Histol Histopathol (2017) 32: 413-431

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

Review

# The emerging role of mTOR up-regulation in brain astrocytoma

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**Summary.** The present manuscript is an overview of various effects of mTOR up-regulation in astrocytoma with an emphasis on its deleterious effects on the proliferation of Glioblastoma Multiforme. The manuscript reports consistent evidence indicating the occurrence of mTOR up-regulation both in experimental and human astrocytoma. The grading of human astrocytoma is discussed in relationship with mTOR up-regulation. In the second part of the manuscript, the biochemical pathways under the influence of mTOR are translated to cell phenotypes which are generated by mTOR up-regulation and reverted by its inhibition. A special section is dedicated to the prominent role of autophagy in mediating the effects of mTOR in glioblastoma. In detail, autophagy inhibition produced by mTOR up-regulation determines the fate of cancer stem cells. On the other hand, biochemical findings disclose the remarkable effects of autophagy activators as powerful inducers of cell differentiation with a strong prevalence towards neuronal phenotypes. Thus, mTOR modulation acts on the neurobiology of glioblastoma just like it operates *in vivo* at the level of brain stem cell niches by altering autophagy-dependent cell differentiation. In the light of such a critical role of autophagy we analyzed the ubiquitin proteasome system. The merging between autophagy and proteasome generates a novel organelle, named autophagoproteasome which is strongly induced by mTOR inhibitors in glioblastoma cells. Remarkably, when mTOR is maximally inhibited the proteasome component selectively moves within autophagy vacuoles, thus making the proteasome activity dependent on the entry within autophagy compartment.

**Key words:** Autophagy, Autophagoproteasome, Cancer stem cells, Glioblastoma, Stem cell differentiation

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DOI: 10.14670/HH-11-835

## Introducing the role of mTOR in astrocytoma

Gliomas are the most common primary brain tumors, accounting approximately for 32% of all primary CNS tumors and they represent 80% of malignant CNS tumors (Agnihotri et al., 2013). About 76% of all gliomas are astrocytomas. This is a group of neoplasms which can be divided into four prognostic grades (grade I-IV, Louis et al., 2007; CBTRUS, 2010). In particular, grade IV refers to the most malignant glioma, known as Glioblastoma Multiforme (GBM). This is the most common (about 54% of all astrocytic tumors), aggressive and lethal primary brain tumor. The high invasiveness and a lack of effective cures make GBM significantly inauspicious, with mean survival rate ranging from 9 to 12 months from diagnosis (Persson et al., 2007; Agnihotri et al., 2013).

A growing body of data indicates that the mammalian Target of Rapamycin (mTOR), a molecular complex placed downstream to PI3K/PTEN/Akt pathway (Fig. 1), is involved in GBM just as it happens in a variety of human diseases including type II diabetes

and neurodegeneration (Shaw and Cantley, 2006; Akhavan et al., 2010; Fan and Weiss, 2012; Laplante and Sabatini, 2012). In fact, among all molecular and cell alterations occurring in GBM, the aberrant signaling of AKT-mTOR is key in developing the malignant phenotype. This occurs via stimulation of cell migration (Catalano et al., 2015), infiltration (Palumbo et al., 2014; Chandrika et al., 2016), tumor relapse and capability to elude current treatments (Zhuang et al., 2011; Garros-Regulez et al., 2016).

Recently, evidence indicated that a fraction of cells within tumors share the biological properties of normal stem cells, such as the potential of self-renewing and maintaining proliferation; thus, this cell fraction may initiate and propagate the tumor (Rosen and Jordan, 2009; Zhao et al., 2010; Goffart et al., 2013). These cells may also promote tumor progression in orthotopic xenograft (Venere et al., 2011). Nowadays this cell fraction identified in GBM is known as Glioma Stem/Progenitor Cells (GSPCs) (Galli et al., 2004; Singh et al., 2004). GSPCs represent the amplification of stem cell niches which are placed in perivascular areas of the CNS in baseline conditions. For instance, in the normal brain the most active stem cell niche is placed within the subependymal ventricular zone of cornu temporalis within the lateral ventricle, just close to the Cornu Ammonis and Dentatus Gyrus of hippocampus (Basak and Taylor, 2009). Remarkably, when a GBM develops, a number of atypical and ectopic stem cell niches is generated by strong angiogenetic stimulation (Calabrese et al., 2007). Thus, the tumor-induced support to microvascular proliferation is fundamental to perpetuate GSPCs, which in turn promote tumor growth by infiltration within surrounding tissue. Thus, it is likely that GSPCs are responsible for GBM progression, radio- and chemo-resistance, and relapse (Singh et al., 2004; Bao et al., 2006; Zhuang et al., 2009). All this evidence calls for an in depth characterization of those molecular pathways which generate and characterize GSPCs. In detail, GSPCs are characterized by marked up-regulation of mTOR. Some studies demonstrate the involvement of PTEN/Akt/PI3K signaling in the maintenance and viability of GSPCs population (Zeng and Zhou, 2008). In fact, the relationship between GBM and mTOR can be traced back to the level of GSPCs, which may be considered as an early, though persistent stage in tumor development.

### Up-regulation of mTOR in human astrocytoma

Human studies demonstated mTOR up-regulation in a variety of astrocytoma, such as subependymal giant cell astrocytoma (SEGA), which is a rare, slowly-growing, benign tumor (WHO grade I). This tumor mainly occurs in patients under 20 years of age, but it can also be found in infants and fetus (Hahn et al., 1991; Medhkour et al., 2002). Subependymal giant cell astrocytoma is usually found in patients with tuberous sclerosis complex (TSC), in which the increase in mTOR activity is a constant hallmark (Giorgi et al., 2015). The SEGA is generally non-infiltrating and poorly vascular. This is often bound to the wall of the lateral ventricle near the Monroe foramen. Surgical resection is the main option, although there are specific sites that make the mass difficult to be removed surgically. As mentioned above, the occurrence of this tumor often happens in the context of TSC, which is a genetic, multisystem disorder caused by mutations in tumor suppressor genes TSC1 and TSC2 (Tyburczy et al., 2010; Giorgi et al., 2015; Jóźwiak et al., 2015). However, solitary SEGAs (without TSC-related lesions) have also been reported (Ichikawa et al., 2005). Franz et al. (2006) provided the first morphological evidence for a successful pharmacological treatment with rapamycin in SEGA patients. By using MRI scans performed at regular time intervals, they demonstrated that rapamycin (sirolimus) when administered orally, at standard immunosuppressive doses (serum levels ranging between 5-15 ng/ml) for 2.5 up to 20 months, markedly reduces tumor volume. At that time, the authors, who were driven by an empirical approach, could not be aware of the molecular mechanisms underlying their results (Franz et al., 2006). Nowadays, the relationship between mTOR and TSC complex is better characterized. TCS complex is composed of TSC1 and TSC2 (Fig. 1). TSC1 codes for the protein hamartin, which acts via stabilizing TSC2 (Chong-Kopera et al., 2006). TSC2 codes for the protein tuberin, which is a GTPase activating RHEB (Ras homolog enriched in brain) (Zhang et al., 2003) (Fig. 1). In normal conditions, TSC complex inhibits the mammalian Target of Rapamycin Complex 1 (mTORC1). Following mutations of these tumor suppressing genes (TSC1/2) the complex does not work properly, therefore mTORC1 is activated by high levels of RHEB-GTP (Fig. 1). This leads to a downstream cascade which produces cell overgrowth, abnormal cell differentiation, and cell migration which cause brain neoplasms (Fingar and Blenis, 2004; Wullschlege et al., 2006). In line with this, several studies demonstrate that mTOR activation is crucial for SEGA's pathogenesis. In particular, immune-histochemical and genetic analyses demonstrate that a bi-allelic mutation of TSC genes (through a two-hit mechanism of bi-allelic inactivation of TSC1 or TSC2), leads to activation of mTOR kinase, which triggers mTOR activity (Chan et al., 2004; Astrinidis and Henske, 2005). In this way, SEGA TSC patients lose the functional interplay within the tuberin-hamartin complex, which fosters tumor development. Evidence on altered mTOR signaling in the pathogenesis of SEGA was provided in cultured TSC1 and TSC2 null cells and it was further confirmed in primary cell cultures derived from SEGA TSC patients (Tyburczy et al., 2010). Based on these observations, pharmacological treatments with mTOR inhibitors (sirolimus and everolimus) were tried (Koenig et al., 2008; Krueger et al., 2010). In fact, the mTOR inhibitor everolimus has been approved by FDA (2010) as the first alternative treatment to surgical resection in SEGA patients. This

becomes crucial for those patients in which tumor placement does not allow to perform invasive therapeutic approaches. Recently, Moavero et al. (2013) showed a significant volume reduction in SEGA during the first 3 months of everolimus administration. These effects persisted up to 12-18 months of continuous treatment with no relapse. This is critical since the neoplasm often relapses when the therapy is withdrawn. Further studies are needed to assess whether the pharmacological inhibition of mTOR can be protracted in a long-term therapy. In the study of Franz et al. (2013) 117 patients with TSC-related SEGA were treated with oral everolimus (to achieve blood levels of 5-15 ng/mL) which led to more than 50% tumor reduction in 35% of patients (Franz et al., 2013). These studies suggest the potential of mTOR inhibitors to be considered as valid therapeutic approach in astrocytoma (Roth et al., 2013).

A potential role of mTOR signaling in the biology of Pediatric Low-Grade Glioma (PLGG), including Pilocytic Astrocytoma (PA), has been evaluated by Hütt-Cabezas and colleagues (Hütt-Cabezas et al., 2013). They analyzed 177 whole tissue sections of PLGGs by immune-histochemistry and western blot analysis to assess different substrates of mTOR pathway (i.e. pS6, p4EBP1, RAPTOR, RICTOR, pAkt). The results show a consistent increase in mTOR-related antigens in PLGGs. When the mTOR pathway is inhibited by using the rapalog MK8669 (ridaforolimus) in PLGGs-derived cell lines, a marked suppression of cell proliferation is observed, as revealed by BrdU incorporation (Hütt-Cabezas et al., 2013). In low-grade gliomas mTOR alters morphological phenotype. This was investigated by Jentoft et al. (2011) by analysing 22 cases of low grade astrocytomas. These patients were affected by pilocytic astrocytoma (PA) and "low-grade astrocytoma with subtype indeterminate" (LGSI). By studying LGSI it was demonstrated that mTOR activation was responsible for specific morophological variations, such as increased cytoplasmic size and occurrence of macronuclei. A significant correlation exists between histopathological variations and increased mTOR signaling which is confirmed by phospho-S6, a marker used to evaluate mTOR activation, which increases in astrocytoma (Jentoft et al., 2011).

Only a few morphological studies were carried out on the role of mTOR on anaplastic astrocytoma (AA). One study demonstrates the occurrence of mutation in the gene coding for PTEN, a tumor suppressing gene, which is the most important endogenous inhibitor of mTOR (Smith et al., 2001). PI3K/Akt and mTOR pathways need to be tightly regulated and to interact properly to sustain normal development through a large number of physiological processes, such as proliferation, metabolism, angiogenesis, survival and differentiation. Dysregulation of these pathways leads to a number of pathological conditions including GBM (Akhavan et al., 2010; Fan and Weiss, 2012). In fact, an aberrant AKT-mTOR signaling in GBM correlates with malignancy. In particular, mTOR up-regulation is related with high proliferation, invasiveness, relapse and resistance to current treatments (Zhuang et al., 2011; Palumbo et al., 2014; Catalano et al., 2015; Garros-Regulez et al., 2016).

The occurrence of phosphatase and tensin homolog (PTEN) mutations occurs in roughly 70% of GBM. In fact, PTEN possess anti-tumoral effects by activating several downstream pathways such as PI3K/Akt/mTOR (Rasheed et al., 1997; Chakravarti et al., 2004; Parsons et al., 2008; Cancer Genome Atlas Research Network, 2008) (Fig. 1).

## Up-regulation of mTOR suppresses autophagy (Fig. 1)

The over-expression/-activity of mTOR, in turn leads to the inhibition of the autophagy pathway (ATG), which by itself triggers a variety of biochemical cascades known to be crucial for the neoplasm and in particular, for the fate of GSPCs (Ji et al., 2006; Zhang et al., 2006).

In fact, mTOR is known to suppress ATG, while mTOR inhibition (induced for instance by rapamycin or rapalogs) strongly activates ATG (Codogno and Meijer, 2005; Jung et al., 2010). Recent evidence suggests that activation of ATG plays a beneficial role in a variety of gliomas (Jiang et al., 2009; Liu et al., 2014), and clinical trials are currently in progress to assess the efficacy of therapies which combine TOR inhibitors with classic temozolomide chemotherapy and/or radiotherapy (Burckel et al., 2005; Chheda et al., 2015; Ma et al., 2015; Wen et al., 2015; Yan et al., 2016).

In line with this, activation of ATG, a major intracellular pathway which quickly activates cell catabolism, leads to a wide spectrum of antitumoral effects (Mariño et al., 2007; Levine and Kroemer, 2008; Yang and Klionsky, 2010). Indeed, when looking at classic literature on tumors, one of the most consistent metabolic findings is the slowing down of cell metabolism, which switches from aerobic to anaerobic. This old concept in oncology reminds us "there is nothing new under the sun"; we are just detailing previous classic knowledge in the light of current terminology and improved understanding of cell metabolism.

The present manuscript represents a short overview aimed to focus on key molecular mechanisms operating in astrocytoma and GBM, and to provide experimental evidence on the role of mTOR-dependent ATG inhibition and its reversal by targeting mTOR and PIP2 to impair the development of GBM. Altogether, the review of the literature joined with novel experimental data, provides updated evidence on the morphological findings, which connect mTOR, ATG and the various facets of GBM malignancy. In fact, in the second part of the manuscript we report recent data and add original pictures produced by our joint laboratories (University

of Pisa and IRCCs Neuromed), which lend substance to ATG status in GBM cells. In this paper we also report fascinating evidence which represents the powerful properties of rapamycin to promote cell differentiation *in vivo* in a brain glioblastoma xenograft.

