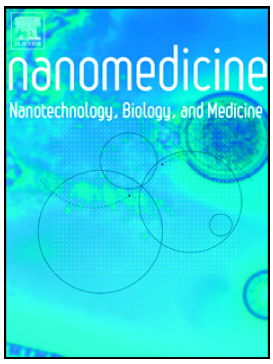
Journal Pre-proof



Nano delivery of natural substances as prospective autophagy modulators in glioblastoma

Srishti Agarwal, Toru Maekawa

PII: S1549-9634(20)30124-6

DOI: https://doi.org/10.1016/j.nano.2020.102270

Reference: NANO 102270

To appear in: *Nanomedicine: Nanotechnology, Biology, and Medicine*

Revised date: 9 July 2020

Please cite this article as: S. Agarwal and T. Maekawa, Nano delivery of natural substances as prospective autophagy modulators in glioblastoma, *Nanomedicine: Nanotechnology, Biology, and Medicine* (2020), https://doi.org/10.1016/j.nano.2020.102270

Nano delivery of natural substances as prospective autophagy modulators in glioblastoma

Srishti Agarwal , Toru Maekawa

Bio-Nano Electronics Research Center, Graduate School of Interdisciplinary New Science, Toyo University, Kawagoe, Saitama, 350 - 8585, Japan,

*Corresponding Author

Srishti Agarwal
Bio-Nano Electronics Research Centre, Toyo University, Kawagoe, Saitama-350-8585, Japan
Email: srishagarwal2011@gmail.com
Phone: +81-7031642244

Conflicts of interest

The authors declare no conflict of interest.

Abstract word count: 149

Manuscript word count: 10, 274 (body text and figure legends)

Number of figures: 3

Number of references: 231

Abstract

Glioblastoma is the most destructive type of malignant brain tumor in humans due to cancer relapse. Latest studies have indicated that cancer cells are more reliant on autophagy for survival than non-cancer cells. Autophagy is entitled as programmed cell death type II and studies imply that it is a comeback of cancer cells to innumerable anti-cancer therapies. To diminish the adverse consequences of chemotherapeutics, numerous herbs of natural origin have been retained in cancer treatments. Additionally, autophagy induction occurs via their tumor suppressive actions that could cause cell senescence and increase apoptosis-independent cell death. However, most of the drugs have poor solubility and thus nano drug delivery systems possess excessive potential to improve the aqueous solubility and bioavailability of encapsulated drugs. There is a pronounced need for more therapies for glioblastoma treatment and hereby, the fundamental mechanisms of natural autophagy modulators in glioblastoma are prudently reviewed in this article.

Keywords: Autophagy, Glioblastoma Multiforme, Natural substances, Nanotechnology, Drug delivery

1. Introduction

Brain cancers form an assembly of extremely destructive and fatal neoplastic diseases, in which gliomas are a major group distressing the central nervous system (CNS)[1]. Gliomas originate from glial cells and the World Health Organization (WHO) grades Glioblastoma Multiforme (GBM), as grade IV being the most aggressive[2]. One of the key contributors of GBM defiance to chemotherapy/radiotherapy is the deregulation of cell death pathways like apoptosis and autophagy, which causes overexpression of anti-apoptotic proteins and cell survival proteins along with diminished levels of pro-apoptotic proteins [3]. Based on apoptosis induction, intensification in autophagy has been associated with a superior therapeutic efficacy. Autophagy, also known, as macroautophagy is a degradative mechanism stimulated mainly by stresses, like starvation, instigating the capture of intracellular organelles and proteins by autophagosomes [4]. The cargo is delivered for degradation upon lysosomal fusion that enables biomolecules recycling. Autophagy is known to play a major role in control and degradation of damaged organelles and proteins as well as regulating homeostasis. Hence, dysfunctional autophagy can trigger pathogenesis of numerous diseases like neurodegenerative disorders, cancer, etc. [5, 6]. Many human ailments, including cancer, have autophagy deregulation and it has been reported that inhibition or stimulation of autophagic pathways has a therapeutic benefit in cancer deterrence. In addition to many lysosomal inhibitors like hydroxychloroquine (HCQ), chloroquine (CQ) etc., latest evidence has implied that naturally derived substances are involved in autophagy regulation and can regulate the autophagic process both *in vitro* and *in vivo* by operating on numerous transcription factors and cellular signaling pathways [4, 7]. Phytochemicals originated from medicinal and dietary plants can decrease cancer occurrence by curbing activation of several

oncogenic molecules. However, the efficacy is limited due to poor bioavailability as well as rapid clearance by the reticuloendothelial system (RES). Phytochemicals have comparatively low bioavailability as the body treats them as xenobiotics therefore the existence in the body is brief [8]. Chemical structure and dietary intake forms are the main factors affecting the bioavailabilty of phytochemicals. For example, polyphenols are conjugated by glucuronidation (addition of glucuronic acid), methylation (addition of a methyl group), or sulfurylation (addition of a sulfo-group) after intestinal hydrolysis that frequently causes their urinary elimination [9]. Therefore, making use of nanotechnology, high therapeutic action can be achieved due to site-specific delivery and active targeting thereby limiting off-target effects. In the present review, we synopsize the molecular mechanism of autophagy and its role in glioblastoma using delivery of natural products/derivatives via nano-mediated carriers. As far to our information only limited reviews are available on nano-mediated delivery of natural products for autophagy modulation in GBM. Autophagy, playing a fundamental role in cancer therapy, herein, we deliberate the innumerable phytochemicals that might have the capability to control cellular signaling pathways and offer a comprehensive understanding for designing successful treatment approaches.

2. Autophagy as a cell death mechanism

Cell death is an essential process involved in a variety of biological mechanisms regulating development, immune regulation of multicellular organisms, homeostasis and its imbalance is linked with many pathologies [10]. Cell death can be characterized either as apoptotic or non-apoptotic. Type I cell death or apoptosis is what is called in other terms as “programmed cell death (PCD)” and is the major mechanism by which cells are physiologically eradicated [11]. It is characterized by apoptotic body formation,

