LK, Engedal N, Seglen PO (2015) Novel steps in the autophagic-lysosomal pathway. *FEBS J* **282**: 2202–2214.

Salazar OM, Rubin P, Feldstein ML, Pizzutiello R (1979) High dose radiation therapy in the treatment of malignant gliomas: final report. *Int J Radiat Oncol Biol Phys* **5**: 1733–1740.

Sarkaria JN, Galanis E, Wu W, Peller PJ, Giannini C, Brown PD, Uhm JH, McGraw S, Jaeckle KA, Buckner JC (2011) North Central Cancer Treatment Group Phase I trial N057K of everolimus (RAD001) and temozolomide in combination with radiation therapy in patients with newly diagnosed glioblastoma multiforme. *Int J Radiat Oncol Biol Phys* **81**: 468–475.

Sesen J, Dahan P, Scotland SJ, Saland E, Dang VT, Lemarié A, Tyler BM, Brem H, Toulas C, Cohen-Jonathan Moyal E, Sarry JE, Skuli N (2015) Metformin inhibits growth of human glioblastoma cells and enhances therapeutic response. *PLoS One* **10**: e0123721.

Shapiro WR, Green SB, Burger PC (1989) Randomized trial of three chemotherapy regimens and two radiotherapy regimens and two radiotherapy regimens in postoperative treatment of malignant glioma. Brain Tumour Cooperative Group Trial 8001. *J Neurosurg* **71**: 1–9.

Shen Y, Chen H, Zhang J, Chen Y, Wang M, Ma J, Hong L, Liu N, Fan Q, Lu X, Tian Y, Wang A, Dong J, Lan Q, Huang Q (2015) Increased Notch signaling enhances radioresistance of malignant stromal cells induced by glioma stem/progenitor cells. *PLoS One* **10**(11): e0142594.

Shpilka T, Weidberg H, Pietrokovski S, Elazar Z (2011) Atg8: an autophagy-related ubiquitin-like protein family. *Genome Biol* **12**: 226.

Sivridis E, Koukourakis MI, Zois CE, Ledaki I, Ferguson DJ, Harris AL, Gatter KC, Giatromanolaki A (2010) LC3A-positive light microscopy detected patterns of autophagy and prognosis in operable breast carcinomas. *Am J Pathol* **176**: 2477–2489.

Stupp R (2009) European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* **10**: 459–466.

Stupp R, Mason WP, van den Bent MJ (2005) European Organisation for Research and Treatment of Cancer Brain Tumour and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* **352**: 987–996.

Thomas AA, Omuro A (2014) Current role of anti-angiogenic strategies for glioblastoma. *Curr Treat Options Oncol* **15**: 551–566.

Tsuboi Y, Kurimoto M, Nagai S, Hayakawa Y, Kamiyama H, Hayashi N, Kitajima I, Endo S (2009) Induction of autophagic cell death and radiosensitization by the pharmacological inhibition of nuclear factor-kappa B activation in human glioma cell lines. *J Neurosurg* **110**: 594–604.

Tsurushima H, Yuan X, Dillehay LE, Leong KW (2008) Radiation-inducible caspase-8 gene therapy for malignant brain tumours. *Int J Radiat Oncol Biol Phys* **71**: 517–525.

Ulasov IV, Sonabend AM, Nandi S, Khramtsov A, Han Y, Lesniak MS (2009) Combination of adenoviral virotherapy and temozolomide chemotherapy eradicates malignant glioma through autophagic and apoptotic cell death in vivo. *Br J Cancer* **100**: 1154–1164.

Vakifahmetoglu H, Olsson M, Zhivotovsky B (2008) Death through a tragedy: mitotic catastrophe. *Cell Death Differ* **15**: 1153–1162.

van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, Clement PM, Frenay M, Campone M, Baurain JF, Armand JP, Taphoorn MJ, Tosoni A, Kletzl H, Klughammer B, Lacombe D, Gorlia T (2009) Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumour group study 26034. *J Clin Oncol* **27**: 1268–1274.

Villalonga-Planells R, Coll-Mulet L, Martínez-Soler F, Castaño E, Acebes J, Giménez-Bonafé P, Gil J, Tortosa A (2011) Activation of p53 by nutlin-3a induces apoptosis and cellular senescence in human glioblastoma multiforme. *PLoS One* **6**(4): e18588.10.

Winardi D, Tsai HP, Chai CY, Chung CL, Loh JK, Chen YH, Hsieh CL (2014) Correlation of altered expression of the autophagy marker LC3B with poor prognosis in astrocytoma. *Biomed Res Int* **2014**: 723176.

Wong PM, Puente C, Ganley IG, Jiang X (2013) The ULK1 complex: sensing nutrient signals for autophagy activation. *Autophagy* **9**: 124–137.