## Up-regulation of mTOR in glioblastoma produces a variety of cell and molecular effects

Knowledge of up- and down-stream events involved in PI3K/Akt/mTOR signaling pathways derives

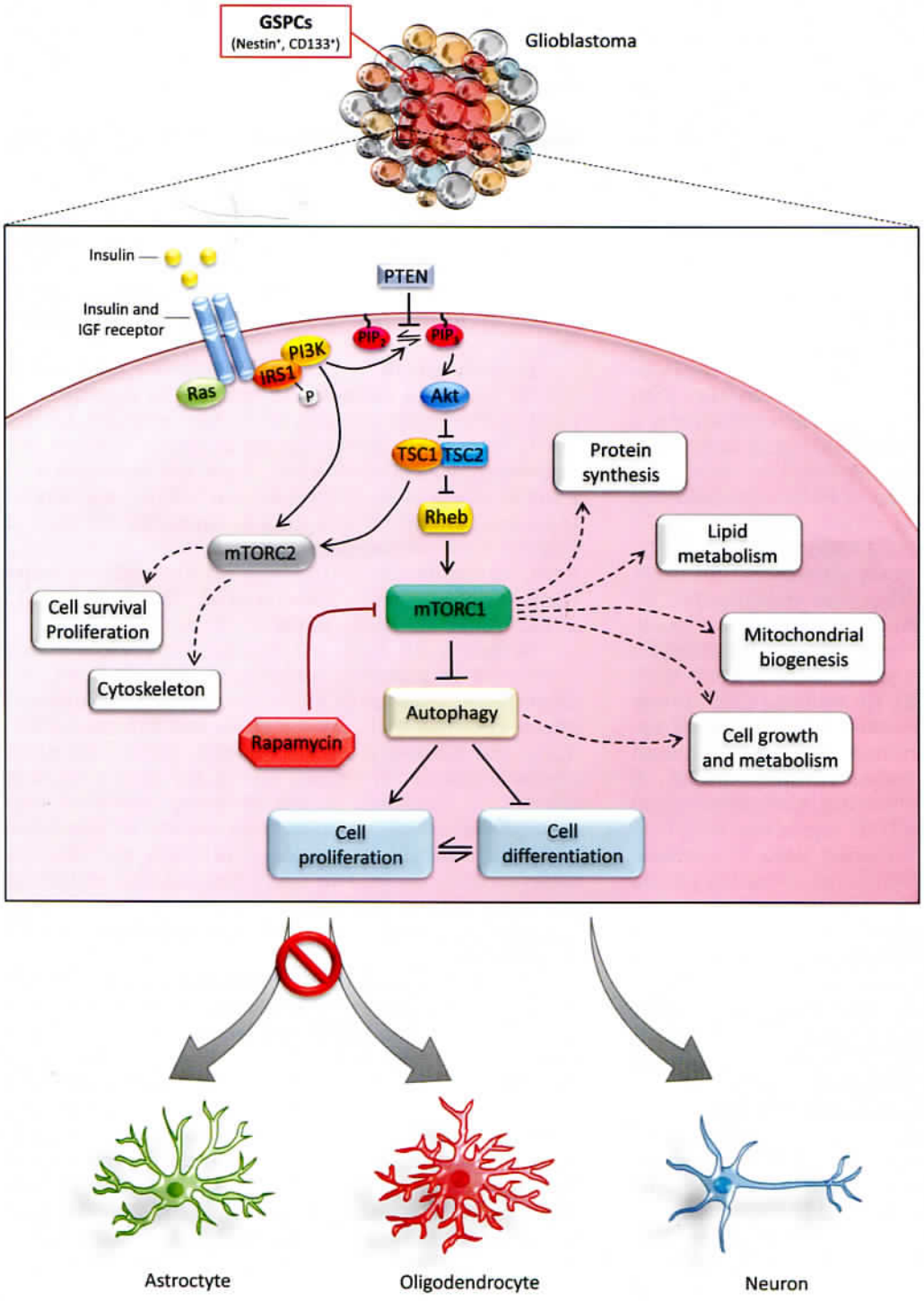


**Fig. 1.** mTOR modulates the fate of GSPCs through a number of pathways including autophagy. This illustration resumes the main upstream and downstream signaling cascades linking mTOR up-regulation and the consequent autophagy (ATG) inhibition, as hallmarks of Glioblastoma Stem/Progenitor Cells (GSPCs). The mTOR up-regulation which occurs within GSPCs leads to an aberrant signaling which suppresses the ATG machinery. Altogether these effects promote cell proliferation and sustain biological properties of stem-like cells. This maintains GBM stem cell niches, which in turn promote tumor growth and infiltration within surrounding tissue. This illustration eventually emphasizes the efficacy of autophagy activators (i.e. rapamycin) as powerful inhibitors of cell proliferation as well as powerful inducers of cell differentiation, with a strong prevalence towards a neuron phenotype. The rapamycin-induced rescue of ATG reduces tumorigenic potential of GSPCs, which are induced to differentiate towards a neuronal cell line.

primarily from *in vitro* studies (Neshat et al., 2001; Choe et al., 2003). Early evidence of the beneficial effects of mTOR inhibition in GBM was obtained by Eshleman and colleagues (Eshleman et al., 2002). They demonstrated that rapamycin inhibits proliferation in various glioma cell lines (A172, U87, U118 and SKMG-3, Eshleman et al., 2002). In particular, incubation with 100 nM rapamycin for 72h produces inhibition of cell proliferation as measured by MTS assay. Moreover, inhibition of mTOR by 100 nM or even lower doses of rapamycin leads to inhibition of a G1-specific cyclin-dependent kinase activity, which arrests the cell cycle in the G0-G1 phase as determined by flow cytometry in two cell lines (U87 and SKMG-3, Eshleman et al., 2002), as well as in U87MG cells and primary patients cells (Arcella et al., 2013). As reported by a number of publications, when mTOR is inhibited by rapamycin, it may produce hyperactive PI3K/Akt pathway, thus leading to drug resistance in tumor cells, which may limit drug efficacy. In contrast, the inhibition of this pathway restores the response to chemotherapy (Shingu et al., 2003). Thus, the effects of rapamycin could be enhanced by combined administration of PTEN/PI3K/Akt pathway inhibitors (Fig. 1). The results obtained by combining mTOR inhibitors with PI3K or Akt inhibitors (LY294002 and UCN-01, respectively) suggest that, in malignant glioma cells, the disruption of the PI3K/Akt signaling pathway enhances the efficacy of rapamycin, especially in rapamycin-resistant glioma cell lines (Takeuchi et al., 2005). In a recent study we found that rapamycin, while inhibiting cell proliferations in various GBM cell lines, may indeed increase Akt. However, if one considers that the active isoform corresponds to phosphorylated Akt (pAkt), then the effects of rapamycin are just the opposite, since it decreases the ratio between pAkt/Akt (Arcella et al., 2013). This suggests that the increase in Akt per se, produced by rapamycin, may not be relevant since the active form of Akt appears to be reduced.

Remarkably, when PI3K/Akt/mTOR pathway is over active the effects of mTOR inhibition are more evident (Kenerson et al., 2002; Kwon et al., 2002; Huang and Houghton, 2003), which lends substance to the role of mTOR inhibitors as a potential adjunct therapy in malignant gliomas even considering its effects on Akt.

The complexity of mTOR pathway and its role as central modulator of cell proliferation in malignant gliomas was originally reviewed by Bjornsti and Houghton (2004). Rapamycin-induced mTOR inhibition produces a variety of downstream effects; among these, the suppression of ATG machinery is crucial. In fact, Takeuchi et al. (2005) found that rapamycin induces autophagy in U87MG and T98G cell lines by inhibiting mTOR. ATG inhibition is likely to be responsible for suppressing development and progression of GBM, as shown *in vivo* and *in vitro* (Mills et al., 2001; Podsypanina et al., 2001; Chang et al., 2007; Weppler et al., 2007; Arcella et al., 2013). *In vivo*, rapamycin inhibits the growth of a peripheral xenograft, in subcutaneously implanted mouse, as described by Weppler et al. (2007). In this work, rapamycin was administered as a single agent to nude mice (i.p., 1 mg/kg, for 2 days) and the xenograft was immune-stained for phospho-S6, as a marker for mTOR activity. In rapamycin treated tumors, there was a ~50% decrease of phospho-S6 immune-staining, and rapamycin reduced the rate of tumor growth. The peripheral xenograft carries the inherent limit of being placed outside the blood brain barrier and it grows in a "milieu" which is way different from the CNS, which is the natural seat of GBM. Therefore, the effectiveness of rapamycin needed to be assessed within CNS xenografts. Thus, in 2013 we administered rapamycin to mice which were implanted human GBM cells in the left striatum. In these experimental conditions, rapamycin was administered either immediately, or at 1 week following tumor implantation. In both cases, 3 weeks of systemic rapamycin administration (i.p., 5 mg/Kg, daily) produced a massive reduction of tumor volume, which exceeded 95% of controls. These data were produced even withdrawing rapamycin weeks before sacrifice. These data were intriguing since it remains to be established whether a complete tumor regression may take place when the drug administration is reiterated for longer times. In this study, we also observed *in vitro* that the anti-proliferative effects of rapamycin measured in U87MG cell lines occurred with a dose-response curve, which overlaps exactly with what we measured in primary cell cultures derived from different patients. At low doses, the pharmacological treatment had no effects on cell viability, while only the highest doses decreased cell proliferation up to a marked cytotoxicity. The inhibition of cell proliferation was dose- and time-dependent (measured with direct cell count, MTT assay, and FACS analysis). Transmission electron microscopy (TEM), confirmed that rapamycin toxicity may involve an excess of ATG activation, rather than apoptosis. This study generated solid evidence showing the efficacy of rapamycin *in vivo* in the brain. Moreover, these data suggested further mechanisms which are presently under scrutiny for the beneficial effects of mTOR inhibition in GBM. For instance, we found that the tumor volume reached at 7 days following implantation was way greater than the volume which was measured following rapamycin even when the drug was started just at 7 days following tumor implantation. This suggests that rapamycin did not simply inhibit tumor growth but it also generated tumor regression. If this hypothesis is correct, one should expect the presence of necrotic or apoptotic areas within or around the original tumor volume measured at 7 days following implantation; however, we never detected any altered brain areas around or within the small tumor size in rapamycin treated tumors. Thus, neoplastic cells composing the greatest part of the tumor volume, when measured at 7 days following implantation, disappeared at 21 days following rapamycin, but we still do not know their fate, which indeed remains a dilemma. This is why in the

following paragraph we discuss and review hypotheses on further mechanisms, which may operate in GBM during mTOR inhibition.

### Additional mechanisms recruited *in vivo* by mTOR inhibition

Although mTOR inhibitors and ATG activators were reported to induce in vitro autophagy cell death, a previous report (Zhuang et al., 2011) failed to document cell death when rapamycin was administered in a single dose *in vivo*, focally within the tumor injection site, at the dose of 3 nmol in 15 μL. Despite the invasive procedure to administer rapamycin which may have produced mechanical bias, this study does not report any cell death. In another *in vivo* study carried out in subcutaneously transplanted xenograft in nude mice, rapamycin was administered at the dose of 1 mg/Kg i.p, daily, up to 6 days (Weppler et al., 2007). In this study rapamycin produced a delay in tumor growth. However, even in this study no evidence of cell death was reported. In a previous study, rapamycin was administered at the dose of 5 mg/Kg daily, i.p., for 21 or 28 days but no cell death was observed (Arcella et al., 2013).

Thus, despite various experimental protocols, no study has documented *in vivo* the occurrence of cell death induced by rapamycin within GBM xenografts. Remarkably, these studies demonstrated that rapamycin reduces tumor development. In detail, when rapamycin administration was reiterated daily at the dose of 5 mg/Kg, the reduction in tumor volume was surprising being above 95% (which was accompanied by a significant increase in survival rate). Such a massive effect induced by rapamycin *in vivo* when administered systemically calls for an in situ characterization of cell phenotypes in the tissue surrounding and within the tumor. This is crucial, since only one study carried out in 2011 analyzed the phenotype *in vivo*, but this was made following an invasive injection of rapamycin within the tumor (Zhuang et al., 2011). Since rapamycin suppresses the amount of GSPCs *in vitro*, it would be interesting to investigate whether a comparable cell differentiation occurs *in vivo* following systemic rapamycin injections. This is shown in (Fig. 2), showing nestin immune-staining along with the early neuronal marker, beta-III tubulin, and the glial marker, GFAP, in brain tissue, which was stained for H&E to assess tumor volume. These original pictures we report here (Fig. 2) feed the hypothesis that the pro-differentiating effects of rapamycin may switch the cells from neoplastic ones to differentiated neurons, in the absence of any cell death. In fact, if the antitumoral effects of rapamycin were based on cell toxicity, this could have been documented within and/or around the remnant tumoral tissue. Again, if the antitumoral effects of rapamycin were solely based on slowing down the cell growth, then the volume of the tumor measured at 7 days following implantation could not regress of nearly 10 fold when compared to what measured at 28 days following implantation (21 days of treatment). In fact, we detected a 50% decrease in Ki67 positive cells *in vivo* following rapamycin, but this anti-proliferative effect is expected only to slow down the tumor growth. Instead, we measured a regression of tumor size in the absence of cell death, which represents the crucial dilemma. In fact, the reduction of roughly 50% of Ki67 immune-staining that we detected corresponds to the analogous suppression of BrdU immune-staining measured by Weppler et al. (2007) in subcutaneously implanted GBM, treated with 1 mg/Kg rapamycin up to 20 days. This effect, despite reducing tumor growth, cannot account for a regression in tumor size. This assumption generates the working hypothesis that rapamycin acts on GBM both reducing cell proliferation and by promoting GSPCs differentiation *in vivo* (Figs. 1, 2). As reported in original pictures of Fig. 2, brain tissue sections from the left striatum of vehicle and rapamycin-treated mice bearing a GBM xenograft differ markedly (Fig. 2A,B). In fact, when stained with hematoxylin/eosin, the tumoral tissue of a vehicle-treated mouse is intensely stained with hematoxylin, as expected due to the high basophilia. In the same area of a rapamycin-treated mouse, the border of the strong hematoxylin-stained tissue is evident. This corresponds to the boundary of the tumor as conventionally described in this experimental model. Thus, the occurrence of intensely hematoxylin-stained cells is substituted by eosinophilic brain cells with no cell death. Remarkably, when this area is stained for the stemness marker nestin the difference made by rapamycin is remarkable, being lost the brownish peroxidase staining which marks the presence of the stem cell antigen nestin (Fig. 2C,D). At the same time, the early neuronal marker beta-III tubulin, which is absent in the hematoxylin-stained tissue of the vehicle administered mouse, is well evident in the presence of rapamycin (Fig. 2E,F). Remarkably, the occurrence of such an early neuronal marker suggests an ongoing differentiation, since such immune-staining does not occur in the striatum in baseline conditions. This further suggests that the fate of the tumor under the effects of rapamycin *in vivo* consists of a decrease in cell proliferation and progressive loss of the stem cell-like phenotype towards a neuronal differentiation. Of course, such a hypothesis needs to be validated by research papers detailing the continuum progression from GSPCs towards early and post-mitotic neurons, which possibly integrates in the trim of the brain hosting the xenograft. In line with this, experimental procedures aimed at staining the brain areas previously recruited by the tumor with the post-mitotic neuronal antigen NeuN are much needed. In the very same experimental conditions, when the brain tissue was probed for the differentiated glial protein GFAP antigen, we failed to document an increase in the amount of immune-positive cells following rapamycin. Nonetheless, rapamycin alters the distribution of GFAP immune-positive cells, which were clearly evident as an intense border around the reduced tumor mass. This

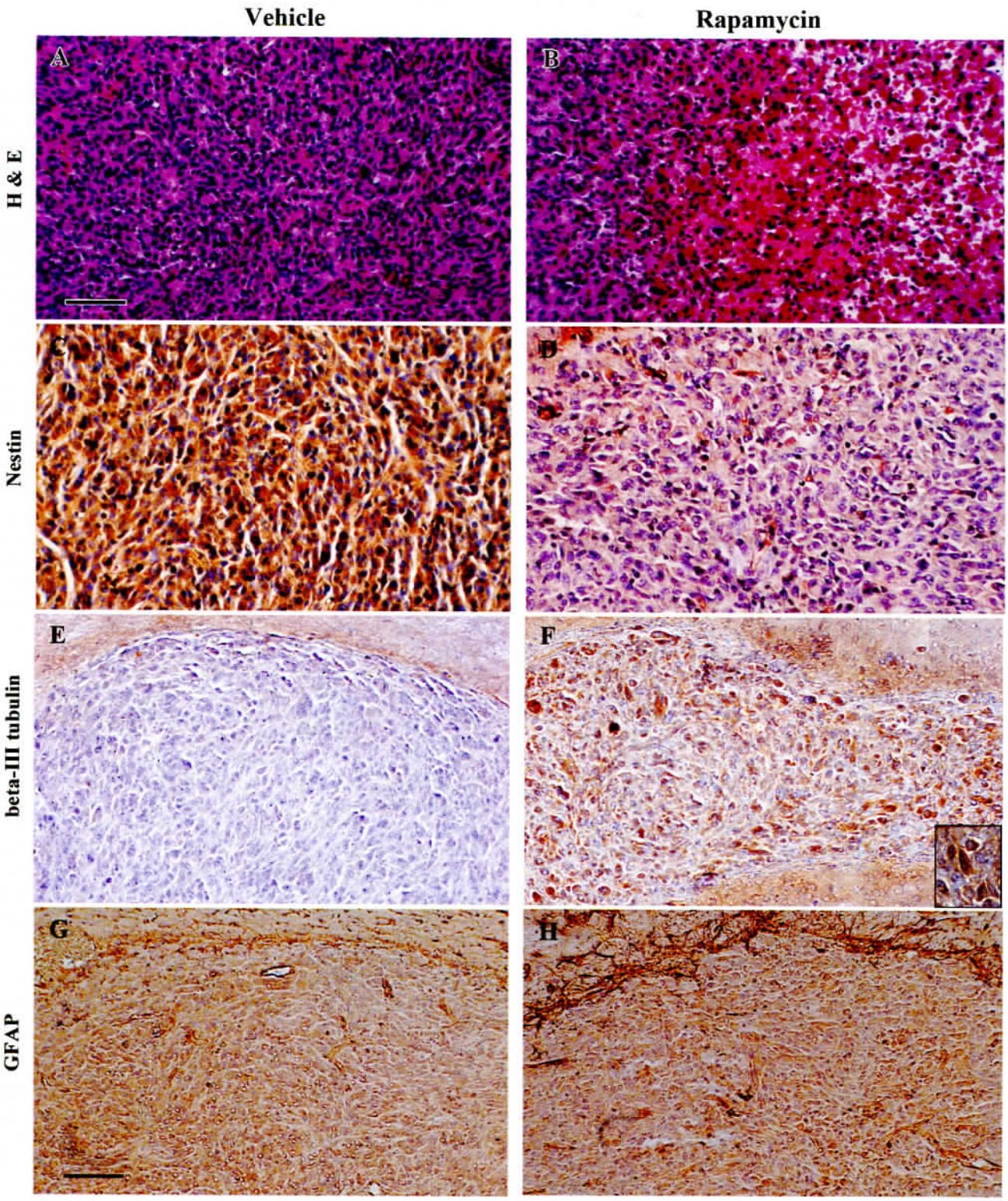


**Fig. 2.** Rapamycin induces cell differentiation in glioblastoma brain xenograft. The Figure reports an original micrograph from an *in vivo* study carried out in male CD1 nude mice stereotaxically implanted U87MG cells (400000 cells/5 µl/5 min) into the left striatum. The control mouse was treated with a water solution of DMSO10%, i.p. (vehicle). Rapamycin (5 mg/Kg, i.p.) was administered daily starting at 7 days following U87MG cell implantation (when the brain tumor already reaches a conspicuous volume). At the end of the treatment (day 21) mice were sacrificed under deep anesthesia and the brain was processed for immune-histochemical analysis. Representative pictures show brain tissue sections of vehicle and rapamycin-treated mice bearing GBM xenograft (respectively **A, B**) stained with hematoxylin/eosin. In the vehicle treated mouse an intensive hematoxylin staining is observed, while a clear cut hematoxynophilic tumoral area is present in rapamycin treated mouse. Immune-histochemistry for the stem cell marker nestin shows rapamycin-induced reduction of nestin positive cells in glioblastoma xenograft **(C, D)**. A significant increase of the early neuron marker beta-III tubulin is evident after rapamycin treatment **(E, F)**. The GFAP-positivity following treatment with rapamycin is only increased in the tumor boundary, as shown in representative pictures of immune-histochemistry against the glial marker GFAP **(G, H)**. Scale bars: A-D, G, H, 50 µm; E, F, 100 µm.