cell shrinkage (pyknosis), chromatin condensation, etc.[12]. On the other hand, necrosis is type III cell death, which occurs due to failure of normal signaling pathways, which are vital for sustaining cellular homeostasis [11, 12]. A necrotic cell death is characteristic of large malignant tumors like GBM in which there is hypoxia, radical exhaustion of oxygen and other trophic factors as well as swelling of subcellular organelles [13]. Since long, necrosis has been contemplated as an uncontrolled type of cell death. However over the last few decades, numerous new forms of non-apoptotic regulated cell death (RCD) have been recognized like necroptosis, pyroptosis, netotic cell death, autophagy dependent cell death, etc.[12]. Necroptosis, which displays morphological features alike to necrosis (cell swelling, plasma membrane rupture) happens in a controlled manner and is triggered by multiple stimuli like toll like receptors, death receptors, Fas ligand etc.[12]. Pyroptosis is another form of programmed non-apoptotic cell death, which occurs due to inflammasome activation and is morphologically different from apoptosis [12]. There is nuclear condensation, cell swelling, absence of deoxyribonucleic acid (DNA) fragmentation *in vitro* and formation of large bubbles at plasma membrane that eventually ruptures. Numerous current breakthroughs specify that gasdermin D (GSDMD) is the main effector of pyroptosis and many tumor cells are undergoing pyroptosis as GSDME expression is suppressed in many cancers [14, 15]. Another type is netotic cell death is a type of RCD stimulated by NET, which are extracellular net-like DNA-protein structures discharged by cells in response to infection or injury [12]. NETosis is a dynamic process and can be stimulated by matrix metalloproteinases (MMPs), granular enzymes, autophagy, etc. A mechanism for non-apoptotic programmed cell death that is separate from apoptosis and necrosis is autophagy, which is type II cell death [12]. The main difference between apoptosis and autophagy is the engagement of caspases in apoptosis while autophagy is a caspase-

independent process [16]. Around 40 autophagy-related genes/proteins (ATGs) play important functions in autophagic membrane dynamics and processes [17]. There is cytoplasm condensation and organelle preservation in apoptosis whereas in autophagy there is widespread autophagic degradation of the golgi apparatus, polyribosomes, and endoplasmic reticulum, with all these features leading to the annihilation of the nucleus [18, 19]. Autophagy activation is known to play a biphasic role in cancer. On one side, it stimulates the effectiveness of anti-cancer strategies while on the other hand; it could encourage cancer advancement through the augmentation of cell survival [20]. Autophagic progression can be sectioned into five stages: initiation, autophagosome nucleation, elongation, autophagosome maturation, docking to lysosome, and lysosomal fusion **(Figure 1)**.

Phagophore initiation and nucleation

Microtubule-associated protein light chain 3 (LC3-I) is a copious cytoplasmic protein that is split and lipidated thru beginning of autophagy (forming LC3-II), translocating to and combining with the autophagosome in a punctate pattern [21]. Therefore, autophagy assists the cell to eradicate and reutilize proteins and organelles to bear metabolism and can be documented in part by creation of LC3-II punctae [22]. The autophagic molecular machinery is fairly multifaceted and comprises numerous kinases, phosphatases, and GTPases, which are determined by ATGs[23]. Autophagy regulation relies on many signaling pathways but mammalian target of rapamycin (mTOR) is one of the major pathway, which regulates many functions like cytoskeletal reorganization, ribosome regulation, cell proliferation, etc. and appears to be involved in Type II autophagic programmed cell death and its inactivation can prompt autophagy [23]. mTOR is formed by the complexes TORC1 suppressing factor

by destroying damaged organelles, protein aggregates and TORC2. P13K pathway regulates mTORC1 [24]. The plasma membrane lipid phosphatidylinositol-4, 5-bisphosphate (PIP2) is converted into PIP3 by activated P13K which then recruits the serine/threonine kinases phosphoinositide-dependent kinase 1 (PDK1) and AKT/PKB to the plasma membrane [25]. The rictor-mTOR complex activates AKT by phosphorylating it, which in turn causes activation of tuberous sclerosis complex (TSC), a TSC1/TSC2 heterodimer, and the most significant regulator upstream of mTOR [26]. AKT causes phosphorylation of TSC2, inhibiting formation of TSC1/TSC2 complex and TORC1 stimulation. TSC1/TSC2 function as a GTPase for Rheb, a GTP-binding protein that stimulates TORC1. AMP-activated kinase (AMPK) and ribosomal S6 kinase 1 (RSK1) are two other kinases which control mTORC1 by phosphorylating TSC2 which further activates TSC1/2 heterodimer activity. AMPK is activated by LKB1 in nutrient or energy deprivation when the ATP/AMP ratio drops. AMPK activates ULK1 [27] and induces nuclear translocation of FOXO which is important for regulating autophagic genes - Atg12, VPS3, Atg4B, MAPLC3B and Beclin-1 [28]. Phosphorylation of Atg13 by TORC1 kinase represses autophagy, averting Atg17 and Atg1 interplay during a homeostatic situation [23]. However during rapamycin treatment or starvation, TORC1 kinase is deactivated, with consequential hypophosphorylation of Atg13, causing contact of Atg1 with Atg17, thus initiating autophagy. In a similar fashion, phosphorylation of ULK1/2 by mTORC1 deactivates the pathway. Upon autophagy induction, mTORC1 is constrained and detached from ULK1/2, causing phosphorylation of FIP200 and mAtg13 and and prompts autophagosome development as shown in **figure 1** [29].

The instigation of VPs34, a class-III PI3K, in mammals relies on the creation of a compound that comprises Beclin-1, the beclin-1-associated autophagy-related key

regulator or Barkor (Atg14), and Vps15 (p150 in humans)[30]. Beclin-1, another important regulator of autophagy binds to AMBRA-1 (activating molecule in Beclin-1 regulated autophagy-1), the UV irradiation resistance-associated tumor suppressor gene (UVRAG), and Bax-interacting factor-1 (Bif-1) causes autophagy stimulation [23]. However, autophagy is inhibited when beclin-1 attaches to Bcl-xL or Bcl-2. It has also been verified that by phosphorylation of beclin-1 at Ser-14, ULK1 galvanizes the VPs34 complex, which comprises Atg14L, aiding the autophagy instigation [31].

Phagophore expansion and completion of autophagosome

The initial compartment swells to form the double-membrane vesicle called autophagosome after initiation and nucleation step. The transport of membrane components from the trans-golgi network to the phagophore is carried out by Atg9 protein. Atg8-phosphatidylethanolamine (PE) and Atg5-Atg12-Atg16 are the two ubiquitin-like protein conjugation systems that regulate the expansion process **(figure 1)**. In the first expansion step, Atg12, an ubiquitin like protein binds to Atg5, and then both proteins align to form a complex with Atg16L1 followed by the Atg12-Atg5-Atg16 complex that causes autophagosome development and is detached when the procedure is finished. The protein microtubule-associated protein 1 light-chain 3 (MAP1-LC3/LC3/Atg8) carries out the second step in which Atg4B cleaves LC3 into the cytosolic isoform LC3-I, which is then attached to phosphatidylethanolamine (PE) by Atg7 and Atg3 to form LC3-II, which stays in the autophagosome till it is destroyed in the autolysosomes [32].