Yacoub A, Hamed H, Emdad L, Dos Santos W, Gupta P, Broaddus WC, Ramakrishnan V, Sarkar D, Shah K, Curiel DT, Grant S, Fisher PB, Dent P (2008) MDA-7/IL-24 plus radiation enhance survival in animals with intracranial primary human GBM tumours. *Cancer Biol Ther* **7**: 917–933.

Yao KC, Komata T, Kondo Y (2003) Molecular response of human glioblastoma multiforme cells to ionizing radiation: cell cycle arrest, modulation of the expression of cyclin-dependent kinase inhibitors, and autophagy. *J Neurosurg* **98**: 378–384.

Yao KC, Komata T, Kondo Y, Kanzawa T, Kondo S, Germano IM (2003) Molecular response of human glioblastoma multiforme cells to ionizing radiation: cell cycle arrest, modulation of the expression of cyclin-dependent kinase inhibitors, and autophagy. *J Neurosurg* **98**: 378–384.

Ye F, Zhang Y, Liu Y, Yamada K, Tso JL, Menjivar JC, Tian JY, Yong WH, Schaue D, Mischel PS, Cloughesy TF, Nelson SF, Liau LM, McBride W, Tso CL (2013) Protective properties of radio chemoresistant glioblastoma stem cell clones are associated with metabolic adaptation to reduced glucose dependence. *PLoS One* **8**: e80397.

Yokoyama T, Iwado E, Kondo Y, Aoki H, Hayashi Y, Georgescu MM, Sawaya R, Hess KR, Mills GB, Kawamura H, Hashimoto Y, Urata Y, Fujiwara T, Kondo S (2008) Autophagy-inducing agents augment the antitumour effect of telerase-selve oncolytic adenovirus OBP-405 on glioblastoma cells. *Gene Therapy* **15**: 1233–1239.

Yorimitsu T, Klionsky DJ (2005) Autophagy: molecular machinery for self eating. *Cell Death Differ.* **2**: 1542–1552.

You WC, Chiou SH, Huang CY, Chiang SF, Yang CL, Sudhakar JN, Lin TY, Chiang IP, Shen CC, Cheng WY, Lin JC, Shieh SH, Chow KC (2013) Mitochondrial protein ATPase family, AAA domain containing 3A correlates with radioresistance in glioblastoma. *Neuro-Oncology* **15**: 1342–1352.

Yu VY, Nguyen D, Pajonk F, Kupelian P, Kaprealian T, Selch M, Low DA, Sheng K (2015) Incorporating cancer stem cells in radiation therapy treatment response modeling and the implication in glioblastoma multiforme treatment resistance. *Int J Radiat Oncol Biol Phys* **91**: 866–875.

Yuan X, Du J, Hua S, Zhang H, Gu C, Wang J, Yang L, Huang J, Yu J, Liu F (2015) Suppression of autophagy augments the radiosensitizing effects of STAT3 inhibition on human glioma cells. *Exp Cell Res* **330**: 267–276.

Zanotto-Filho A, Braganhol E, Klafke K, Figueiró F, Terra SR, Paludo FJ, Morrone M, Bristot IJ, Battastini AM, Forcelini CM, Bishop AJ, Gelain DP, Moreira JC (2015) Autophagy inhibition improves the efficacy of curcumin/temozolomide combination therapy in glioblastomas. *Cancer Lett* **358**: 220–231.

Zhivotovsky B, Joseph B, Orrenius S (1999) Tumour radiosensitivity and apoptosis. *Exp Cell Res* **248**: 10–17.

Zhuang W, Li B, Long L, Chen L, Huang Q, Liang Z (2011a) Induction of autophagy promotes differentiation of glioma-initiating cells and their radiosensitivity. *Int J Cancer* **129**: 2720–2731.

Zhuang W, Li B, Long L, Chen L, Huang Q, Liang ZQ (2011b) Knockdown of the DNA-dependent protein kinase catalytic subunit radiosensitizes glioma-initiating cells by inducing autophagy. *Brain Res* **1371**: 7–15.

Ziegler DS, Wright RD, Kesari S, Lemieux ME, Tran MA, Jain M, Zawel L, Kung AL (2008) Resistance of human glioblastoma multiforme cells to growth factor inhibitors is overcome by blockade of inhibitor of apoptosis proteins. *J Clin Invest* **118**: 3109–3122.

Zois CE, Giatromanolaki A, Kainulainen H, Botaitis S, Torvinen S, Simopoulos C, Kortsaris A, Sivridis E, Koukourakis MI (2011) Lung autophagic response following exposure of mice to whole body irradiation, with and without amifostine. *Biochem Biophys Res Commun* **404**: 552–558.

Zois CE, Giatromanolaki A, Sivridis E, Papaiakovou M, Kainulainen H, Koukourakis MI (2011) 'Autophagic flux' in normal mouse tissues: focus on endogenous LC3A processing. *Autophagy* **7**: 1371–1378.

Zois CE, Koukourakis MI (2009) Radiation-induced autophagy in normal and cancer cells: towards novel cytoprotection and radio-sensitization policies? *Autophagy* **5**: 442–450.