magnifies what is already evident in tumors following vehicle (Fig. 2G,H). Remarkably, promoting stem cell differentiation towards a neuron vs a glia phenotype is typical for compounds which induce autophagy even in normal brain tissue (Chen et al., 2000; Kim et al., 2004; Fornai et al., 2005, 2008; Wada et al., 2005; Yucel et al., 2008; Boku et al., 2009, 2011; Pasquali et al., 2009; Su et al., 2009; Wada, 2009; Fiorentini et al., 2010; Pasquali et al., 2010; Hanson et al., 2011; Huo et al., 2012; Jeerage et al., 2012; Fornai et al., 2014; Rodolfo et al., 2016).

## Recent findings on GSPCs differentiation induced by rescue of ATG

In the light of recent findings obtained *in vitro*, the hypothesis we made based on *in vivo* data becomes more and more plausible. In fact, GBM relapse and infiltration correlates with up-regulation of the molecular complex mTOR, which in turn leads to the inhibition of ATG pathway, which is a hallmark in GBM cells (Jiang et al., 2009; Olsen et al., 2012; Liu et al., 2014; Ma et al., 2015).

The gold standard to detect ATG still remains transmission electron microscopy (TEM). Remarkably, Zhao et al. (2010), applied such a method to examine the ultrastructure of GSPCs. These cells feature a suppressed ATG activity when compared to NSPCs (Neural Stem/Progenitor Cells). In fact, double membrane vesicles (i.e. ATG-like vacuoles, autophagosomes) were rarely observed in GSPCs, contrarily to NSPCs. When measured in the presence of fetal calf serum (FCS, which induces cell differentiation) ATG activity in GSPCs was increased. This is counteracted by ATG inhibitors (3-methyladenine, 3-MA, or bafillomycin A1, BFA), while it is enhanced/reproduced by ATG activators (rapamycin). Thus, ATG activation leads to GSPCs differentiation. This suggests that, in general, ATG produces cell differentiation or *vice-versa*, cell differentiation elevates ATG or, alternatively, these events are randomly associated. It is likely that the process of cell differentiation derives from ATG induction as witnessed by a number of recent studies (Fornai et al., 2008; Delk and Farach-Carson, 2012; Zogovic et al., 2015; Rodolfo et al., 2016). For instance, in a seminal study Jang et al. (2016) demonstrate that ATG is key in inducing neuroectodermal differentiation in human embryonic mesodermal/neuroectodermal stem cells. Amongst the multiple ATG regulators, p62 plays a fundamental role in neural stem cell differentiation (Wang et al., 2016). Altogether, these data confirm that a lack of differentiation, which characterizes GSPCs (Gam et al., 2004), strongly correlates with ATG depression which is measured within the same cells. *Vice-versa*, ATG activation leads to GSPCs differentiation. Sunayama et al. (2010) found that inhibiting mTOR with rapamycin, or knocking down mTOR by RNA interference in Cancer Stem-Like Cells (CSLCs), markedly inhibits neuro-spheres formation, while reducing the expression of NSC/progenitor markers (Nestin, CD133, Bmi1, Sox2). The differentiating effects produced by mTOR inhibition are enhanced by a concomitant inhibition of PI3K, since the dual mTOR-PI3K inhibition (with rapamycin and LY294002) abolishes stemness markers while increasing neuronal differentiation markers (beta-III tubulin). However, such a dual effect was also reported to occur following the sole mTOR inhibition (rapamycin, 200 nM, Zhuang et al., 2011), which by itself down-regulates stem cell markers while increasing markers of cell differentiation in human GSPCs and *in vivo* in nude mice bearing orthotopic SU-2 cell xenograft. These effects were reversed by the ATG inhibitor 3-MA, confirming that GSPC differentiation promoted by rapamycin is bound to ATG induction. On the other hand, the prominent role of ATG in mediating GSPCs differentiation induced by mTOR inhibition is confirmed by alternative experiments. In fact, one may stimulate ATG by-passing the mTOR pathway, either by inhibiting phosphatidyl-inositol turn-over or inducing the Wnt/beta catenin pathway, which in turn induces stem cell differentiation (Wang et al., 2009, 2012; Jeerage et al., 2012; Walasek et al., 2012; Fornai et al., 2014; Dong et al., 2015), both in normal brain (Yan et al., 2007; Wexler et al., 2008; Fiorentini et al., 2010) as well as in GBM (Korur et al., 2009; Elmaci and Altinoz, 2016).

Similarly to GSPCs differentiation, mTOR activity is likely to rely on ATG pathway for its effects on cell migration and infiltration, which are hallmarks of GBM aggressiveness and lethality (Galavotti et al., 2013; Chandrika et al., 2016). In particular, the results obtained by Catalano et al. (2015) extend the effects of ATG modulation to GBM cell migration. In fact, Beclin 1-depleted GBM cells (GL15 cells infected with a lentivirus carrying Beclin 1 shRNA) show increased migration and invasion concomitantly to up-regulation of two epithelial-mesenchimal transition process regulators (SNAIL and SLUG). The translational value of these fine molecular mechanisms bridging mTOR up-regulation, ATG inhibition, stemness, and migration, is confirmed by pathological studies from human patients. When 62 tumor samples from different grade astrocytoma were analyzed, the expression of Beclin 1 and LC3B-II in high-grade astrocytoma (WHO grade III, IV) was lower than in low grade astrocytoma (WHO grade I, II), which confirms the correlation between ATG suppression and tumor grading. In line with this, beclin-1 and LC3B-II exhibit a correlation with survival rates (Huang et al., 2010).

## Baseline suppression and rapamycin-induced rescue of autophagoproteasomes in GBM

In the light of recent findings showing the contamination of the ATG pathway by other cell clearing mechanisms, it is worth mentioning the integrated scenario of cell clearing pathways in GBM cells (Fig. 3). While ATG inhibition is well established as a marker of

GBM cells, the activity of another clearing mechanism, the ubiquitin-proteasome (UP) pathway is less characterized. In fact, although proteasome inhibitors have been suggested as an adjunct therapy in GBM (Friday et al., 2012; Lin et al., 2014), all studies aimed to measure baseline activity of UP in GBM, report a UP depression, which recapitulates what described for ATG (Vlashi et al., 2009; Lagadec et al., 2014; Stacer et al., 2015). In this latter manuscript the activity of UP was related to classic phenotypes of GSPCs such as tumor-initiation, expression of stem cell markers, relapse, and neuro-sphere formation. In addition, Stacer et al. (2015) found that primary human GBM cell cultures produce tumors in mouse xenografts to an extent which is related to the loss of UP activity. Similarly, disulfiram may work as an adjunct therapy in GBM just for its proteasome-dependent stimulation of degradation of the chemo-resistance protein MGMT (Paranjpe et al., 2014).

In line with this, Sato et al. (2013) found that proteasome activation degrades Nanog in GBM, thus reducing Nanog-related detrimental effects on tumor progression, such as proliferation rate and amount of GSPCs within the tumor. The beneficial effects of proteasome-dependent Nanog degradation can be elicited by proteasome activating compounds such as resveratrol. In fact, resveratrol-induced suppression of Nanog within GBM is due to UP activation (Sato et al., 2013). It is remarkable that a specific interplay may act within GBM between ATG and UP (Fig. 3). In fact, as shown by Ding et al. (2012) in GBM cells, when ATG is suppressed by knocking down beclin-1, a sudden increased sensitivity to proteasome inhibition occurs. This suggests a functional interplay and reciprocal compensation between ATG and UP in GBM (Ding et al., 2012), analogous to that described in other systems (Pandey et al., 2007; Rubinsztein, 2007; Rüssmann et al., 2010; Sheng et al., 2012; Lee et al., 2016). On the other hand, proteasome may compensate when ATG is impaired (Giuliano et al., 2015). In keeping with this, there are a number of proteins, which represent both ATG and UP substrates (Webb et al., 2003; Ebrahimi-Fakhari et al., 2011; Engelender, 2012). This is

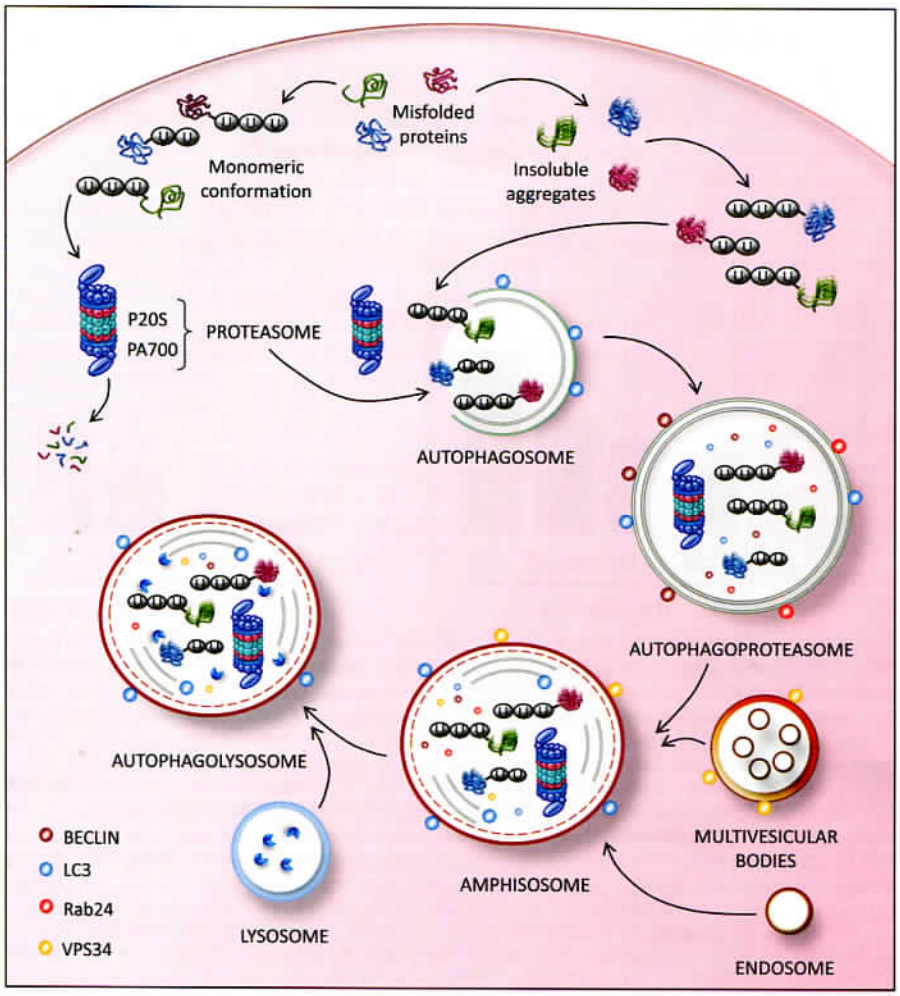


**Fig. 3.** Cell clearing pathways in GBM include the autphagoproteasome. This illustration reports the main clearing pathways within Glioblastoma Stem/Progenitor Cells (GSPCs). The GSPCs are characterized by marked mTOR up-regulation, which in turn suppresses autophagy, the main way of removing damaged organelles and macromolecules. This produces a suppression of ubiquitin proteasome system (UP) as well. These combined effects reduce the autophago-proteasome. This is a unique novel organelle where ATG and UP morphological and biochemical cascades converge. The classic ATG pathway occurs within a double-layered cytosolic vacuole named autophagosome, gifted with a rich enzymatic apparatus aimed at clearing various cell cargoes and waste compounds. In contrast, the classic UP pathway, which does not imply a well-defined cell organelle, occurs within dispersed cytosolic domains where UP subunits (P20S) interact to recognize altered ubiquitinated substrates and provide proteolitic clearance. The autophago-proteasome, an ultimate sophisticated clearing apparatus, is derived from the inclusion of UP structures within either early or late ATG vesicles (i.e amphisome) containing cytoplasmic material at various stages of degradation. (Lenzi et al 2016). This adds to enzymatic activity of amphisome leading to a novel extremely powerful eukariotic clearing system.