Maturation of autophagosome and fusion with lysosomes

Autophagosome is formed, which is then delivered to lysosomes where it forms autophagolysosome upon fusion; thereby releasing the contents in the lumen, which

are then recycled or degraded **(figure 1)**. The cytoskeleton (particularly microtubules and motor proteins, like dynein) is predominantly significant for the movement of autophagosomes [33]. ESCRT, SNAREs, Rab7, and class-C Vps proteins also contribute in the fusion step [34]. Autophagosome fusion is also regulated by UVRAG, which supports C-vacuolar protein (C-VPS) recruitment to the autophagosome. Rab7-GTPase and LAMP-1, LAMP-2 activity is promoted via contact of UVRAG with the C-VPS complex which ultimately causes autophagosome and lysosome fusion [35]. Upon fruitful fusion, cargo proteins brought by autophagosomes are destroyed inside the single-membrane autolysosome by the help of lysosomal hydrolases.

2.1. Autophagy in tumorigenesis

According to recent studies, the function of autophagy in tumorigenesis is that it can either be a tumor promoter or suppressor depending on tumor type and stage[36]. Genetic evidence proposes that tumor suppressive functions of autophagy act throughout tumor initiation however there is also persuasive evidence advocating autophagy as a mean of survival mechanism opted by tumors to manage with various stresses in the tumor microenvironment [37].

2.1.1. Tumor suppression by autophagy

The remark that Beclin1 (BECN1) is monoallelically deleted in a high proportion of human breast, ovarian and prostate cancers specified the primary genetic link amid autophagy and cancer [38]. Consequently, heterozygous disruption of the Beclin*1* gene in mice was revealed to result in augmented cell proliferation and tumorigenesis, signifying that Beclin1 is a haploinsufficient tumor suppressor gene [39]. In case of brain tumors, cytoplasmic levels of Beclin1 protein and mRNA were discovered to be lesser in GBMs compared to lower grade astrocytomas and normal brain tissue [40].

Also, enhanced survival rates were seen in GBM patients having poor performance scores with high LC3 expression whereas in patients with normal performance scores low LC3 expression was seen [41]. Due to low lovels of LC3B-II and Beclin-1 proteins in higher-grade astrocytomas, it has been proposed that a decline in autophagic activity could push advancement of astrocytic tumors [36]. Monoallelic loss of UVRAG has been reported in various types of cancers- colon, gastric and breast resulting in autophagy inactivation and could have a similar role in brain tumors but no direct studies have addressed this [36]. Genes like EGFR, NF1, PTEN, Akt and p53 whose mutations are regularly allied with brain tumors are identified to be involved in autophagy regulation thereby implying that autophagy may be extremely pertinent to gliomas [36]. Receptor tyrosine kinase EGFR is often amplified in gliomas and is known to subdue autophagy by sustaining the basal intracellular glucose level by physically interacting with the sodium/glucose cotransporter (SGLT)[42]. Tumor suppressor gene, PTEN is also known to positively regulate autophagy by inhibiting PI3-K/Akt pathway [43]. However in brain gliomas generally there is mutation of PTEN and NF1 genes resultant in constitutive activation of the PI3-K/Akt/TOR signaling that could suppress autophagy [36]. One of the major mechanisms of tumor suppression is maintenance of genomic stability by autophagy [44]. p53 is an important tumor suppressor gene in retaining genomic integrity and loss of p53 is a mutual discovery in numerous tumors. It has also been implied to have dual roles in autophagy regulation. Nuclear p53 has been exhibited to stimulate autophagy via transcriptional regulation of DRAM (damage regulated autophagy modulator)[45] while cytoplasmic p53 can negatively control autophagy [46]. Frameshift mutations of ATG genes- Atg2b, Atg5, Atg12 have been reported in gastric and colorectal cancers but need to be assessed in brain tumors [36]. Due to recurrent mutations of tumor

suppressors known to positively regulate autophagy especially in case of brain tumors, a tumor suppressor role of autophagy has been suggested. Other mechanisms by which autophagy suppresses tumorigenesis is necrosis as well as oncogene induced senescence [47, 48].

2.1.2. Autophagy as Tumor promoter

While abridged autophagic capacity has been revealed to be related with tumor advancement and poor prognosis in countless tumors, it is extensively appreciated that cancer cells can use autophagy as a endurance mechanism to survive varied stresses in the tumor microenvironment. After detaching from the extracellular matrix (ECM), autophagy has been known to protect cells from anoikis [49]. This capability to dodge anoikis delivers the tumor cells the chance to attack neighboring tissue and form dispersed tumors, a typical feature of GBMs. A new model for autophagy in tumor promotion identified as "autophagic tumor stroma model of cancer" has been anticipated by current studies [50]. According to the model, tumor cells bring autophagy and oxidative stress in neighboring stromal fibroblasts causing stromal overproduction of recycled nutrients, which are then used by tumor cells for their growth. Another way in which autophagy promotes tumorigenesis is by facilitating glycolysis [36]. Inhibition of autophagy resulted in diminished multiplying of Ras-transformed cells and autophagy deficient cells were seen to have reduced glycolytic capacity [51]. Solid tumors have central regions, which are often hypoxic due to continuous growing tumors. Autophagy is produced as a survival mechanism by tumor cells in the hypoxic core [52]. Upregulation of BNIP3 (Bcl-2/adenovirus E1B 19 kDa-interacting protein) and BNIP3L occur due to overexpression of HIF1α (hypoxia inducible factor-1α) in the hypoxic regions of the tumor. BNIP3 then stimulates autophagy via disruption of the Beclin1-Bcl-2 complex, which is reported to be an

adaptive survival response during prolonged hypoxia [53]. Since autophagy has a vital role in tumor growth, management of autophagy levels will have substantial effects on tumor outcome. Both death-inducing as well as cytoprotective properties of autophagy can be exploited for therapeutic purposes according to a number of preclinical studies [37].

2.2. Autophagy in glioma

GBM is the most belligerent and malignant type of brain tumor due to high resistance to apoptosis as well as moderate resistance to autophagic cell death. Autophagic cell death is a substitute to overcome glioblastoma resistance to pro-apoptosis related therapies [23]. Autophagy is stated to be both as cell endurance and a cell death mechanism in GBM dependent on the cellular milieu, tumor stage, activity of oncogenes and tumor suppressor genes [54]. Autophagy promotes tumor progression by making energy and nutrients accessible for cell survival under stressful condition while it might work as a tumor-suppressing factor by destroying damaged organelles, protein aggregates, and oxidized products, constraining the preliminary stage of the carcinogenic progression [55].

2.2.1. Suppressive action of autophagy in GBM

Autophagy has been known to show tumor suppressive activity in glioma as the advancement of astrocytic tumors is linked with a decline in autophagic activity [56]. It has been established that low expression of autophagy related proteins is found in higher-grade glioma and various studies carried on tumors obtained from GBM patients have depicted altered expression of numerous proteins vital for autophagy comprising LC3B, p62, Beclin 1, ULK1 and ULK2 [57]. Shukla *et al*. stated that lesser ULK1/2 expression owing to p53 inactivation in gliomas supported astrocytic

conversion by restraining autophagy [58]. Autophagy was also known to restrain tumor progression by reducing inflammatory response, which supports necrosis-associated tumor growth[23]. Also, autophagy causes glioma cell senescence [59]. The proliferation of U343 glioma cells was condensed by adenovirus strains expressing shMet (an inhibitor of c-met, receptor of the tyrosine kinase of hepatocyte growth factor) by autophagy activation (enhancing the levels of Beclin-1, LC3-II, and autophagic vacuoles) and senescence (decreasing SM22 (smooth muscle protein of 22kDa), TGase II (transglutaminase-II), and PAI-1 (plasminogen activator inhibitor-1) mRNA), thereby obstructing the instigation of the PI3K/AKT/mTOR signaling pathway. The virus also provoked a G2/M cell cycle arrest and augmented the expression of the cyclin-dependent kinases Cdc2 and Cdc25C [60]. Temozolomide (TMZ) provoked autophagy after senescence in glioma cells, and when autophagy 3-Methyladenine (3-MA) inhibited autophagy, senescence was blocked implying that autophagy is prerequisite for senescence initiation [61]. Lastly, autophagy has been verified to curb glioma cell motility and invasion [62]. Combination of autophagy with EGFR lessened cell migration and heightened radiosensitivity in human glioblastoma cells [63]

2.2.2. Tumor promoting actions of autophagy in GBM

Autophagy has also been known to have positive effects on GBM tumor growth, as well as reducing apoptosis [64]. Autophagy was reported to provide energy to GBM tumor cells via metabolic substrates in order to overcome insufficient oxygen supply as well as vascularization [65]. Autophagy could also be stimulated by reactive oxygen species (ROS) to augment tumor cell survival by increasing actetoacetate, lactate as well as promoting glycolysis [66]. According to Hue *et a*l, amplified levels of the BNIP3 (a HIF-1α downstream target protein) in U87 and T96G cells could trigger

autophagy and stimulate cell survival under hypoxic conditions [67]. Hypoxia-inducible factor-1α (HIF-1α) and NF-κB are known to be activated via oxidative stress, which lead to degradation of caveolin-1 (Cav-1), the tumor suppressor through autophagy [68]. Metastasis of tumor cells is also facilitated by autophagy, which helps in detachment of cancer cells from the ECM [69]. Additionally, autophagy inhibitors can improve chemotherapy against GBM [70, 71]. Combination therapy using CQ and ZD6474, a small molecule that blocks the VEGF receptor on GBM cell lines could considerably upsurge cell apoptosis [72].

2.2.3. Autophagy related molecules affecting GBM

Beclin 1 and LC3:

Expression of key autophagy proteins- LC3A, LC3B, Beclin 1 and ULK1, 2 proteins in GBM was studied by Giatromanolaki *et al.*[73]. LC3 staining was escalated in GBM and undigested trace stone like structures (SLS) were seen in the cytoplasmic space in GBM never notable in normal brain glia. An extreme expression of both forms (LC3 and SLS) - both membrane bound (I and II) and cytoplasmic was observed in U87 cells but faintly expressed in T98 cells and upon valuation of LC3A and LC3B in U87 and T98 cells. This discovery reveals the immunohistochemical expression intensity disparity of LC3s noted among human glioblastomas [73]. Expression of LC3A-I and pro-LC3A forms while absence of LC3A-II, and all forms of LC3B protein was noted in normal human brain inferring that unlike LC3B, LC3A plays vital roles in human brain. The expression of Beclin-1 was also upregulated in tumor cells in roughly half of GBM cases in comparison to normal brain [73]. Similar to LC3s expression, Beclin-1 was overexpressed in U87 cells but not in T98 cell however ULK1/ULK2 was overexpressed in both cells at comparable levels compared to normal brain. In another study by Huang *et al.*[74], glioma cell viability was considerably decreased with Beclin-

1 overexpression. The proposed mechanism of action for Beclin-1 activity was it's binding to Bcl-2 and Bcl-xL, causing their inactivation, thus permitting the stimulation of the pro-apoptotic proteins Bax and Bak, discharging mitochondrial cytochrome c into the cytosol via permeability transition pores and consequently galvanizing caspase-9 and -3, advocating that Beclin-1 controls cell death mechanisms like apoptosis and autophagy by complex formation with Bcl-2 family. Low Beclin-1 and LC3B-II expression levels were observed by Huang *et al.* in GBM signifying that the progression of astrocytic tumors could be favored due to a drop in autophagy induction [56]. It is striking that greater LC3 expression levels were related with an enhanced survival in GBM patients with meager performance scores, while a low LC3 expression associated with superior survival rates in patients with standard performance scores [41].

P62:

p62 (SQSTM1) is an autophagy cargo receptor and susbtrate which notably aggregates with autophagic flux impairment [75]. Higher p62 expression levels have been testified to relate with tumor grade and an inferior diagnosis in adult glioblastoma patients [76]. p62 promotes invasion and migration in glioblastoma stem cells by metabolic deregulation of the RAS/MAPK pathway. During GBM initiation, autophagy is important to avert accumulation of protein aggregates and impaired mitochondria encompassing p62 and ubiquitin [77]. In autophagy-defective cells, accretion of p62 complemented by the collection of undecomposed proteins and damaged organelles, could upsurge oxidative stress, chronic inflammation, DNA damage and cell death, subsequent in tumor commencement through AKT/mTOR pathway [78, 79]. On the opposing end, augmenting the removal of p62 has been evidenced to subdue tumorigenesis by *in-vitro* and *in-vivo* tests [79, 80]. Expression

levels of other proteins like mTOR, NRF2, NFκB as well as anti-apoptotic proteins like Bcl-2 and Bcl-xL was also affected by p62 ensuing in upsurge of tumor cell proliferation and survival [81].