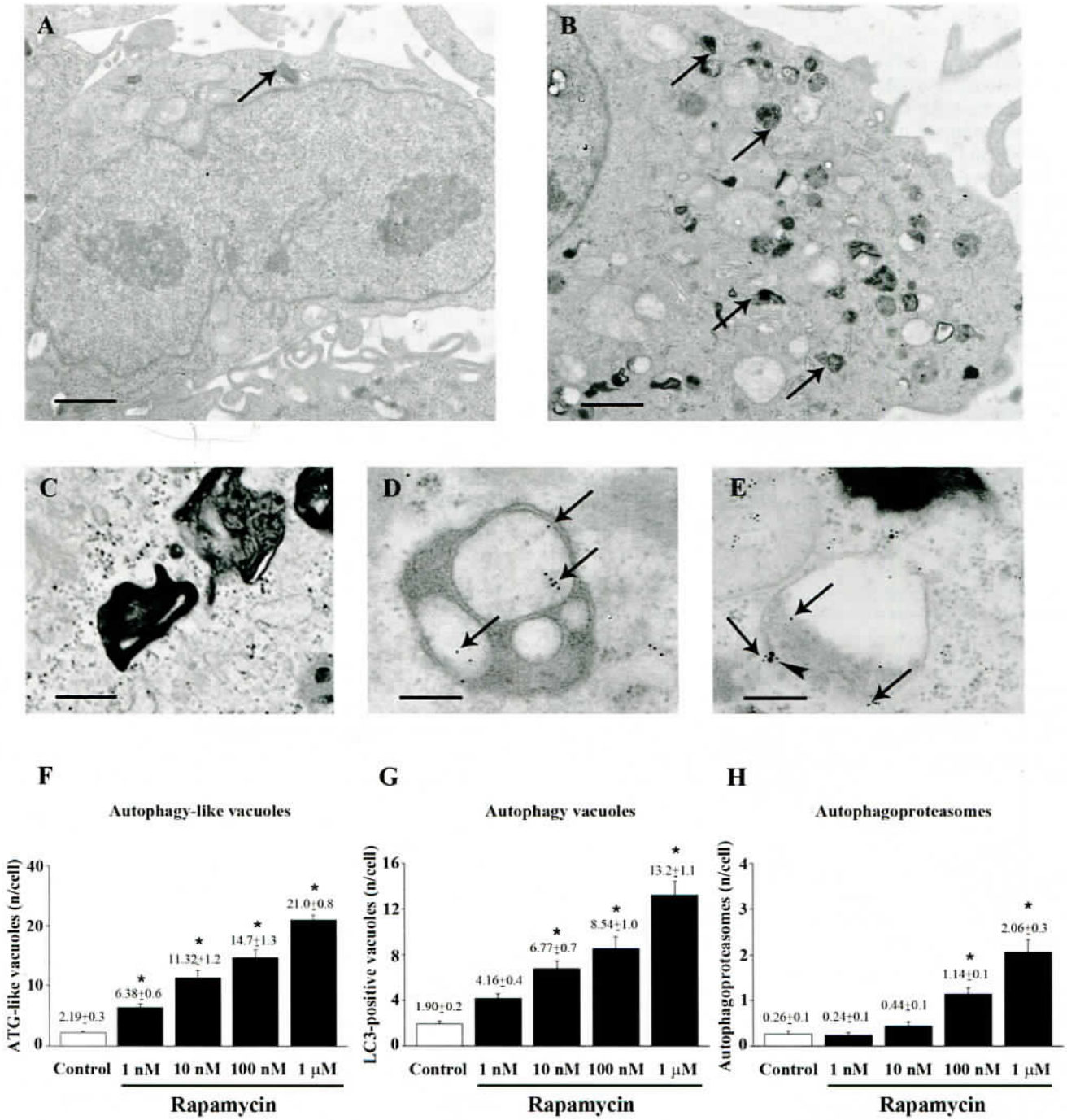


**Fig. 4.** Plain electron microscopy and immune-cytochemistry of U87MG cells. Representative pictures from human glioblastoma cell lines U87MG which were treated with rapamycin (1, 10, 100 and 1000 nM for 24h). Control cells were maintained in culture medium containing 0.01% DMSO. After 24h U87MG cells were pre-fixed in a solution containing 2.0% paraformaldehyde/0.1% glutaraldehyde in 0.1M PBS (pH 7.4), post-fixed in 1% $OsO_4$ and embedded in Epoxy-resin. For ultrastructural morphometry, ultrathin sections were examined directly at TEM at a magnification of 8,000x. For immune-cytochemistry, sections were incubated with primary antibodies (anti-LC3 and anti-p20S) and then with gold-coniugated secondary antibodies. Finally, samples were fixed with 1.0% glutaraldehyde, contrasted with uranyl acetate and lead citrate and then observed using electron microscopy. Representative pictures show ATG-like vacuoles (arrows) in the cytoplasm in control condition **(A)** and after 100 nM of rapamycin **(B, C)**. The number of cytosolic ATG-like vacuoles significantly increases dose-dependently after rapamycin compared with vehicle (control) as reported in graph **(F)**. As shown in representative picture **(C)** ATG vacuoles were stained with LC3 immune-gold particles (shown by arrows, **D**). ATG LC3-positive vacuoles increase dose-dependently after rapamycin (1 nM; 10 nM; 100 nM and 1 μM) compared with control as reported in graph. In the representative picture **(E)** is shown an ATG vacuole harboring both ubiquitin proteasome (UP) component revealed by P20S particles (arrowhead) and ATG particles (LC3 particles, arrows). This very new organelle named autophagoproteasome (Lenzi et al., 2016) represents the merging of UP and ATG system. Treatment with rapamycin induces a dose-dependent increase of autophagoproteasomes (LC3+P20S positive vacuoles) **(H)**. Values are given as the mean±SEM (N=20). Comparisons between groups were made by using one-way ANOVA followed by Scheffé post-hoc test. *P≤0.05 compared with baseline conditions. Scale bars: A, B, 0.8 μm; C, 0.38 μm; D, 0.25 μm; E, 0.22 μm.

remarkable since in a recent study ATG and UP components were demonstrated to be hosted in the same organelles named autophagoproteasomes (Klionsky et al., 2016; Lenzi et al., 2016) (Fig. 3). In this way, the double/multiple membrane vacuoles, which were reported to contain also the endosomal enzymatic apparatus in the form of amphisomes, are now considered as ultimate clearing organelles in which an enriched catalytic pattern occurs, thus providing the merging of all main clearing pathways. Within these autophagoproteasomes, mTOR acts like a fine modulator on both UP and ATG (Fig. 4), though providing a stronger stimulus for UP when mTOR is markedly suppressed, whereas ATG activity prevails following milder mTOR inhibition (Lenzi et al., 2016). This ultimate clearing system was shown to occur in GBM cell lines where a marked, dose-dependent activation occurs under the effects of rapamycin. Within this system almost all cytosolic UP particles are sequestered upon strong mTOR inhibition (Lenzi et al., 2016), whereas the rich ATG compartment remains partly independent. In all cases, when rapamycin is administered, a concomitant increase occurs in double membrane vacuoles, LC3-immune-positive vacuoles and double LC3+P20S immune-positive vacuoles (Fig. 4). This novel organelle apart from generating a new scenario in cell biology and general mechanisms of cell histopathology, also provides a remarkable target for specific drugs. In fact, resveratrol, apart from being a UP activator, is known to exert beneficial effects in glioma cells by also activating the ATG machinery (Filippi-Chiela et al., 2011). Thus, although certain drugs are preferential activators of ATG or UP, others may activate both pathways. Interestingly, recent studies have shown that a variety of natural dietary compounds such as curcumin and quercetin, in addition to resveratrol, regulate both protein quality control systems (Murakami, 2013). This might provide novel adjunct therapies in astocytoma and GBM.

*Acknowledgements.* This work was supported by a grant of the Ministry of Health (Ricerca Corrente 2016/FF). The authors wish to thank Dr Antonella Arcella for the fruitful collaboration in the research topic.

## References

Agnihotri S., Burrell K.E., Wolf A., Jalali S., Hawkins C., Rutka J.T. and Zadeh G. (2013). Glioblastoma, a brief review of history, molecular genetics, animal models and novel therapeutic strategies. Arch. Immunol. Ther. Exp. 61, 25-41.

Akhavan D., Cloughesy T.F. and Mischel P.S. (2010). mTOR signaling in glioblastoma: lessons learned from bench to bedside. NeuroOncol. 12, 882-889.

Arcella A., Biagioni F., Oliva M.A., Bucci D., Frati A., Escposito V., Cantore G., Giangaspero F. and Fornai F. (2013). Rapamycin inhibits the growth of glioblastoma. Brain Res. 1495, 37-51.

Astrinidis A. and Henske E.P. (2005). Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. Oncogene 24, 7475-7481.

Bao S., Wu Q., McLendon R.E., Hao Y., Shi Q., Hielmeland A.B., Dewhirst M.W., Bigner D.D. and Rich J.N. (2006). Glioma stem cells promote radioresistance by preferential activation ofthe DNA damage response. Nature 444, 756-760.

Basak O. and Taylor V. (2009). Stem cells of the adult mammalian brain and their niche. Cell. Mol. Life Sci. 66, 1057-1072.

Bjornsti M.A. and Houghton P.J. (2004). The TOR pathway: a target for cancer therapy. Nat. Rev. Cancer 4, 335-348.

Boku S., Nakagawa S., Masuda T., Nishikawa H., Kato A., Kitaichi Y., Inoue T. and Koyama T. (2009). Glucocorticoids and lithium reciprocally regulate the proliferation of adult dentate gyrus-derived neural precursor cells through GSK-3beta and beta-catenin/TCF pathway. Neuropsychopharmacology 34, 805-815.

Boku S., Nakagawa S., Masuda T., Nishikawa H., Kato A., Toda H., Song N. and Kitaichi Y. (2011). Effects of mood stabilizers on adult dentate gyrus-derived neural precursor cells. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 111-117.

Burckel H., Josset E., Denis J.M., Gueulette J., Slabbert J., Noël G. and Bischoff P. (2015). Combination of the mTOR inhibitor RAD001 with temozolomide and radiation effectively inhibits the growth of glioblastoma cells in culture. Oncol. Rep. 33, 471-477.

Calabrese C., Poppleton H., Kocak M., Hogg T.L., Fuller C., Hamner B., Oh E.Y., Gaber M.W., Finklestein D., Allen M., Frank A., Bayazitov I.T., Zakharenko S.S., Gaijar A., Davidoff A. and Gilbertson R.J. (2007). A perivascular niche for brain tumor stem cells. Cancer Cell 11, 69-82.

Cancer Genome Atlas Research Network (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455, 1061-1068.

Catalano M., D'Alessandro G., Lepore F., Corazzari M., Caldarola S., Valacca C., Faienza F., Esposito V., Limatola C., Cecconi F. and Di Bartolomeo S. (2015). Autophagy induction impairs migration and invasion by reversing EMT in glioblastoma cells. Mol. Oncol. 9, 1612-1625.

CBTRUS (2010). CBTRUS Statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004-2006. Source: Central Brain Tumor Registry of the United States, Hinsdale, IL.

Chakravarti A., Zhai G., Suzuki Y., Sarkesh S., Black P.M., Muzikansky A. and Loeffler J.S. (2004). The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. J. Clin. Oncol. 22, 1926-1933.

Chan J.A., Zhang H., Roberts P.S., Jozwiak S., Wieslawa G., Lewin-Kowalik J., Kotulska K. and Kwiatkowski D.J. (2004). Pathogenesis of tuberous sclerosis subependymal giant cell astrocytomas: biallelic inactivation of TSC1 or TSC2 leads to mTOR activation. J. Neuropathol. Exp. Neurol. 63, 1236-1242.

Chang J.E., Khuntia D., Robins H.I. and Metha M.P. (2007). Radiotherapy and radiosensitizers in the treatment of glioblastoma multiforme. Clin. Adv. Hematol. Oncol. 5, 907-915.

Chandrika G., Natesh K., Ranade D., Chugh A. and Shastry P. (2016). Suppression of the invasive potential of glioblastoma cells by mTOR inhibitors involves modulation of NFκB and PKC-α signaling. Sci. Rep. 6: 22455.

Chen G., Rajkowska G., Du F., Seraji-Bozorgzad N. and Manji H.K. (2000). Enhancement of hippocampal neurogenesis by lithium. J. Neurochem. 75, 1729-1734.

Chheda M.G., Wen P.Y., Hochberg F.H., Chi A.S., Drappatz J., Eichler

A.F., Yang D., Beroukhim R., Norden A.D., Gerstner E.R., Betensky R.A. and Batchelor T.T. (2015). Vandetanib plus sirolimus in adults with recurrent glioblastoma: results of a phase I and dose expansion cohort study. J. Neurooncol. 121, 627-634.

Choe G., Horvath S., Cloughesy T.F., Crosby K., Seligson D., Palotie A., Inge L., Smith B.L., Sawyers C.L. and Mischel P.S. (2003). Analysis of the Phosphatidylinositol 3-Kinase signaling pathway in glioblastoma patients *in vivo*. Cancer Res. 63, 2742-2746.

Chong-Kopera H., Inoki K., Li Y., Zhu T., Garcia-Gonzalo F.R., Rosa J.L. and Guan K.L. (2006). TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. J. Biol. Chem. 281, 8313-8316.

Codogno P. and Meijer A.J. (2005). Autophagy and signaling: their role in cell survival and cell death. Cell. Death Differ. 12, 1509-1518.

Delk N.A. and Farach-Carson M.C. (2012). Interleukin-6: a bone marrow stromal cell paracrine signal that induces neuroendocrine differentiation and modulates autophagy in bone metastatic PCa cells. Autophagy 8, 650-663.

Ding W.X., Chen X. and Yin X.M. (2012). Tumor cells can evade dependence on autophagy through adaptation. Biochem. Biophys. Res. Commun. 425, 684-688.

Dong B.T., Tu G.J., Han Y.X. and Chen Y. (2015). Lithium enhanced cell proliferation and differentiation of mesenchymal stem cells to neural cells in rat spinal cord. Int. J. Clin. Exp. Pathol. 8, 2473-2483.

Ebrahimi-Fakhari D., Cantuti-Castelvetri I., Fan Z., Rockenstein E., Masliah E., Hyman B.T., McLean P.J. and Unni V.K. (2011). Distinct roles *in vivo* for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of α-synuclein. J. Neurosci. 31, 14508-14520.

Elmaci I. and Altinoz M.A. (2016). A metabolic inhibitory cocktail for grave cancers: Metformin, Pioglitazone and Lithium combination in treatment of pancreatic cancer and Glioblastoma Multiforme. Biochem. Genet. 5, 573-618.

Engelender S. (2012). α-Synuclein fate: proteasome or autophagy? Autophagy 8, 418-420.

Eshleman J.S., Brett L.C., Mladek A.C., Kastner D., Shide K.L and Sarkaria J.N. (2002). Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy. Cancer Res. 62, 7291-7297.

Fan Q.W. and Weiss W.A. (2012). Inhibition of PI3K-Akt-mTOR signaling in glioblastoma by mTORC1/2 inhibitors. Methods Mol. Biol. 821, 349-359.

Filippi-Chiela E.C., Villodre E.S., Zamin L.L. and Lenz G. (2011). Autophagy interplay with apoptosis and cell cycle regulation in the growth inhibiting effect of resveratrol in glioma cells. PLoS One 6:e20849.

Fingar D.C. and Blenis J. (2004). Target of rapamyicn (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 23, 3151-3171.

Fiorentini A., Rosi M.C., Grossi C., Luccarini I. and Casamenti F. (2010). Lithium improves hippocampal neurogenesis, neuropathology and cognitive functions in APP mutant mice. PLoS One 5:e14382.

Fornai F., Schlüter O.M., Lenzi P., Gesi M., Ruffoli R., Ferrucci M., Lazzeri G., Busceti C.L., Pontarelli F., Battaglia G., Pellegrini A., Nicoletti F., Ruggieri S., Paparelli A. and Südhof T.C. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. Proc. Natl. Acad. Sci. USA 102, 3413-3418.

Fornai F., Longone P., Ferrucci M., Lenzi P., Isidoro C., Ruggieri S. and Paparelli A. (2008). Autophagy and amyotrophic lateral sclerosis: The multiple roles of lithium. Autophagy 4, 527-530.

Fornai F., Ferrucci M., Lenzi P., Falleni A., Biagioni F., Flaibani M., Siciliano G., Giannessi F. and Paparelli A. (2014). Plastic changes in the spinal cord in motor neuron disease. Biomed. Res. Int. 2014, 670756.

Franz DN., Leonard J., Tudor C. Chuck G., Care M., Sethuraman G., Dinopoulos A., Thomas G. and Crone K.R. (2006). Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. Ann. Neurol. 59, 490-498.

Franz D.N., Belousova E., Sparagana S., Bebin E.M., Frost M., Kuperman R., Witt O., Kohrman M.H., Flamini J.R., Wu J.Y., Curatolo P., de Vries P.J., Whittemore V.H., Thiele E.A., Ford J.P., Shah G., Cauwel H., Lebwohl D., Sahmound T. and Jozwiak S. (2013). Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomized, placebo-controlled phase 3 trial. Lancet 381, 125-132.

Friday B.B., S. Anderson K., Buckner J., Yu C., Giannini C., Geoffroy F., Schwerkoske J., Mazurczak M., Gross H., Pajon E., Jaeckle K. and Galanis E. (2012). Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. Neuro Oncol. 14, 215-221.

Galavotti S., Bartesaghi S., Faccenda D., Shaked-Rabi M., Sanzone S., McEvoy A., Dinsdale D., Condorelli F., Brandner S., Campanella M., Grose R., Jones C. and Salomoni P. (2013). The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. Oncogene 32, 699-712.

Galli R., Binda E., Orfanelli U., Cipelletti B., Gritti A., De Vitis S., Fiocco R., Foroni C., Dimeco F. and Vescovi A. (2004). Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 64, 7011-7021.

Gam R., Binda E., Orfanelli U., Cipelletti B., Gritti A., Vitis S.D., Fiocco R., Foroni C., Dimeco F. and Vescovi A. (2004). Isolation and characterization of tumorigenic, stem-like neural precursor from human glioblastoma. Cancer Res. 64, 7011-7021.

Garros-Regulez L., Aldaz P., Arrizabalaga O., Moncho-Amor V., Carrasco-Garcia E., Manterola L., Moreno-Cugnon L., Barrena C., Villanua J., Ruiz I., Pollard S., Lovell-Badge R., Sampron N., Garcia I. and Matheu A. (2016). mTOR inhibition decreasesSOX2-SOX9 mediated glioma stem cell activity and temozolomide resistance. Exp. Opin. Therapeutic Targets 20, 393-405.