PTEN:

The AKT/mTOR pathway is antagonized by the phosphatase encoded by PTEN, which plays a significant part in autophagy, and it has been stated that U87MG glioma cells lack a functional PTEN activity [82]. Giatromanolaki *et al.* [73] also confirmed this wherein autophagy suppression was seen with enhanced mTOR activity. The strong presence of LC3A,B-II forms in U87, as found in the study, suggests an mTOR independent autophagy regulation pathway in U87. For example, autophagy is regulated by ULK1–Atg13–FIP200 complex by signaling downstream of mTORC1 to initiate autophagosomal formation [83]. Reviewing the different signaling pathways amid human glioblastomas may be imperative in the organization of tumors according to their autophagic profile. A direct association of PTEN with LC3B expression but not with LC3A was found upon examination of PTEN immunohistochemical expression in series of glioblastomas [73]. PTEN was also associated with supplementary autophagy and lysosomal related proteins like ULK1/2, TFEB atg5 and atg12, in gene expression analysis. In most glioblastomas, there was an intensification of lysosomes as seen by overexpression of LAMP2a membrane and cathepsin D intra-lysosomal protein expression. Furthermore, the main transcription factor TFEB controlling the expression of proteins involved in lysosomal biogenesis, and also of autophagy related proteins, was overexpressed in a subset of tumors and directly connected with autophagy markers. Overexpression of TFEB and lysososomal proteins in U87 glioblastoma cell line was confirmed by

western blot analysis however it was not that prominent in T98 thereby verifying the presence of different autophagy profiles amid glioblastomas [73].

Beclin 1:

As discussed earlier, allelic loss of BECN1, an additional autophagy-related gene in tumorigenesis has been shown via examination of genomic information on human cancers [84]. As BECN1 is positioned in adjacent vicinity to BRCA1 (a tumor suppressor), it is conceivable that BECN1 might be a candidate tumor suppressor gene [85]. For example, mice with allelic loss of BECN1 were more disposed to to lymphomas, liver tumors and mammary hyperplasia [84]. It is also seen that BECN1 may also play a role in GBM suppression mechanism. In lower grade of glioma, there were decreased levels of BECN1 [76]while in glioma cells A172 and T98G, BECN1 expression inhibited by miR-34-5p and miR-5195-3p led to glioma cell invasion and migration [86]. By binding to members of Bcl-2 family, BECN1 could control autophagy further activating Bak and Bax which lead to release of mitochondrial cyt c and activate caspase-3 [74].

ATG proteins:

ATG proteins are another class of proteins aiding in autophagy. Atg5 has been known to network with the FADD death domain, stimulating apoptotic cell death [87]. Jo GH *et al.*[88] validated that exposing (10 Gy) glioma cell lines at days 3 and 5 prompted autophagy trailed by apoptosis, and an atg5 knockdown in U373 and LN229 glioma cells after radiation drastically weakened both apoptosis and autophagy signifying that atg5 is obligatory to prompt apoptosis.

Signaling pathways that also regulate tumorigenesis controls autophagy regulation and thus deletion or inactivation of tumor suppressor genes, whose products are often linked with autophagy regulation in tumor may influence on blocking the autophagy's

protective function in GBM [54]. Frequent dysregulation of the PIK3-AKT-mTOR signaling pathway and deficiency in expression of autophagy-regulating genes like Bif-1, Atg5, Beclin 1 promotes GBM development [89].

3. Natural products as autophagy modulators

Epidemiological studies established that there is a robust connection amid diet and human cancer mortality and thus daily ingestion of phytochemicals decreases the occurrence of multiple cancer types [90]. Natural compounds prompt cell death pathways, typically apoptosis and autophagy, and are contemplated as a foremost reserve for drug development and refining innovative anticancer strategies [90, 91]. Though autophagic consequences of natural products have been stated in a few current reviews, their mode of action is very multifaceted, and thus, the complete beneficial potential of phytochemicals still needs to be deliberated. Given the significance of autophagy in cancer therapy, we review the characteristic examples of phytochemicals, which displayed substantial activity in controlling autophagy-signaling pathways thereby inducing death **(Figure 2)**.

Resveratrol

Resveratrol (3, 4′, 5-tri-hydroxy-trans-stilbene) is a polyphenolic compound isolated from grapes, red wine, berries etc. possessing high antioxidant action and chemo-preventive properties [92]. It is known to have anticancer, anti-inflammatory, antimetastatic properties as well as regulation of cell division in different cancer types [93, 94]. Resveratrol has been described to provoke autophagy in multiple cancer types as reported by numerous studies [95-97]. In a study reported by Opipari *et al.*[98], resveratrol constrained growth and induced apoptosis in five human ovarian carcinoma cell lines via mitochondrial release of cytochrome c, apoptosome complex formation, and

caspase activation. In another study, resveratrol treatment induced cell death and repressed growth in U373 glioma cells [99] as well as it enhanced the anticancer effects of temozolomide by controlling its autophagic actions [54]. In case of human colorectal cancer cells, resveratrol sulfates were reconverted by steroid sulfatases accordingly bringing senescence and autophagy in cancer cells [100]. Resveratrol repressed NF-κB activation and augmented lysosomal permeability thereby triggering autophagy in cervical cancer cells [101]. Augmented quantities of LC3-II protein, autophagosomes formation as well as dependency on LKB1/AMPK/mTOR pathway was the apoptosis mechanism induced by resveratrol in HL-60 promyelocytic leukemia cells in a study reported by Fan *et al.*[97] while in case of PC3 and DU-145 androgen independent prostrate cancer cells[102], downregulation of stromal interaction molecule (STIM1) expression, and inhibition of AKT/mTOR pathways led to autophagy mediated cell death. Also, combination therapy of mTORC1 inhibitor, rapamycin along with resveratrol drastically repressed the growth of estrogen receptor-positive and estrogen receptor-negative breast cancer cells by blocking stimulation of AKT pathway [103]. Trincheri *et al.*[104] reported that in human colorectal DLD1 cancer cells, autophagy was triggered with acute exposure to resveratrol however prolonged exposure led to activation of caspase-mediated cell death. A novel pathway for resveratrol toxicity was uncovered in which autophagy had dual roles: a pro-survival stress response that later switched to a caspase-dependent apoptosis pathway. In glioblastoma cells, resveratrol prompted the autophagosomes formation via up regulation of Atg5, Beclin-1 and LC3-11 [105]. Thus, resveratrol can trigger early senescence that is linked with a blockade of autolysosome materialization and was also seen to down regulate the levels of Rictor, an important constituent of the mTORC2 complex, thereby reducing Rho-GTPase activity [106]. Reseveratrol suggestively improved the therapeutic

effect of temozolomide against malignant glioma *in vivo* and *in vitro* by inhibiting autophagy in another study [107]. In combination with radiotherapy, resveratrol considerably amplified autophagy and apoptosis levels in both *in vitro* glioma stem cells and nude mouse model [108]. Since resveratrol has depicted usefulness in provoking autophagy in multiple cancer types, additional clinical studies are necessitated to completely explicate the usefulness and bioactivity of resveratrol in autophagic killing of cancer cells.

Curcumin

Curcumin (1,7-bis[4-hydroxy-3 methoxyphenyl]-1, 6-heptadiene-3,5-dione) is a biologically active phenolic compound obtained from rhizome of the plant *Curcuma longa*. It has been established to possess chemopreventive, anti-inflammatory, hepatoprotective, anticarcinogenic properties [109, 110]. It addition, it also acts as an inhibitor of NF-κB, STAT3, PI3K and AKT pathways and induces apoptosis in multiple cancer types both *in vivo* and *in vitro* [111]. Molecular mechanisms of curcumin-induced autophagy are miscellaneous and autophagy initiation by curcumin by utilizing distinct molecular mechanisms has been established by many studies [112]. It was proposed that the autophagy modulation could deliver benefits for the patients with glioblastoma since countless anti-glioma approaches produce autophagy induction [113]. Aoki *et al.*[114] reported that curcumin brought G(2)/M arrest and non-apoptotic autophagic cell death in U87-MG and U373-MG glioma cells. It also repressed the Akt/mTOR/p70S6K pathway and stimulated the ERK1/2 pathway, resultant in autophagy generation both *in viv*o and *in vitro*. In another study, curcumin prompted the differentiation of glioblastoma cells from glioma-initiating cells *in vivo* and *in vitro* following autophagy induction through the inhibition of PI3K/Akt/mTOR

signaling pathway [115]. Curcumin inhibited growth of lung adenocarcinoma cells by inducing autophagy through activation of AMPK signaling pathway [116] while in case of human A375 and C8161 melanoma cells, autophagy was induced via deactivation of PI3K/ Akt/mTOR/P70S6K pathway [117]. Zanotto-Filho *et al.*[118] proved that curcumin in amalgamation with TMZ strongly constrained autophagy and enhanced effectiveness against glioblastomas. The effect of Curcumin on lysosome was reported by another study in which curcumin heightened autophagic flux in human colon cancer HCT116 cells and mouse embryonic fibroblasts. It additionally stimulated transcription factor EB (TFEB), a vital nuclear transcription factor in control of autophagy as well as lysosome biogenesis and function. Inhibition of autophagy and lysosome led to added cell death in Curcumin-treated HCT116 cells via the activation of TFEB inhibition [119]. In mesothelioma cells, curcumin induced autophagy as denoted by enhanced conversion of LC3-1 to LC3-II and autophagosomes formation, which was condensed by RNA silencing of Atg5 [120].

Morusin

Morusin is a natural product derived from the root bark of mulberry tree (Morus specie, Moraceae) used as a conventional chinese medicine for antiphlogistic, antipyretic, antiheadache, and diuretic effects [121]. Morusin has been known to provoke autophagy via AMPK activation and mTOR activity inhibition [122]. In a study reported by Srishti *et al.* morusin induced autophagy in human glioma cell lines (U87 and GI-1) but not in normal neuronal cells (HCN-1A). The precise mechanism by which morusin induces autophagy still remains to be elucidated by future studies.

Artemisinin

Another chinese herbal medicine, commonly used for malaria treatment is artemisinin, derived from the medicinal plant Artemisia annua or sweet wormwood [123]. Latest studies have specified that artemisinin selectively kills cancer cells by controlling autophagy [4]. Dihydroartemisinin (DHA), a key active metabolite of artemisinin, brought autophagy in multiple cancer cells by subduing nuclear factor-κB (NF-κB) stimulation and the accretion of ROS as reported by Hu *et al.*[124]. DHA constrained cell proliferation and triggered autophagy-mediated apoptosis in esophageal cancer cells [125] as well as stimulated apoptosis and autophagy in SKMG-4 glioma cells [124]. Artesunate, a water-soluble semisynthetic derivative of artemisinin, provoked autophagy and apoptosis in GBM cells and was allied with DNA damage. Also, artesunate pretreatment prompted TMZ-induced cell death *in vitro* and in *vivo* in GBM cells [126]. Dihydroartemisinin increased the efficacy of temozolomide by stimulating expression of autophagy molecular markers, Beclin-1 and LC3-B, *in vitro* and *in vivo* in glioma cells [127]. In Burkitt lymphoma cells, growth was repressed in a concentration and time dependent manner by artesunate thereby inducing apoptosis as well as expression of Beclin-1, LC3-I/LC3-II, and caspase-3 autophagy markers in these cells [128].

Thymoquinone

Thymoquinone (TQ) is a phytochemical isolated from *Nigella sativa* L. or black cumin known to exhibit anticancer effects against many cancers, like brain, breast, colon, liver, lung, and prostate [129, 130]. In a CPT-11-resistant colon cancer cell line, thymoquinone tempted caspase-independent, autophagic cell death via mitochondrial dysfunction and instigation of JNK and p38 [131]. It was also established that TQ

constrained the growth of irinotecan-resistant LoVo colon cancer cells by primarily triggering apoptosis before autophagy-induced autophagosome formation. Hence, TQ-induced, caspase-independent, autophagic cell death was linked with activation of the JNK and p38 MAPK pathways as well as mitochondrial outer membrane permeability. In another study reported by Racoma *et al.*[132], TQ specifically subdued glioblastoma cell growth, and along with a lysosomal inhibitor, considerably prompted apoptosis, signifying the connection of autophagy in TQ-induced cell death. LC3-II and p62 protein levels were also enhanced upon exposure to TQ. In combination with TMZ, TQ repressed the growth of U87MG cells by transcriptionally impeding autophagy and stimulating apoptosis [133] while in case of breast cancer cells, it enhanced the anti-cancer activity of gemcitabine by moderating its apoptotic and autophagic activities [134].

Capsaicin

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a naturally occurring phytochemical as well as the main piquant component of hot chili peppers of the genus *Capsicum* (family Solanaceae), which are widely utilized as food additives [135]. Various pharmaceutical formulations and clinical applications have confirmed the importance of capsaicin [136]. Capsaicin has many antitumor effects [137] and prompts apoptosis in many malignant cell lines. It is generally known to induce apoptosis via increased intracellular Ca^{2+} levels[138], ROS generation [138, 139], interruption of mitochondrial membrane potential [140] and stimulation of transcription factors [139]. Lately, it was reported that capsaicin might bring autophagy, implying a likely therapeutic strategy for cancer [139, 141, 142]. Lin *et al.*[135] explained autophagy and apoptosis induced in human asopharyngeal carcinoma cells by capsaicin via down

regulation of the PI3K/AKT/mTOR Pathway. In another study, capsaicin provoked prostate cancer cell death in a concentration and time dependent manner, augmented microtubule-associated protein light chain 3-II (LC3-II, a marker of autophagy) levels and the accretion of the cargo protein p62 signifying an autophagy blockage [143]. Also, there was an increase in lysosomes, which co-localized with LC3 positive vesicles in a comparable degree to that made by the lysosomal protease inhibitors E64 and pepstatin directing to an autophagolysosomes breakdown inhibition. Capsaicin also induced autophagy in U251 glioma cells by increasing the expression of LC3 in cytoplasm after treatment [144].