Giorgi F.S., Biagioni F., Lenzi P. Frati A. and Fornai F. (2015). The role of autophagy in epileptogenesis and in epilepsy-induced neuronal alterations. J. Neural. Transm. (Vienna) 122, 849-862.

Giuliano S., Cormerais Y., Dufies M., Grépin R., Colosetti P., Belaid A., Parola J., Martin A., Lacas-Gervais S., Mazure N.M., Benhida R., Auberger P., Mograbi B. and Pagès G. (2015). Resistance to sunitinib in renal clear cell carcinoma results from sequestration in lysosomes and inhibition of the autophagic flux. Autophagy 11, 1891-1904.

Goffart N., Kroonen J. and Rogister B. (2013). Glioblastoma-Initiating Cells: relationship with Neural Stem Cells and the micro-environment. Cancers 5, 1049-1071.

Hahn J.S., Bejar R. and Gladson C.L. (1991). Neonatal subependymal giant cell astrocytoma associated with tuberous sclerosis: MRI, CT and ultrasound correlation. Neurology 41, 124-128.

Hanson N.D., Nemeroff C.B. and Owens M.J. (2011). Lithium, but not fluoxetine or the corticotropin-releasing factor receptor 1 receptor

antagonist R121919, increases cell proliferation in the adult dentate gyrus. J. Pharmacol. Exp. Ther. 337, 180-186.

Huang S. and Houghton P.J. (2003). Targeting mTOR signaling for cancer therapy. Curr. Opin. Pharmacol. 3, 371-377.

Huang X., Bai H.M., Chen L., Li B. and Lu Y.C. (2010). Reduced expression of LC3B-II and Beclin 1 in glioblastoma multiforme indicates a down-regulated autophagic capacity that relates to the progression of astrocytic tumors. J. Clin. Neurosci. 17, 1515-1519.

Huo K., Sun Y., Li H., Du X., Wang X., Karlsson N., Zhu C. and Blomgren K. (2012). Lithium reduced neural progenitor apoptosis in the hippocampus and ameliorated functional deficits after irradiation to the immature mouse brain. Mol. Cell. Neurosci. 51, 32-42.

Hütt-Cabezas M., Karajannis M.A., Zagzag D., Shah S., Horkayne-Szakaly I., Rushing E.J., Cameron J.D., Jain D., Eberhart C.G., Raabe E.H. and Rodriguez F.J. (2013). Activation of mTORC1/mTORC2 signaling in pediatric low-grade and pilocytic astrocytoma reveals mTOR as a therapeutic target. Neuro. Oncol. 15, 1604-1614.

Ichikawa T., Wakisaka A., Daido S., Takao S., Tamiya T. and Date I. (2005). A case of solitary subependymal giant cell astrocytoma: two somatic hits of TSC2 in the tumor, without evidence of somatic mosaicism. J. Mol. Diagn. 7, 544-549.

Jang J., Wang Y., Lalli M.A., Guzman E., Godshalk S.E., Zhou H. and Kosik K.S. (2016). Primary cilium-autophagy-Nrf2 (PAN) axis activation commits Human Embryonic Stem Cells to a neuroectoderm fate. Cell 165, 410-420.

Jentoft M., Giannini C., Cen L., Scheithauer B.W., Hoesley B., Sarkaria J.N., Abell-Aleff P.C., Rodriguez E.F., Li Y. and Rodriguez F.J. (2011). Phenotypic variations in NF1-associated low grade-astrocytomas: possible role for increased mTOR activation in a subset. Int. J. Clin. Exp. Pathol. 4, 43-57.

Jeerage K.M., Oreskovic T.L. and Hume S.L. (2012). Neurite outgrowth and differentiation of rat cortex progenitor cells are sensitive to lithium chloride at non-cytotoxic exposures. Neurotoxicology 33, 1170-1179.

Ji X.Y., Huang Q., Dong J., Zhu Y.D., Wang A.D. and Lan Q. (2006). Characteristics of morphology, differentiation related markers, and proliferation dynamics of differentiated brain tumor stem cells *in vitro*. Zhonghua Yi xue Za zhi. 86, 1604-1609.

Jiang H., White E.J., Conrad C., Gomez-Manzano C. and Fueyo J. (2009). Autophagy pathways in glioblastoma. Methods Enzymol. 453, 273-286.

Jóźwiak S., Mandera M. and Młynarski W. (2015). Natural history and current treatment options for subependymal giant cell astrocytoma in tuberous sclerosis complex. Semin. Pediatr. Neurol. 22, 274-281.

Jung C.H., Ro S.H., Cao J., Otto N.M. and Kim D.H. (2010). mTOR regulation of autophagy. FEBS Lett. 584, 1287-1295.

Kenerson H.L., Aicher L.D., True L.D. and Yeung R.S. (2002). Activated mammalian target of rapamycin pathway in the pathogenesis of tuberous sclerosis complex renal tumors. Cancer Res. 62, 5645-5650.

Kim J.S., Chang M.Y., Yu I.T., Kim J.H., Lee S.H., Lee Y.S. and Son H. (2004). Lithium selectively increases neuronal differentiation of hippocampal neural progenitor cells both *in vitro* and *in vivo*. J. Neurochem. 89, 324-336

Klionsky D.J., Abdelmohsen K., Abe A., Abedin M.J., Abeliovich H., Acevedo Arozena A., Adachi H., Adams C.M., Adams P.D., Adeli K., Adhihetty P.J., Adler S.G., Agam G., Agarwal R., Aghi M.K., Agnello M., Agostinis P., Aguilar P.V., Aguirre-Ghiso J., Airoldi E.M., Ait-Si-Ali S., Akematsu T., Akporiaye E.T., Al-Rubeai M., Albaiceta G.M., Albanese C., Albani D., Albert M.L., Aldudo J., Algül H., Alirezaei M., Alloza I., Almasan A., Almonte-Beceril M., Alnemri E.S., Alonso C., Altan-Bonnet N., Altieri D.C., Alvarez S., Alvarez-Erviti L., Alves S., Amadoro G., Amano A., Amantini C., Ambrosio S., Amelio I., Amer A.O., Amessou M., Amon A., An Z., Anania F.A., Andersen S.U., Andley U.P., Andreadi C.K., Andrieu-Abadie N., Anel A., Ann D.K., Anoopkumar-Dukie S., Antonioli M., Aoki H., Apostolova N., Aquila S., Aquilano K., Araki K., Arama E., Aranda A., Araya J., Arcaro A., Arias E., Arimoto H., Ariosa A.R., Armstrong J.L., Arnould T., Arsov I., Asanuma K., Askanas V., Asselin E., Atarashi R., Atherton S.S., Atkin J.D., Attardi L.D., Auberger P., Auburger G., Aurelian L., Autelli R., Avagliano L., Avantaggiati M.L., Avrahami L., Awale S., Azad N., Bachetti T., Backer J.M., Bae D.H., Bae J.S., Bae O.N., Bae S.H., Baehrecke E.H., Baek S.H., Baghdiguian S., Bagniewska-Zadworna A., Bai H., Bai J., Bai X.Y., Bailly Y., Balaji K.N., Balduini W., Ballabio A., Balzan R., Banerjee R., Bánhegyi G., Bao H., Barbeau B., Barrachina M.D., Barreiro E., Bartel B., Bartolomé A., Bassham D.C., Bassi M.T., Bast R.C. Jr, Basu A., Batista M.T., Batoko H., Battino M., Bauckman K., Baumgarner B.L., Bayer K.U., Beale R., Beaulieu J.F., Beck G.R. Jr, Becker C., Beckham J.D., Bédard P.A., Bednarski P.J., Begley T.J., Behl C., Behrends C., Behrens G.M., Behrns K.E., Bejarano E., Belaid A., Belleudi F., Bénard G., Berchem G., Bergamaschi D., Bergami M., Berkhout B., Berliocchi L., Bernard A., Bernard M., Bernassola F., Bertolotti A., Bess A.S., Besteiro S., Bettuzzi S., Bhalla S., Bhattacharyya S., Bhutia S.K., Biagosch C., Bianchi M.W., Biard-Piechaczyk M., Billes V., Bincoletto C., Bingol B., Bird S.W., Bitoun M., Bjedov I., Blackstone C., Blanc L., Blanco G.A., Blomhoff H.K., Boada-Romero E., Böckler S., Boes M., Boesze-Battaglia K., Boise L.H., Bolino A., Boman A., Bonaldo P., Bordi M., Bosch J., Botana L.M., Botti J., Bou G., Bouché M., Bouchecareilh M., Boucher M.J., Boulton M.E., Bouret S.G., Boya P., Boyer-Guittaut M., Bozhkov P.V., Brady N., Braga V.M., Brancolini C., Braus G.H., Bravo-San Pedro J.M., Brennan L.A., Bresnick E.H., Brest P., Bridges D., Bringer M.A., Brini M., Brito G.C., Brodin B., Brookes P.S., Brown E.J., Brown K., Broxmeyer H.E., Bruhat A., Brum P.C., Brumell J.H., Brunetti-Pierri N., Bryson-Richardson R.J., Buch S., Buchan A.M., Budak H., Bulavin D.V., Bultman S.J., Bultynck G., Bumbasirevic V., Burelle Y., Burke R.E., Burmeister M., Bütikofer P., Caberlotto L., Cadwell K., Cahova M., Cai D., Cai J., Cai Q., Calatayud S., Camougrand N., Campanella M., Campbell G.R., Campbell M., Campello S., Candau R., Caniggia I., Cantoni L., Cao L., Caplan A.B., Caraglia M., Cardinali C., Cardoso S.M., Carew J.S., Carleton L.A., Carlin C.R., Carloni S., Carlsson S.R., Carmona-Gutierrez D., Carneiro L.A., Carnevali O., Carra S., Carrier A., Carroll B., Casas C., Casas J., Cassinelli G., Castets P., Castro-Obregon S., Cavallini G., Ceccherini I., Cecconi F., Cederbaum A.I., Ceña V., Cenci S., Cerella C., Cervia D., Cetrullo S., Chaachouay H., Chae H.J., Chagin A.S., Chai C.Y., Chakrabarti G., Chamilos G., Chan E.Y., Chan M.T., Chandra D., Chandra P., Chang C.P., Chang R.C., Chang T.Y., Chatham J.C., Chatterjee S., Chauhan S., Che Y., Cheetham M.E., Cheluvappa R., Chen C.J., Chen G., Chen G.C., Chen G., Chen H., Chen J.W., Chen J.K., Chen M., Chen M., Chen P., Chen Q., Chen Q., Chen S.D., Chen S., Chen S.S., Chen W., Chen W.J., Chen W.Q., Chen W., Chen X., Chen Y.H., Chen Y.G., Chen Y., Chen Y., Chen Y., Chen Y.J., Chen Y.Q., Chen Y., Chen Z., Chen Z., Cheng A., Cheng C.H., Cheng H., Cheong H., Cherry S., Chesney J., Cheung C.H., Chevet E., Chi H.C., Chi S.G., Chiacchiera F., Chiang H.L., Chiarelli

R., Chiariello M., Chieppa M., Chin L.S., Chiong M., Chiu G.N., Cho D.H., Cho S.G., Cho W.C., Cho Y.Y., Cho Y.S., Choi A.M., Choi E.J., Choi E.K., Choi J., Choi M.E., Choi S.I., Chou T.F., Chouaib S., Choubey D., Choubey V., Chow K.C., Chowdhury K., Chu C.T., Chuang T.H., Chun T., Chung H., Chung T., Chung Y.L., Chwae Y.J., Cianfanelli V., Ciarcia R., Ciechomska I.A., Ciriolo M.R., Cirone M., Claerhout S., Clague M.J., Clària J., Clarke P.G., Clarke R., Clementi E., Cleyrat C., Cnop M., Coccia E.M., Cocco T., Codogno P., Coers J., Cohen E.E., Colecchia D., Coletto L., Coll N.S., Colucci-Guyon E., Comincini S., Condello M., Cook K.L., Coombs G.H., Cooper C.D., Cooper J.M., Coppens I., Corasaniti M.T., Corazzari M., Corbalan R., Corcelle-Termeau E., Cordero M.D., Corral-Ramos C., Corti O., Cossarizza A., Costelli P., Costes S., Cotman S.L., Coto-Montes A., Cottet S., Couve E., Covey L.R., Cowart L.A., Cox J.S., Coxon F.P., Coyne C.B., Cragg M.S., Craven R.J., Crepaldi T., Crespo J.L., Criollo A., Crippa V., Cruz M.T., Cuervo A.M., Cuezva J.M., Cui T., Cutillas P.R., Czaja M.J., Czyzyk-Krzeska M.F., Dagda R.K., Dahmen U., Dai C., Dai W., Dai Y., Dalby K.N., Dalla Valle L., Dalmasso G., D'Amelio M., Damme M., Darfeuille-Michaud A., Dargemont C., Darley-Usmar V.M., Dasarathy S., Dasgupta B., Dash S., Dass C.R., Davey H.M., Davids L.M., Dávila D., Davis R.J., Dawson T.M., Dawson V.L., Daza P., de Belleroche J., de Figueiredo P., de Figueiredo R.C., de la Fuente J., De Martino L., De Matteis A., De Meyer G.R., De Milito A., De Santi M., de Souza W., De Tata V., De Zio D., Debnath J., Dechant R., Decuypere J.P., Deegan S., Dehay B., Del Bello B., Del Re D.P., Delage-Mourroux R., Delbridge L.M., Deldicque L., Delorme-Axford E., Deng Y., Dengjel J., Denizot M., Dent P., Der C.J., Deretic V., Derrien B., Deutsch E., Devarenne T.P., Devenish R.J., Di Bartolomeo S., Di Daniele N., Di Domenico F., Di Nardo A., Di Paola S., Di Pietro A., Di Renzo L., DiAntonio A., Díaz-Araya G., Diaz-Laviada I., Diaz-Meco M.T., Diaz-Nido J., Dickey C.A., Dickson R.C., Diederich M., Digard P., Dikic I., Dinesh-Kumar S.P., Ding C., Ding W.X., Ding Z., Dini L., Distler J.H., Diwan A., Djavaheri-Mergny M., Dmytruk K., Dobson R.C., Doetsch V., Dokladny K., Dokudovskaya S., Donadelli M., Dong X.C., Dong X., Dong Z., Donohue T.M. Jr, Doran K.S., D'Orazi G., Dorn G.W. 2nd., Dosenko V., Dridi S., Drucker L., Du J., Du L.L., Du L., du Toit A., Dua P., Duan L., Duann P., Dubey V.K., Duchen M.R., Duchosal M.A., Duez H., Dugail I., Dumit V.I., Duncan M.C., Dunlop E.A., Dunn W.A. Jr, Dupont N., Dupuis L., Durán R.V., Durcan T.M., Duvezin-Caubet S., Duvvuri U., Eapen V., Ebrahimi-Fakhari D., Echard A., Eckhart L., Edelstein C.L., Edinger A.L., Eichinger L., Eisenberg T., Eisenberg-Lerner A., Eissa N.T., El-Deiry W.S., El-Khoury V., Elazar Z., Eldar-Finkelman H., Elliott C.J., Emanuele E., Emmenegger U., Engedal N., Engelbrecht A.M., Engelender S., Enserink J.M., Erdmann R., Erenpreisa J., Eri R., Eriksen J.L., Erman A., Escalante R., Eskelinen E.L., Espert L., Esteban-Martínez L., Evans T.J., Fabri M., Fabrias G., Fabrizi C., Facchiano A., Færgeman N.J., Faggioni A., Fairlie W.D., Fan C., Fan D., Fan J., Fang S., Fanto M., Fanzani A., Farkas T., Faure M., Favier F.B., Fearnhead H., Federici M., Fei E., Felizardo T.C., Feng H., Feng Y., Feng Y., Ferguson T.A., Fernández Á.F., Fernandez-Barrena M.G., Fernandez-Checa J.C., Fernández-López A., Fernandez-Zapico M.E., Feron O., Ferraro E., Ferreira-Halder C.V., Fesus L., Feuer R., Fiesel F.C., Filippi-Chiela E.C., Filomeni G., Fimia G.M., Fingert J.H., Finkbeiner S., Finkel T., Fiorito F., Fisher P.B., Flajolet M., Flamigni F., Florey O., Florio S., Floto R.A., Folini M., Follo C., Fon E.A., Fornai F., Fortunato F., Fraldi A., Franco R., Francois A., François A., Frankel L.B., Fraser I.D., Frey N., Freyssenet D.G., Frezza C., Friedman S.L., Frigo D.E., Fu D., Fuentes J.M., Fueyo J., Fujitani Y., Fujiwara Y., Fujiya M., Fukuda M., Fulda S., Fusco C., Gabryel B., Gaestel M., Gailly P., Gajewska M., Galadari S., Galili G., Galindo I., Galindo M.F., Galliciotti G., Galluzzi L., Galluzzi L., Galy V., Gammoh N., Gandy S., Ganesan A.K., Ganesan S., Ganley I.G., Gannagé M., Gao F.B., Gao F., Gao J.X., García Nannig L., García Véscovi E., Garcia-Macía M., Garcia-Ruiz C., Garg A.D., Garg P.K., Gargini R., Gassen N.C., Gatica D., Gatti E., Gavard J., Gavathiotis E., Ge L., Ge P., Ge S., Gean P.W., Gelmetti V., Genazzani A.A., Geng J., Genschik P., Gerner L., Gestwicki J.E., Gewirtz D.A., Ghavami S., Ghigo E., Ghosh D., Giammarioli A.M., Giampieri F., Giampietri C., Giatromanolaki A., Gibbings D.J., Gibellini L., Gibson S.B., Ginet V., Giordano A., Giorgini F., Giovannetti E., Girardin S.E., Gispert S., Giuliano S., Gladson C.L., Glavic A., Gleave M., Godefroy N., Gogal R.M. 0, Gokulan K., Goldman G.H., Goletti D., Goligorsky M.S., Gomes A.V., Gomes L.C., Gomez H., Gomez-Manzano C., Gómez-Sánchez R., Gonçalves D.A., Goncu E., Gong Q., Gongora C., Gonzalez C.B., Gonzalez-Alegre P., Gonzalez-Cabo P., González-Polo R.A., Goping I.S., Gorbea C., Gorbunov N.V., Goring D.R., Gorman A.M., Gorski S.M., Goruppi S., Goto-Yamada S., Gotor C., Gottlieb R.A., Gozes I., Gozuacik D., Graba Y., Graef M., Granato G.E., Grant G.D., Grant S., Gravina G.L., Green D.R., Greenhough A., Greenwood M.T., Grimaldi B., Gros F., Grose C., Groulx J.F., Gruber F., Grumati P., Grune T., Guan J.L., Guan K.L., Guerra B., Guillen C., Gulshan K., Gunst J., Guo C., Guo L., Guo M., Guo W., Guo X.G., Gust A.A., Gustafsson Å.B., Gutierrez E., Gutierrez M.G., Gwak H.S., Haas A., Haber J.E., Hadano S., Hagedorn M., Hahn D.R., Halayko A.J., Hamacher-Brady A., Hamada K., Hamai A., Hamann A., Hamasaki M., Hamer I., Hamid Q., Hammond E.M., Han F., Han W., Handa J.T., Hanover J.A., Hansen M., Harada M., Harhaji-Trajkovic L., Harper J.W., Harrath A.H., Harris A.L., Harris J., Hasler U., Hasselblatt P., Hasui K., Hawley R.G., Hawley T.S., He C., He C.Y., He F., He G., He R.R., He X.H., He Y.W., He Y.Y., Heath J.K., Hébert M.J., Heinzen R.A., Helgason G.V., Hensel M., Henske E.P., Her C., Herman P.K., Hernández A., Hernandez C., Hernández-Tiedra S., Hetz C., Hiesinger P.R., Higaki K., Hilfiker S., Hill B.G., Hill J.A., Hill W.D., Hino K., Hofius D., Hofman P., Höglinger G.U., Höhfeld J., Holz M.K., Hong Y., Hood D.A., Hoozemans J.J., Hoppe T., Hsu C., Hsu C.Y., Hsu L.C., Hu D., Hu G., Hu H.M., Hu H., Hu M.C., Hu Y.C., Hu Z.W., Hua F., Hua Y., Huang C., Huang H.L., Huang K.H., Huang K.Y., Huang S., Huang S., Huang W.P., Huang Y.R., Huang Y., Huang Y., Huber T.B., Huebbe P., Huh W.K., Hulmi J.J., Hur G.M., Hurley J.H., Husak Z., Hussain S.N., Hussain S., Hwang J.J., Hwang S., Hwang T.I., Ichihara A., Imai Y., Imbriano C., Inomata M., Into T., Iovane V., Iovanna J.L., Iozzo R.V., Ip N.Y., Irazoqui J.E., Iribarren P., Isaka Y., Isakovic A.J., Ischiropoulos H., Isenberg J.S., Ishaq M., Ishida H., Ishii I., Ishmael J.E., Isidoro C., Isobe K., Isono E., Issazadeh-Navikas S., Itahana K., Itakura E., Ivanov A.I., Iyer A.K., Izquierdo J.M., Izumi Y., Izzo V., Jäättelä M., Jaber N., Jackson D.J., Jackson W.T., Jacob T.G., Jacques T.S., Jagannath C., Jain A., Jana N.R., Jang B.K., Jani A., Janji B., Jannig P.R., Jansson P.J., Jean S., Jendrach M., Jeon J.H., Jessen N., Jeung E.B., Jia K., Jia L., Jiang H., Jiang H., Jiang L., Jiang T., Jiang X., Jiang X., Jiang X., Jiang Y., Jiang Y., Jiménez A., Jin C., Jin H., Jin L., Jin M., Jin S., Jinwal U.K., Jo E.K., Johansen T., Johnson D.E., Johnson G.V., Johnson J.D., Jonasch E., Jones C., Joosten L.A., Jordan J., Joseph A.M., Joseph B., Joubert A.M., Ju D., Ju J., Juan H.F., Juenemann K., Juhász G.,