Crocetin/Crocin

Crocetin is a main component of saffron, the dried dark-red stigma of *Crocus sativus L*. which is a conventional Chinese medicine formerly been used to increase blood circulation [145]. Saffron has been extensively used in the treatment of numerous diseases comprising cancers for its antioxidative, antiproliferative, and anti-inflammatory properties [146, 147]. Crocetin and its glucosidic derivatives like crocin, safranal, and flavonoids are the major components of saffron [148]. Crocin is known to possess most efficient anticancer activity. Apoptosis induction and the allied proliferation inhibition are the foremost mechanisms of crocin in overturning cancer advancement [147]. Crocin was stated to induce autophagic apoptosis in hepatocellular carcinoma by deterring Akt/mTOR activity [145]. In case of breast cancer cells, crocetin drastically enhanced the tumor suppressive activity of fluorouracil on breast cancer cell growth as well as enhanced autophagic cell death in fluorouracil-treated breast cancer cells feasibly through modulation of Atg1 and Beclin-1 expression [149]. Giakoumettis *et al.*[150] in their study on C6 glioma cells conveyed the synergistic

effect of crocin with temozolomide. The results advocated that the cells experienced calpain-dependent programmed cell death while co-exposure to Crocus extract and TMZ enhanced the antineoplastic effect of TMZ.

Honokiol

Another chinese and Japanese medicine isolated from the magnolia tree is Honokiol (HNK), a biphenolic compound used for treatment of anxiety, thrombotic stroke and gastrointestinal symptoms [151]. Various activities displayed by HNK are anti-inflammatory, anti-microbial, cardioprotective, antiangiogenic and anticancer activities [152-157]. Also, the therapeutic effects were significantly enhanced upon amalgamation of honokiol with numerous other chemotherapeutic drugs both *in vivo* and *in vitro* [155, 158]. It is recommended that through stimulation of different apoptotic mechanisms, the cytotoxicity of honokiol increased drastically[151]. In a study reported by Huang *et al.*[151] honokiol produced apoptosis and autophagy through the ROS/ERK1/2 signaling pathway in human osteosarcoma cells *in vitro* and *in vivo.* There was an increased production of intracellular ROS, activation of the MAP kinase ERK and up regulation of Atg7, all hallmarks of honokiol-induced autophagy. Similar sets of results were reported by Yeh *et al.*[159] in neuroblastoma cells wherein honokiol induced autophagy via activation of the PI3K/Akt/mTOR and ERS/ROS/ERK1/2 signaling pathways and repressed cell migration. In another study, combined treatment of honokiol with TMZ significantly enhanced cell death, provoked superior caspase-3 activation, DNA fragmentation, cell apoptosis in U87-MG glioma cells [160] and also induced autophagy of glioma cells *in vivo* and *in vitro* [161]. In combination with magnolol, honokiol induced autophagy and apoptosis in glioma cells *in vivo* and *in vitro* [162]. In human thyroid cancer cells, honokiol treatment suppressed cell growth,

prompted cell cycle arrest, and augmented the stimulation of caspase-dependent apoptosis and autophagy in cancer cells. Honokiol treatment also controlled Akt/mTOR, ERK, JNK, and p38, suppressed cell growth, induced cell cycle arrest, and enhanced the induction of caspase-dependent apoptosis and autophagy in cancer cells [163].

Paclitaxel (taxol)

A very common and widely used chemotherapeutic drug is paclitaxel, derived from Pacific yew tree (Taxus brevifolia Nutt)[4]. Paclitaxel (PTX) is a taxane class diterpenoid that has a powerful cytotoxic effect against multiple cancer types [164]. Recent studies on breast cancer cells have confirmed that paclitaxel elicits premature autophagy in both normoxic and hypoxic conditions and is linked with apoptosis [165]. Even in combination therapy with another drug, pristimerin the autophagy effect was additively enhanced via ERK1/2 regulation [166]. In another study reported, paclitaxel triggered autophagy and curbed proliferation of gastric cancer cells [167] while in A549 lung cancer cells, paclitaxel prompted autophagy, increased the formation of acidic vesicular organelles, and enhanced Atg5, Beclin-1, and LC3 expression [168]. In a recent study reported by Zhou *et al.*[169] on SiHa cerivical cancer cells, inhibition of cell proliferation along with autophagy induction via lncRNARP11-381N20.2 was observed. Increase in autophagic markers has been stated in multiple cancer cells like U87, HT-29, A549 when exposed to taxol [168]. Yet, its poor solubility confines taxol efficacy in clinical scenario. As we converse in the following section, this problem can be resolved by nano-based strategies that intend to upsurge taxol bioavalibility.

Ursolic acid

Ursolic acid (UA) is a naturally occurring plant triterpenoid stated to deter cell proliferation with distinctive anticancer mechanisms in various cancer types [170, 171]. The mechanisms by which UA is known to induce apoptosis consist of Stat5/Akt, ERK1/2 MAPK, and PI3K/Akt/mTOR, pathways [172, 173]. In oral squamous cell carcinoma (OSCC), UA triggered caspase-dependent apoptosis via downregulation of various signaling pathways like Akt/mTOR/NF-κB signaling, ERK, and p38 [174]. Autophagy induction by UA was via LC3B-II conversion, amplified p62 expression and accretion of autophagosomes. In cervical cancer cells, UA induced autophagy and curbed the cell growth in a concentration-dependent manner by modulating LC3-II and Atg5 [175]. In U87MG glioma cells, UA provoked both G1-phase arrest and autophagy [176]. The major hallmarks of UA-induced autophagy were the formation of acidic vesicular organelles, upsurge of autophagolysosomes and LC3-II accumulation. The increased free cytosolic calcium produced by UA that activated the CaMKK-AMPK-mTOR kinase-signaling cascade eventually elicited autophagy. Thus, basically UA induced autophagy by 3 dissimilar pathways: phosphorylated extracellular signal-regulated kinase/eukaryotic initiation factor 2/C/ EBP homologous protein (PERK/eIF2α/CHOP), calmodulin-dependent kinase protein kinase (CaMMK)/ AMPK/mTOR, and inositol-requiring enzyme 1α (IRE1α)/JNK signaling in U87MG cells. In a study reported by Wu *et al.*[177], UA considerably provoked autophagy in human osteosarcoma cells along with a third generation, nitrogen-containing bisphosphonate, zoledronic acid. The viability of breast cancer cells was reduced by UA via modulation of the PI3K/AKT-regulated GSK autophagy pathway and caspase-3 escorted by the NF-κB signaling pathway [178].