Jung H.S., Jung J.U., Jung Y.K., Jungbluth H., Justice M.J., Jutten B., Kaakoush N.O., Kaarniranta K., Kaasik A., Kabuta T., Kaeffer B., Kågedal K., Kahana A., Kajimura S., Kakhlon O., Kalia M., Kalvakolanu D.V., Kamada Y., Kambas K., Kaminskyy V.O., Kampinga H.H., Kandouz M., Kang C., Kang R., Kang T.C., Kanki T., Kanneganti T.D., Kanno H., Kanthasamy A.G., Kantorow M., Kaparakis-Liaskos M., Kapuy O., Karantza V., Karim M.R., Karmakar P., Kaser A., Kaushik S., Kawula T., Kaynar A.M., Ke P.Y., Ke Z.J., Kehrl J.H., Keller K.E., Kemper J.K., Kenworthy A.K., Kepp O., Kern A., Kesari S., Kessel D., Ketteler R., Kettelhut Ido C., Khambu B., Khan M.M., Khandelwal V.K., Khare S., Kiang J.G., Kiger A.A., Kihara A., Kim A.L., Kim C.H., Kim D.R., Kim D.H., Kim E.K., Kim H.Y., Kim H.R., Kim J.S., Kim J.H., Kim J.C., Kim J.H., Kim K.W., Kim M.D., Kim M.M., Kim P.K., Kim S.W., Kim S.Y., Kim Y.S., Kim Y., Kimchi A., Kimmelman A.C., Kimura T., King J.S., Kirkegaard K., Kirkin V., Kirshenbaum L.A., Kishi S., Kitajima Y., Kitamoto K., Kitaoka Y., Kitazato K., Kley R.A., Klimecki W.T., Klinkenberg M., Klucken J., Knævelsrud H., Knecht E., Knuppertz L., Ko J.L., Kobayashi S., Koch J.C., Koechlin-Ramonatxo C., Koenig U., Koh Y.H., Köhler K., Kohlwein S.D., Koike M., Komatsu M., Kominami E., Kong D., Kong H.J., Konstantakou E.G., Kopp B.T., Korcsmaros T., Korhonen L., Korolchuk V.I., Koshkina N.V., Kou Y., Koukourakis M.I., Koumenis C., Kovács A.L., Kovács T., Kovacs W.J., Koya D., Kraft C., Krainc D., Kramer H., Kravic-Stevovic T., Krek W., Kretz-Remy C., Krick R., Krishnamurthy M., Kriston-Vizi J., Kroemer G., Kruer M.C., Kruger R., Ktistakis N.T., Kuchitsu K., Kuhn C., Kumar A.P., Kumar A., Kumar A., Kumar D., Kumar D., Kumar R., Kumar S., Kundu M., Kung H.J., Kuno A., Kuo S.H., Kuret J., Kurz T., Kwok T., Kwon T.K., Kwon Y.T., Kyrmizi I., La Spada A.R., Lafont F., Lahm T., Lakkaraju A., Lam T., Lamark T., Lancel S., Landowski T.H., Lane D.J., Lane J.D., Lanzi C., Lapaquette P., Lapierre L.R., Laporte J., Laukkarinen J., Laurie G.W., Lavandero S., Lavie L., LaVoie M.J., Law B.Y., Law H.K., Law K.B., Layfield R., Lazo P.A., Le Cam L., Le Roch K.G., Le Stunff H., Leardkamolkarn V., Lecuit M., Lee B.H., Lee C.H., Lee E.F., Lee G.M., Lee H.J., Lee H., Lee J.K., Lee J., Lee J.H., Lee J.H., Lee M., Lee M.S., Lee P.J., Lee S.W., Lee S.J., Lee S.J., Lee S.Y., Lee S.H., Lee S.S., Lee S.J., Lee S., Lee Y.R., Lee Y.J., Lee Y.H., Leeuwenburgh C., Lefort S., Legouis R., Lei J., Lei Q.Y., Leib D.A., Leibowitz G., Lekli I., Lemaire S.D., Lemasters J.J., Lemberg M.K., Lemoine A., Leng S., Lenz G., Lenzi P., Lerman L.O., Lettieri Barbato D., Leu J.I., Leung H.Y., Levine B., Lewis P.A., Lezoualc'h F., Li C., Li F., Li F.J., Li J., Li K., Li L., Li M., Li M., Li Q., Li R., Li S., Li W., Li W., Li X., Li Y., Lian J., Liang C., Liang Q., Liao Y., Liberal J., Liberski P.P., Lie P., Lieberman A.P., Lim H.J., Lim K.L., Lim K., Lima R.T., Lin C.S., Lin C.F., Lin F., Lin F., Lin F.C., Lin K., Lin K.H., Lin P.H., Lin T., Lin W.W., Lin Y.S., Lin Y., Linden R., Lindholm D., Lindqvist L.M., Lingor P., Linkermann A., Liotta L.A., Lipinski M.M., Lira V.A., Lisanti M.P., Liton P.B., Liu B., Liu C., Liu C.F., Liu F., Liu H.J., Liu J., Liu J.J., Liu J.L., Liu K., Liu L., Liu L., Liu Q., Liu R.Y., Liu S., Liu S., Liu W., Liu X.D., Liu X., Liu X.H., Liu X., Liu X., Liu X., Liu Y., Liu Y., Liu Z., Liu Z., Liuzzi J.P., Lizard G., Ljujic M., Lodhi I.J., Logue S.E., Lokeshwar B.L., Long Y.C., Lonial S., Loos B., López-Otín C., López-Vicario C., Lorente M., Lorenzi P.L., Lõrincz P., Los M., Lotze M.T., Lovat P.E., Lu B., Lu B., Lu J., Lu Q., Lu S.M., Lu S., Lu Y., Luciano F., Luckhart S., Lucocq J.M., Ludovico P., Lugea A., Lukacs N.W., Lum J.J., Lund A.H., Luo H., Luo J., Luo S., Luparello C., Lyons T., Ma J., M.a. Y., Ma Y., Ma Z., Machado J., Machado-Santelli G.M., Macian F., MacIntosh G.C., MacKeigan J.P., Macleod K.F., MacMicking J.D., MacMillan-Crow L.A., Madeo F., Madesh M., Madrigal-Matute J., Maeda A., Maeda T., Maegawa G., Maellaro E., Maes H., Magariños M., Maiese K., Maiti T.K., Maiuri L., Maiuri M.C., Maki C.G., Malli R., Malorni W., Maloyan A., Mami-Chouaib F., Man N., Mancias J.D., Mandelkow E.M., Mandell M.A., Manfredi A.A., Manié S.N., Manzoni C., Mao K., Mao Z., Mao Z.W., Marambaud P., Marconi A.M., Marelja Z., Marfe G., Margeta M., Margittai E., Mari M., Mariani F.V., Marin C., Marinelli S., Mariño G., Markovic I., Marquez R., Martelli A.M., Martens S., Martin K.R., Martin S.J., Martin S., Martin-Acebes M.A., Martin-Sanz P., Martinand-Mari C., Martinet W., Martinez J., Martinez-Lopez N., Martinez-Outschoorn U., Martinez-Velázquez M., Martinez-Vicente M., Martins W.K., Mashima H., Mastrianni J.A., Matarese G., Matarrese P., Mateo R., Matoba S., Matsumoto N., Matsushita T., Matsuura A., Matsuzawa T., Mattson M.P., Matus S., Maugeri N., Mauvezin C., Mayer A., Maysinger D., Mazzolini G.D., McBrayer M.K., McCall K., McCormick C., McInerney G.M., McIver S.C., McKenna S., McMahon J.J., McNeish I.A., Mechta-Grigoriou F., Medema J.P., Medina D.L., Megyeri K., Mehrpour M., Mehta J.L., Mei Y., Meier U.C., Meijer A.J., Meléndez A., Melino G., Melino S., de Melo E.J., Mena M.A., Meneghini M.D., Menendez J.A., Menezes R., Meng L., Meng L.H., Meng S., Menghini R., Menko A.S., Menna-Barreto R.F., Menon M.B., Meraz-Ríos M.A., Merla G., Merlini L., Merlot A.M., Meryk A., Meschini S., Meyer J.N., Mi M.T., Miao C.Y., Micale L., Michaeli S., Michiels C., Migliaccio A.R., Mihailidou A.S., Mijaljica D., Mikoshiba K., Milan E., Miller-Fleming L., Mills G.B., Mills I.G., Minakaki G., Minassian B.A., Ming X.F., Minibayeva F., Minina E.A., Mintern J.D., Minucci S., Miranda-Vizuete A., Mitchell C.H., Miyamoto S., Miyazawa K., Mizushima N., Mnich K., Mograbi B., Mohseni S., Moita L.F., Molinari M., Molinari M., Møller A.B., Mollereau B., Mollinedo F., Mongillo M., Monick M.M., Montagnaro S., Montell C., Moore D.J., Moore M.N., Mora-Rodriguez R., Moreira P.I., Morel E., Morelli M.B., Moreno S., Morgan M.J., Moris A., Moriyasu Y., Morrison J.L., Morrison L.A., Morselli E., Moscat J., Moseley P.L., Mostowy S., Motori E., Mottet D., Mottram J.C., Moussa C.E., Mpakou V.E., Mukhtar H., Mulcahy Levy J.M., Muller S., Muñoz-Moreno R., Muñoz-Pinedo C., Münz C., Murphy M.E., Murray J.T., Murthy A., Mysorekar I.U., Nabi I.R., Nabissi M., Nader G.A., Nagahara Y., Nagai Y., Nagata K., Nagelkerke A., Nagy P., Naidu S.R., Nair S., Nakano H., Nakatogawa H., Nanjundan M., Napolitano G., Naqvi N.I., Nardacci R., Narendra D.P., Narita M., Nascimbeni A.C., Natarajan R., Navegantes L.C., Nawrocki S.T., Nazarko T.Y., Nazarko V.Y., Neill T., Neri L.M., Netea M.G., Netea-Maier R.T., Neves B.M., Ney P.A., Nezis I.P., Nguyen H.T., Nguyen H.P., Nicot A.S., Nilsen H., Nilsson P., Nishimura M., Nishino I., Niso-Santano M., Niu H., Nixon R.A., Njar V.C., Noda T., Noegel A.A., Nolte E.M., Norberg E., Norga K.K., Noureini S.K., Notomi S., Notterpek L., Nowikovsky K., Nukina N., Nürnberger T., O'Donnell V.B., O'Donovan T., O'Dwyer P.J., Oehme I., Oeste C.L., Ogawa M., Ogretmen B., Ogura Y., Oh Y.J., Ohmuraya M., Ohshima T., Ojha R., Okamoto K., Okazaki T., Oliver F.J., Ollinger K., Olsson S., Orban D.P., Ordonez P., Orhon I., Orosz L., O'Rourke E.J., Orozco H., Ortega A.L., Ortona E., Osellame L.D., Oshima J., Oshima S., Osiewacz H.D., Otomo T., Otsu K., Ou J.H., Outeiro T.F., Ouyang D.Y., Ouyang H., Overholtzer M., Ozbun M.A., Ozdinler P.H., Ozpolat B., Pacelli C., Paganetti P., Page G., Pages G., Pagnini U., Pajak B., Pak S.C., Pakos-Zebrucka K., Pakpour N., Palková Z., Palladino F., Pallauf K., Pallet N., Palmieri M., Paludan S.R., Palumbo C., Palumbo S., Pampliega O., Pan H., Pan W.,

Panaretakis T., Pandey A., Pantazopoulou A., Papackova Z., Papademetrio D.L., Papassideri I., Papini A., Parajuli N., Pardo J., Parekh V.V., Parenti G., Park J.I., Park J., Park O.K., Parker R., Parlato R., Parys J.B., Parzych K.R., Pasquet J.M., Pasquier B., Pasumarthi K.B., Patschan D., Patterson C., Pattingre S., Pattison S., Pause A., Pavenstädt H., Pavone F., Pedrozo Z., Peña F.J., Peñalva M.A., Pende M., Peng J., Penna F., Penninger J.M., Pensalfini A., Pepe S., Pereira G.J., Pereira P.C., Pérez-de la Cruz V., Pérez-Pérez M.E., Pérez-Rodríguez D., Pérez-Sala D., Perier C., Perl A., Perlmutter D.H., Perrotta I., Pervaiz S., Pesonen M., Pessin J.E., Peters G.J., Petersen M., Petrache I., Petrof B.J., Petrovski G., Phang J.M., Piacentini M., Pierdominici M., Pierre P., Pierrefite-Carle V., Pietrocola F., Pimentel-Muiños F.X., Pinar M., Pineda B., Pinkas-Kramarski R., Pinti M., Pinton P., Piperdi B., Piret J.M., Platanias L.C., Platta H.W., Plowey E.D., Pöggeler S., Poirot M., Polčic P., Poletti A., Poon A.H., Popelka H., Popova B., Poprawa I., Poulose S.M., Poulton J., Powers S.K., Powers T., Pozuelo-Rubio M., Prak K., Prange R., Prescott M., Priault M., Prince S., Proia R.L., Proikas-Cezanne T., Prokisch H., Promponas V.J., Przyklenk K., Puertollano R., Pugazhenthi S., Puglielli L., Pujol A., Puyal J., Pyeon D., Qi X., Qian W.B., Qin Z.H., Qiu Y., Qu Z., Quadrilatero J., Quinn F., Raben N., Rabinowich H., Radogna F., Ragusa M.J., Rahmani M., Raina K., Ramanadham S., Ramesh R., Rami A., Randall-Demllo S., Randow F., Rao H., Rao V.A., Rasmussen B.B., Rasse T.M., Ratovitski E.A., Rautou P.E., Ray S.K., Razani B., Reed B.H., Reggiori F., Rehm M., Reichert A.S., Rein T., Reiner D.J., Reits E., Ren J., Ren X., Renna M., Reusch J.E., Revuelta J.L., Reyes L., Rezaie A.R., Richards R.I., Richardson D.R., Richetta C., Riehle M.A., Rihn B.H., Rikihisa Y., Riley B.E., Rimbach G., Rippo M.R., Ritis K., Rizzi F., Rizzo E., Roach P.J., Robbins J., Roberge M., Roca G., Roccheri M.C., Rocha S., Rodrigues C.M., Rodríguez C.I., de Cordoba S.R., Rodriguez-Muela N., Roelofs J., Rogov V.V., Rohn T.T., Rohrer B., Romanelli D., Romani L., Romano P.S., Roncero M.I., Rosa J.L., Rosello A., Rosen K.V., Rosenstiel P., Rost-Roszkowska M., Roth K.A., Roué G., Rouis M., Rouschop K.M., Ruan D.T., Ruano D., Rubinsztein D.C., Rucker E.B. 3rd., Rudich A., Rudolf E., Rudolf R., Ruegg M.A., Ruiz-Roldan C., Ruparelia A.A., Rusmini P., Russ D.W., Russo G.L., Russo G., Russo R., Rusten T.E., Ryabovol V., Ryan K.M., Ryter S.W., Sabatini D.M., Sacher M., Sachse C., Sack M.N., Sadoshima J., Saftig P., Sagi-Eisenberg R., Sahni S., Saikumar P., Saito T., Saitoh T., Sakakura K., Sakoh-Nakatogawa M., Sakuraba Y., Salazar-Roa M., Salomoni P., Saluja A.K., Salvaterra P.M., Salvioli R., Samali A., Sanchez A.M., Sánchez-Alcázar J.A., Sanchez-Prieto R., Sandri M., Sanjuan M.A., Santaguida S., Santambrogio L., Santoni G., Dos Santos C.N., Saran S., Sardiello M., Sargent G., Sarkar P., Sarkar S., Sarrias M.R., Sarwal M.M., Sasakawa C., Sasaki M., Sass M., Sato K., Sato M., Satriano J., Savaraj N., Saveljeva S., Schaefer L., Schaible U.E., Scharl M., Schatzl H.M., Schekman R., Scheper W., Schiavi A., Schipper H.M., Schmeisser H., Schmidt J., Schmitz I., Schneider B.E., Schneider E.M., Schneider J.L., Schon E.A., Schönenberger M.J., Schönthal A.H., Schorderet D.F., Schröder B., Schuck S., Schulze R.J., Schwarten M., Schwarz T.L., Sciarretta S., Scotto K., Scovassi A.I., Screaton R.A., Screen M., Seca H., Sedej S., Segatori L., Segev N., Seglen P.O., Seguí-Simarro J.M., Segura-Aguilar J., Seki E., Sell C., Seiliez I., Semenkovich C.F., Semenza G.L., Sen U., Serra A.L., Serrano-Puebla A., Sesaki H., Setoguchi T., Settembre C., Shacka J.J., Shajahan-Haq A.N., Shapiro I.M., Sharma S., She H., Shen C.K., Shen C.C., Shen H.M., Shen S., Shen W., Sheng R., Sheng X., Sheng Z.H., Shepherd T.G., Shi J., Shi Q., Shi Q., Shi Y., Shibutani S., Shibuya K., Shidoji Y., Shieh J.J., Shih C.M., Shimada Y., Shimizu S., Shin D.W., Shinohara M.L., Shintani M., Shintani T., Shioi T., Shirabe K., Shiri-Sverdlov R., Shirihai O., Shore G.C., Shu C.W., Shukla D., Sibirny A.A., Sica V., Sigurdson C.J., Sigurdsson E.M., Sijwali P.S., Sikorska B., Silveira W.A., Silvente-Poirot S., Silverman G.A., Simak J., Simmet T., Simon A.K., Simon H.U., Simone C., Simons M., Simonsen A., Singh R., Singh S.V., Singh S.K., Sinha D., Sinha S., Sinicrope F.A., Sirko A., Sirohi K., Sishi B.J., Sittler A., Siu P.M., Sivridis E., Skwarska A., Slack R., Slaninová I., Slavov N., Smaili S.S., Smalley K.S., Smith D.R., Soenen S.J., Soleimanpour S.A., Solhaug A., Somasundaram K., Son J.H., Sonawane A., Song C., Song F., Song H.K., Song J.X., Song W., Soo K.Y., Sood A.K., Soong T.W., Soontornniyomkij V., Sorice M., Sotgia F., Soto-Pantoja D.R., Sotthibundhu A., Sousa M.J., Spaink H.P., Span P.N., Spang A., Sparks J.D., Speck P.G., Spector S.A., Spies C.D., Springer W., Clair D.S., Stacchiotti A., Staels B., Stang M.T., Starczynowski D.T., Starokadomskyy P., Steegborn C., Steele J.W., Stefanis L., Steffan J., Stellrecht C.M., Stenmark H., Stepkowski T.M., Stern S.T., Stevens C., Stockwell B.R., Stoka V., Storchova Z., Stork B., Stratoulias V., Stravopodis D.J., Strnad P., Strohecker A.M., Ström A.L., Stromhaug P., Stulik J., Su Y.X., Su Z., Subauste C.S., Subramaniam S., Sue C.M., Suh S.W., Sui X., Sukseree S., Sulzer D., Sun F.L., Sun J., Sun J., Sun S.Y., Sun Y., Sun Y., Sun Y., Sundaramoorthy V., Sung J., Suzuki H., Suzuki K., Suzuki N., Suzuki T., Suzuki Y.J., Swanson M.S., Swanton C., Swärd K., Swarup G., Sweeney S.T., Sylvester P.W., Szatmari Z., Szegezdi E., Szlosarek P.W., Taegtmeyer H., Tafani M., Taillebourg E., Tait S.W., Takacs-Vellai K., Takahashi Y., Takáts S., Takemura G., Takigawa N., Talbot N.J., Tamagno E., Tamburini J., Tan C.P., Tan L., Tan M.L., Tan M., Tan Y.J., Tanaka K., Tanaka M., Tang D., Tang D., Tang G., Tanida I., Tanji K., Tannous B.A., Tapia J.A., Tasset-Cuevas I., Tatar M., Tavassoly I., Tavernarakis N., Taylor A., Taylor G.S., Taylor G.A., Taylor J.P., Taylor M.J., Tchetina E.V., Tee A.R., Teixeira-Clerc F., Telang S., Tencomnao T., Teng B.B., Teng R.J., Terro F., Tettamanti G., Theiss A.L., Theron A.E., Thomas K.J., Thomé M.P., Thomes P.G., Thorburn A., Thorner J., Thum T., Thumm M., Thurston T.L., Tian L., Till A., Ting J.P., Titorenko V.I., Toker L., Toldo S., Tooze S.A., Topisirovic I., Torgersen M.L., Torosantucci L., Torriglia A., Torrisi M.R., Tournier C., Towns R., Trajkovic V., Travassos L.H., Triola G., Tripathi D.N., Trisciuoglio D., Troncoso R., Trougakos I.P., Truttmann A.C., Tsai K.J., Tschan M.P., Tseng Y.H., Tsukuba T., Tsung A., Tsvetkov A.S., Tu S., Tuan H.Y., Tucci M., Tumbarello D.A., Turk B., Turk V., Turner R.F., Tveita A.A., Tyagi S.C., Ubukata M., Uchiyama Y., Udelnow A., Ueno T., Umekawa M., Umemiya-Shirafuji R., Underwood B.R., Ungermann C., Ureshino R.P., Ushioda R., Uversky V.N., Uzcátegui N.L., Vaccari T., Vaccaro M.I., Váchová L., Vakifahmetoglu-Norberg H., Valdor R., Valente E.M., Vallette F., Valverde A.M., Van den Berghe G., Van Den Bosch L., van den Brink G.R., van der Goot F.G., van der Klei I.J., van der Laan L.J., van Doorn W.G., van Egmond M., van Golen K.L., Van Kaer L., van Lookeren Campagne M., Vandenabeele P., Vandenberghe W., Vanhorebeek I., Varela-Nieto I., Vasconcelos M.H., Vasko R., Vavvas D.G., Vega-Naredo I., Velasco G., Velentzas A.D., Velentzas P.D., Vellai T., Vellenga E., Vendelbo M.H., Venkatachalam K., Ventura N., Ventura S., Veras P.S., Verdier M., Vertessy B.G., Viale A., Vidal M., Vieira H.L., Vierstra R.D., Vigneswaran N., Vij N., Vila M., Villar M., Villar V.H.,

Villarroya J., Vindis C., Viola G., Viscomi M.T., Vitale G., Vogl D.T., Voitsekhovskaja O.V., von Haefen C., von Schwarzenberg K., Voth D.E., Vouret-Craviari V., Vuori K., Vyas J.M., Waeber C., Walker C.L., Walker M.J., Walter J., Wan L., Wan X., Wang B., Wang C., Wang C.Y., Wang C., Wang C., Wang C., Wang D., Wang F., Wang F., Wang G., Wang H.J., Wang H., Wang H.G., Wang H., Wang H.D., Wang J., Wang J., Wang M., Wang M.Q., Wang P.Y., Wang P., Wang R.C., Wang S., Wang T.F., Wang X., Wang X.J., Wang X.W., Wang X., Wang X., Wang Y., Wang Y., Wang Y., Wang Y.J., Wang Y., Wang Y., Wang Y.T., Wang Y., Wang Z.N., Wappner P., Ward C., Ward D.M., Warnes G., Watada H., Watanabe Y., Watase K., Weaver T.E., Weekes C.D., Wei J., Weide T., Weihl C.C., Weindl G., Weis S.N., Wen L., Wen X., Wen Y., Westermann B., Weyand C.M., White A.R., White E., Whitton J.L., Whitworth A.J., Wiels J., Wild F., Wildenberg M.E., Wileman T., Wilkinson D.S., Wilkinson S., Willbold D., Williams C., Williams K., Williamson P.R., Winklhofer K.F., Witkin S.S., Wohlgemuth S.E., Wollert T., Wolvetang E.J., Wong E., Wong G.W., Wong R.W.,Wong V.K., Woodcock E.A., Wright K.L., Wu C., Wu D., Wu G.S., Wu J., Wu J., Wu M., Wu M., Wu S., Wu W.K., Wu Y., Wu Z., Xavier C.P., Xavier R.J., Xia G.X., Xia T., Xia W., Xia Y., Xiao H., Xiao J., Xiao S., Xiao W., Xie C.M., Xie Z., Xie Z., Xilouri M., Xiong Y., Xu C., Xu C., Xu F., Xu H., Xu H., Xu J., Xu J., Xu J., Xu L., Xu X., Xu Y., Xu Y., Xu Z.X., Xu Z., Xue Y., Yamada T., Yamamoto A., Yamanaka K., Yamashina S., Yamashiro S., Yan B., Yan B., Yan X., Yan Z., Yanagi Y., Yang D.S., Yang J.M., Yang L., Yang M., Yang P.M., Yang P., Yang Q., Yang W., Yang W.Y., Yang X., Yang Y., Yang Y., Yang Z., Yang Z., Yao M.C., Yao P.J., Yao X., Yao Z., Yao Z., Yasui L.S., Ye M., Yedvobnick B., Yeganeh B., Yeh E.S., Yeyati P.L., Yi F., Yi L., Yin X.M., Yip C.K., Yoo Y.M., Yoo Y.H., Yoon S.Y., Yoshida K., Yoshimori T., Young K.H., Yu H., Yu J.J., Yu J.T., Yu J., Yu L., Yu W.H., Yu X.F., Yu Z., Yuan J., Yuan Z.M., Yue B.Y., Yue J., Yue Z., Zacks D.N., Zacksenhaus E., Zaffaroni N., Zaglia T., Zakeri Z., Zecchini V., Zeng J., Zeng M., Zeng Q., Zervos A.S., Zhang D.D., Zhang F., Zhang G., Zhang G.C., Zhang H., Zhang H., Zhang H., Zhang H., Zhang J., Zhang J., Zhang J., Zhang J., Zhang J.P., Zhang L., Zhang L., Zhang L., Zhang L., Zhang M.Y., Zhang X., Zhang X.D., Zhang Y., Zhang Y., Zhang Y., Zhang Y., Zhang Y., Zhao M., Zhao W.L., Zhao X., Zhao Y.G., Zhao Y., Zhao Y., Zhao Y.X., Zhao Z., Zhao Z.J., Zheng D., Zheng X.L., Zheng X., Zhivotovsky B., Zhong Q., Zhou G.Z., Zhou G., Zhou H., Zhou S.F., Zhou X.J., Zhu H., Zhu H., Zhu W.G., Zhu W., Zhu X.F., Zhu Y., Zhuang S.M., Zhuang X., Ziparo E., Zois C.E., Zoladek T., Zong W.X., Zorzano A. and Zughaier S.M. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy 12, 1-222.

Koenig M.K., Butler I.J. and Northrup H. (2008). Regression of subependymal giant cell astrocytoma with rapamycin in tuberous sclerosis complex. J. Child. Neurol. 23, 1238-1239.

Korur S., Huber R.M., Sivasankaran B., Petrich M., Morin P., Hemmings B.A., Merlo A. and Lino M.M. (2009). GSK3beta regulates differentiation and growth arrest in glioblastoma. PLoS One 4:e7443

Krueger D.A., Care M.M., Holland K., Agricola K., Tudor C., Mangeshkar P., Wilson K.A., Byars A., Sahmoud T. and Franz D.M. (2010). Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N. Engl. J. Med. 363, 1801-1811.

Kwon H.K, Bae G.U., Yoon J.W., Kim Y.K., Lee H.Y., Lee H.W. and Han J.W. (2002). Constitutive activation of p70S6k in cancer cells. Arch. Pharm. Res. 25, 685-690.

Lagadec C., Vlashi E., Frohnen P., Alhiyari Y., Chan M. and Pajonk F. (2014). The RNA-binding protein Musashi-1 regulates proteasome subunit expression in breast cancer- and glioma-initiating cells. Stem Cells 32, 135-144.

Laplante M. and Sabatini D.M. (2012). mTOR signaling in growth control and disease. Cell 149, 274-293.

Lee T.V., Kamber Kaya H.E., Simin R., Baehrecke E.H. and Bergmann A. (2016). The initiator caspase Dronc is subject of enhanced autophagy upon proteasome impairment in Drosophila. Cell Death Differ. 23, 1555-1564.

Lenzi P., Lazzeri G., Biagioni F., Busceti C.L., Gambardella S., Salvetti A. and Fornai F. (2016). The autophagoproteasome a novel cell clearing organelle in baseline and stimulated conditions. Front. Neuroanat. 10, 78.

Levine B. and Kroemer G. (2008). Autophagy in the pathogenesis of disease. Cell 132, 27-42.

Lin L., Gaut D., Hu K., Yan H., Yin D. and Koeffler H.P. (2014). Dual targeting of glioblastoma multiforme with a proteasome inhibitor (Velcade) and a phosphatidylinositol3-kinase inhibitor (ZSTK474). Int. J. Oncol. 44, 557-562.

Liu R., Li J., Zhang T., Zou L., Chen Y., Wang K., Lei Y., Yuan K., Li Y., Lan J., Cheng L., Xie N., Xiang R., Nice E.C., Huang C. and Wei Y. (2014). Itraconazole suppresses the growth of glioblastoma through induction of autophagy: involvement of abnormal cholesterol trafficking. Autophagy 10, 1241-1255.

Louis D.N., Ohgaki H., Wiestler O.D., Cavenee W.K., Burger P.C., Jouvet A., Scheithauer B.W. and Kleihues P. (2007). WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97-109.

Ma D.J., Galanis E., Anderson S.K., Schiff D., Kaufmann T.J., Peller P.J., Giannini C., Brown P.D., Uhm J.H., McGraw S., Jaeckle K.A., Flynn P.J., Ligon K.L., Buckner J.C. and Sarkaria J.N. (2015). A phase II trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed glioblastoma: NCCTG N057K. Neuro Oncol. 17, 1261-1269.

Mariño G., Salvador-Montoliu N., Fueyo A., Knecht E., Mizushima N. and López-Otín C. (2007). Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. J. Biol. Chem. 282, 18573-18583.

Medhkour A., Traul D. and Hussain N. (2002). Neonatal subependymal giant cell astrocytoma. Pediatr. Neurosurg. 36, 271-274.

Mills G.B., Lu Y. and Kohn E.C. (2001). Linking molecular therapeutics to molecular diagnostics: inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. USA 98, 10031-10033.

Moavero R., Coniglio A., Garaci F. and Curatolo P. (2013). Is mTOR inhibition a systemic treatment for tuberous sclerosis? Ital. J. Pediatr. 39:57.

Murakami A. (2013). Modulation of protein quality control systems by food phytochemicals. J. Clin. Biochem. Nutr. 52, 215-227.

Neshat M.S., Mellinghoff I.K., Tran C., Stiles B., Thomas G., Petersen R., Frost P., Gibbons J.J., Wu H. and Sawyers C.L. (2001). Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc. Natl. Acad. Sci. USA 98, 10314-10319.

Olsen B.B., Svenstrup T.H. and Guerra B. (2012). Downregulation of protein kinase CK2 induces autophagic cell death through modulation of the mTOR and MAPK signaling pathways in human glioblastoma cells. Int. J. Oncol. 41, 1967-1976.

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

Pandey U.B., Nie Z., Batlevi Y., McCray B.A., Ritson G.P., Nedelsky N.B., Schwartz S.L., DiProspero N.A., Knight M.A., Schuldiner O., Padmanabhan R., Hild M., Berry D.L., Garza D., Hubbert C.C., Yao T.P., Baehrecke E.H. and Taylor J.P. (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 447, 859-863.

Paranjpe A., Zhang R., Ali-Osman F., Bobustuc G.C. and Srivenugopal K.S. (2014). Disulfiram is a direct and potent inhibitor of human O6-methylguanine-DNA methyltransferase(MGMT) in brain tumor cells and mouse brain and markedly increases the alkylating DNA damage. Carcinogenesis 35, 692-702.

Parsons D.W., Jones S., Zhang X., Lin J.C., Leary R.J., Angenendt P., Mankoo P., Carter H., Siu I.M., Gallia G.L., Olivi A., McLendon R., Rasheed B.A., Keir S., Nikolskaya T., Nikolsky Y., Busam D.A., Tekleab H., Diaz L.A., Hartigan J., Smith D.R., Strausberg R.L., Marie S.K., Shinjo S.M., Yan H., Riggins G.J., Bigner D.D., Karchin R., Papadopoulos N., Parmigiani G., Vogelstein B., Velculescu V.E. and Kinzler K.W. (2008). An integrated genomic analysis of human glioblastoma multiforme. Science 321, 1807-1812.

Pasquali L., Longone P., Isidoro C., Ruggieri S., Paparelli A. and Fornai F. (2009). Autophagy, lithium, and amyotrophic lateral sclerosis. Muscle Nerve 40, 173-194.

Pasquali L., Busceti C.L., Fulceri F., Paparelli A. and Fornai F. (2010). Intracellular pathways underlying the effects of lithium. Behav. Pharmacol. 21, 473-492.

Persson A., Fan Q.W., Phillips J. and Weiss W.A. (2007). Glioma. Elsevier. San Diego. pp 433-444.

Podsypanina K., Lee R.T., Politis C., Hennessy I., Crane A., Puc J., Neshat M., Wang H., Yang L., Gibbons J., Frost P., Dreisbach V., Blenis J., Gaciong Z., Fisher P., Sawyers C., Hedrick-Ellenson L. and Parsons R. (2001). An inhibition of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. Proc. Natl. Acad. Sci. USA 98, 10320-10325.

Rasheed B.K., Stenzel T.T., McLendon R.E., Parsons R., Friedman A.H., Friedman H.S., Bigner D.D. and Bigner S.H. (1997). PTEN gene mutations are seen in high grade but not in low grade gliomas. Cancer Res. 57, 4187-4190.

Rodolfo C., Di Bartolomeo S. and Cecconi F. (2016). Autophagy in stem and progenitor cells. Cell. Mol. Life Sci. 73, 475-496.

Rosen J.M. and Jordan C.T. (2009). The increasing complexity of the cancer stem cell paradigm. Science 324, 1670-1673.

Roth J., Roach E.S., Bartels U., Jóźwiak S., Koenig M.K., Weiner H.L., Franz D.N. and Wang H.Z. (2013). Subependymal giant cell astrocytoma: diagnosis, screening, and treatment. Recommendations from the International Tuberous Sclerosis Complex Consensus Conference 2012. Pediatr. Neurol. 49, 439-444.

Rubinsztein D.C. (2007). Autophagy induction rescues toxicity mediated by proteasome inhibition. Neuron 54, 854-856.

Rüssmann H., Panthel K., Köhn B., Jellbauer S., Winter S.E., Garbom S., Wolf-Watz H., Hoffmann S., Grauling-Halama S. and Geginat G. (2010). Alternative endogenous protein processing via an autophagy-dependent pathway compensates for Yersinia-mediated inhibition of endosomal major histocompatibility complex class II antigen presentation. Infect. Immun. 78, 5138-5150.

Sato A., Okada M., Shibuya K., Watanabe E., Seino S., Suzuki K., Narita Y., Shibui S., Kayama T. and Kitanaka C. (2013). Resveratrol promotes proteasome-dependent degradation of Nanog via p53 activation and induces differentiation of glioma stem cells. Stem Cell. Res. 11, 601-610.

Shaw R.J. and Cantley L.C. (2006). Ras, PI(3)K and mTOR signaling controls tumor cell growth. Nature 441, 424-430.

Sheng X., Wei Q., Jiang L., Li X., Gao Y. and Wang L. (2012). Different degree in proteasome malfunction has various effects on root growth possibly through preventing cell division and promoting autophagic vacuolization. PLoS One 7, e45673.

Shingu T., Yamada K., Hara N., Moritake K., Osago H., Terashima M., Uemura T., Yamasaki T. and Tsuchiya M. (2003). Growth inhibition of human malignant glioma cells by the PI3K-specific inhibitor. J. Neurosurg. 98, 154-161.

Singh S.K., Hawkins C., Clarke I.D., Squire J.A., Bayani J., Hide T., Henkelman R.M., Cusimano M.D. and Dirks P.B. (2004). Identification of human brain tumor initiating cells. Nature 432, 396-401.

Smith J.S., Tachibana I., Passe S.M., Huntley B.K., Borell T.J., Iturria N., O'Fallon J.R., Schaefer P.L., Scheithauer B.W., James C.D., Buckner J.C. and Jenkins R.B. (2001). PTEN mutation, EGFR amplification, and outcome in patients with Anaplastic Astrocytoma and Glioblastoma Multiforme. J. Natl. Cancer Inst. 93, 1246-1256.

Stacer A.C., Wang H., Fenner J., Dosch J.S., Salomonnson A., Luker K.E., Luker G.D., Rehemtulla A. and Ross B.D. (2015). Imaging reporters for proteasome activity identify tumor- and metastasis-initiating cells. Mol. Imaging. 14, 414-428.

Su H., Zhang W., Guo J., Guo A., Yuan Q. and Wu W. (2009). Lithium enhances the neuronal differentiation of neural progenitor cells *in vitro* and after transplantation into the avulsed ventral horn of adult rats through the secretion of brain-derived neurotrophic factor. J. Neurochem. 108, 1385-1398.

Sunayama J., Sato A., Matsuda K., Tachibana K., Suzuki K., Narita Y., Shibui S., Sakurada K., Kayama T., Tomiyama A. and Kitanada C. (2010). Dual blocking of mTOR and PI3K elicits a prodifferentiation effect on glioblastoma stem-like cells. Neuro. Oncol. 12, 1205-1219.

Takeuchi H., Kondo Y., Fujiwara K., Kanzawa T., Aoki H., Mills G.B. and Konso S. (2005). Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer Res. 65, 3336-3346.

Tyburczy M.E., Kotulska K.,Pokarowski P.,Mieczkowski J.,Kucharska J.,Grajkowska W., Roszkowski M.,Jozwiak S. and Kaminska B. (2010). Novel Proteins Regulated by mTOR in subependymal giant cell astrocytomas of patients with Tuberous Sclerosis Complex and new therapeutic implications. Am. J. Pathol. 176, 1878-1890.

Venere M., Fine H.A., Dirks P.B. and Rich J.N. (2011). Cancer stem cells in gliomas: identifying and understanding the apex cell in cancer's hierarchy. Glia 59, 1148-1154.

Vlashi E., Kim K., Lagadec C., Donna L.D., McDonald J.T., Eghabli M., Sayre J.W., Stefani E., McBride W. and Pajonk F. (2009). *In vivo* imaging, tracking, and targeting of cancer stem cells. J. Natl. Cancer Inst. 101, 350-359.

Wada A., Yokoo H. and Yanagita T. (2005). Lithium: potential therapeutics against acute brain injuries and chronic neurodegenerative diseases. J. Pharmacol. Sci. 99, 307-321.

Wada A. (2009). Lithium and neuropsychiatric therapeutics:

Neuroplasticity via glycogen synthase kinase-3 , -catenin, and neurotrophin cascades. J. Pharmacol. Sci. 110, 14-28.

Walasek M.A., Bystrykh L., van den Boom V., Olthof S., Ausema A., Ritsema M., Huls G., de Haan G. and van Os R. (2012). The combination of valproic acid and lithium delays hematopoietic stem/progenitor cell differentiation. Blood 119, 3050-3059.

Wang Y., Sun Z., Qiu X., Li Y., Qin J. and Han X. (2009). Roles of Wnt/beta-catenin signaling in epithelial differentiation of mesenchymal stem cells. Biochem. Biophys. Res. Commun. 390, 1309-1314.

Wang Y., He W., Bian H., Liu C. and Li S. (2012). Small molecule induction of neural-like cells from bone marrow-mesenchymal stem cells. J. Cell. Biochem. 113, 1527-1536.

Wang C., Chen S., Yeo S., Karsli-Uzunbas G., White E., Mizushima N., Virgin H.W. and Guan J.L. (2016). Elevated p62/SQSTM1 determines the fate of autophagy-deficient neural stem cells by increasing superoxide. J Cell Biol. 212, 545-560.

Webb J.L., Ravikumar B., Atkins J., Skepper J.N. and Rubinsztein D.C. (2003). Alpha-Synuclein is degraded by both autophagy and the proteasome. J. Biol. Chem. 278, 25009-25013.

Wen P.Y., Omuro A., Ahluwalia M.S., Fathallah-Shaykh H.M., Mohile N., Lager J.J., Laird A.D., Tang J., Jiang J., Egile C. and Cloughesy T.F. (2015). Phase I dose-escalation study of the PI3K/mTOR inhibitor voxtalisib (SAR245409, XL765) plus temozolomide with or without radiotherapy in patients with high-grade glioma. Neuro Oncol. 17, 1275-1283.

Weppler S.A., Krause M., Zyromska A., Lambin P., Baumann M. and Wouters B.G. (2007). Response of U87 glioma xenograft treated with concurrent rapamycin and fractionated radiotherapy: possible role for thrombosis. Rad. Oncol. 82, 96-104.

Wexler E.M., Geschwind D.H. and Palmer T.D. (2008). Lithium regulates adult hippocampal progenitor development through canonical Wnt pathway activation. Mol. Psychiatr. 13, 285-292.

Wullschleger S., Loewith R. and Hall M.N. (2006). TOR signaling in growth and metabolism. Cell 124, 471-484.

Yan X.B., Hou H.L., Wu L.M., Liu J. and Zhou J.N. (2007). Lithium regulates hippocampal neurogenesis by ERK pathway and facilitates recovery of spatial learning and memory in rats after transient global cerebral ischemia. Neuropharmacology 53, 487-495.

Yan Y., Xu Z., Dai S., Qian L., Sun L. and Gong Z. (2016). Targeting autophagy to sensitive glioma to temozolomide treatment. J. Exp. Clin. Cancer Res. 35:23.

Yang Z. and Klionsky D.J. (2010). Eaten alive: a history of macroautophagy. Nat. Cell. Biol. 12, 814-822.

Yucel K., Taylor V.H., McKinnon M.C., McDonald K., Alda M., Young L.T. and McQueen G.M. (2008). Bilateral hippocampal volume increase in patients with bipolar disorder and short-term lithium treatment. Neuropsychopharmacology 33, 361-367.

Zeng M. and Zhou J.N. (2008). Roles of autophagy and mTOR signaling in neuronal differentiation of mouse neuroblastoma cells. J. Cell. Signal. 20, 659-665.

Zhang Y., Gao X., Saucedo L.J., Ru B., Edgar B.A. and Pan D. (2003). Rheb is a direct target of the tuberous sclerosis tumor suppressor proteins. Nat. Cell. Biol. 5, 578-581.

Zhang Q.B., Ji X.Y., Huang Q., Dong J., Zhu Y.D. and Lan Q. (2006). Differentiation profile of brain tumor stem cells: a comparative study with neural stem cells. Cell Res. 16, 909-915.

Zhao Y., Huang Q., Yang J., Lou M., Wang A., Dong J., Qin Z. and Zhang T. (2010). Autophagy impairment inhibits differentiation of glioma stem/progenitor cells. Brain. Res. 1313, 250-258.

Zhuang W., Qin Z. and Liang Z. (2009). The role of autophagy in sensitizing malignant glioma cells to radiation therapy. Acta Biochim. Biophys. Sin. 41, 341-351.

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

Zogovic N., Tovilovic-Kovacevic G., Misirkic-Marjanovic M., Vucicevic L., Janjetovic K., Harhaji-Trajkovic L. and Trajkovic V. (2015). Coordinated activation of AMP-activated protein kinase, extracellular signal-regulated kinase, and autophagy regulates phorbol myristate acetate-induced differentiation of SH-SY5Yneuroblastoma cells. J. Neurochem. 133, 223-232.

Accepted October 24, 2016