4. Nano mediated delivery of natural substances as potent autophagy modulators for GBM therapy

Activation of stress-induced autophagy is one of the suggested mechanisms for therapeutic resistance in GBM. Early stage autophagy inhibitors like wortmannin and 3-MA are used to avert initiation and elongation of phagophore while late-stage inhibitors like HCQ, CQ and bafilomycin-A1 target the lysosomal fusion with autophagosomes [179]. Although, most of the chemotherapeutics induce toxicity via provocation of autophagic dependent cell death, autophagy activation in perspective of most therapies has been linked with prosurvival signaling through the preservation of cellular integrity in response to metabolic and hypoxic stress [180]. The likelihood of autophagy induction in GBM as an intermediary of resistance has guided the use of autophagy inhibitors in amalgamation with several treatment strategies. Though initial preclinical and clinical data using CQ and HCQ have been encouraging, these inhibitors lack specificity and might be restricted by toxicity when given in grouping with chemotherapeutic drugs [179]. Targeted drug delivery to tumor tissue is one of the securest means to target the tumor in cancer. The key factors that hinder high oral bioavailability as well as detectable plasma levels of parent compound are poor water solubility, limited intestinal absorption, metabolism by gut microflora, active efflux mechanism, low water stability of phytochemicals and first pass metabolic effects [181, 182]. To tackle this issue, nanoparticles (NPs) have played a significant role in delivery of drugs precisely at the selected site at the prerequisite concentration, dodging immune response minus any undesired effects [183]. They have attained unparalleled triumph as drug-delivery vehicles in cancer therapy owing to their bio physiological properties and their capability to network with cells because of their size similarity

with cellular components [184, 185]. Making use of nanotechnology, it might be possible to achieve [92, 186]:

- Enhanced delivery of poorly water-soluble phytochemicals
- Targeted delivery of phytochemicals in a cell or tissue specific way
- Controlled and sustained release
- Enhanced bioavailability
- Transcytosis of phytochemicals across endothelial barriers and tight junctions like the blood brain barrier (BBB).
- Escaping the RES
- Protecting the drugs from degradation
- Delivery of large macromolecule phytochemicals to intracellular target sites
- Combination therapy using two or more phytochemicals
- Tracking of phytochemicals by incorporation of imaging modalities

Polymeric nanoaprticles, metallic nanoparticles and dendrimers are the most common types of nanoparticles used for drug delivery each having its own benefits and demerits as a drug delivery vehicle. Carbon nanomaterials have also been reported to induce autophagy in different cell types however not much data is available on their application for delivery of natural substances. An important role in autophagy regulation is played by fullerenes and their derivatives. Autophagy was provoked as a protective mechanism against β-amyloid peptide induced cytotoxicity in neuro-2A cells using C60 fullerene-pentoxifylline nanoparticles [187] implying its possible application value in drug design for amyloid-related diseases. An autophagy-dependent chemo sensitization effect on cancer cells has been shown by fullerene C60 and its derivatives, wherein fullerene C60 and its derivative C60 (Nd) nanoparticles could prompt autophagy at very low concentrations in cells [188]. Graphene oxide was

also reported to induce autophagy in CT26 colon cancer cells [189], suppressing tumor activity. Graphene oxide might stimulate the opposite autophagy effect owing to the dissimilar sizes of materials, synthesis methods, surface functional groups in addition to initiating autophagy. Graphene oxide quantum dots (average diameter 3.28 + 1.16 nm) constrained lysosomal degradation by diminishing the action of cathepsin B in GC-2 and TM4 cells, consequently impeding autophagic flux [190]. These different assumptions also exemplify the intricacy of the modulation of autophagy by nanomaterials. A concrete analysis of each specific question needs to be conducted when using nanomaterials to control autophagy. Wu *et al*. methodically premeditated the autophagy effect of 81 multi-walled carbon nanotubes altered by diverse combinations of chemically modified surface ligands and found that multi-walled carbon nanotubes with unlike chemical compositions stimulated autophagy to unusual degrees by stimulating different signaling pathways [191]. Functionalized single-walled carbon nanotubes have also been reported to inverse the irregular activation of mTOR signaling and lysosomal protein hydrolysis effects (a major cause of alzheimer's disease) thereby eliminating autophagic substrates [192]. These discoveries propose that carbon nanotubes could serve as probable neuroprotective therapeutics for neurodegenerative diseases. The major problem is that due to to the non-degradabable nature of most nanomaterials, the accretion of an enormous number of nanomaterials in cells or the toxicity of the materials themselves will trigger damage to the consequent organelles and ultimately lead to the obstruction of autophagic flux. As, most of the studies on using nanomaterials for autophagy regulation are carried out *in vitro* it is difficult to decipher the effect *in vivo*. Since many of these materials show exemplary potential in inducing autophagy, future studies focusing on delivery of

phytochemicals using carbon nanomaterials for glioma therapy may be promising area for research.

To upsurge the targeted delivery of phytochemicals into the brain, which is identified to be usually low due to the existence of the blood brain barrier (BBB), nanotechnologies, and in specific Nano carriers, have been lately made to improve the delivery of therapeutic payloads within the brain tumor mass [193]. Receptor-mediated transcytosis (RMT) to transport cargo through the brain endothelial cells toward brain parenchyma is one such strategy that involves the creation of a complex between the target site and the targeting ligand [194]. Taking into consideration the numerous advantages offered by nanoparticles, they could be exploited to the best of their capability to encapsulate natural substances and modulate autophagy in glioma. **Table 1.** outlines different naturally derived substances and their molecular targets involved in the autophagic process alongside use of nanotechnology for delivery of these substances for GBM therapy.

Curcumin loaded NPs

Polymeric nanoparticles and liposomes have been used as drug carriers for safer and more efficient delivery of countless drugs. Numbers of studies have established liposomes as a promising option for natural products delivery to target GBM and curcumin is one of them [199, 219, 220]. Mukherjee *et al.*[199] utilized liposomes as a therapeutic intervention to target glioblastoma stem cells. They investigated liposomal TriCurin (TrLp), encompassing three natural drugs- curcumin, resveratrol and epicatechin gallate (ECG). TrLp induced apoptosis in GBM cells *in vitro* as well as repolarization of M2-like tumor (GBM)-associated microglia/macrophages to the tumoricidal M1-like phenotype and intra-GBM recruitment of activated natural killer

cells. Poly(lactic-co-glycolic acid) (PLGA) is a polymer-based biocompatible nanoparticle with least toxicity, frequently used in drug-loaded nanoparticles. Orunoglu *et al.*[200] reported the effect of curcumin loaded-PLGA nanoparticles on RG2 rat glioma model. Curcumin induced apotosis/autophagy in glial cells and hence significant toxicity was observed in RG2 cells. Aqueous suspensions of polymeric nanoparticles have been suggested as efficient drug carriers and nanocapsules are one type having an oil core enclosed by a polymeric wall[201]. Cucumin loaded lipid core nanocapsules for glioma treatment were reported by Filho *et al.*[202]. Stimulation of G2/M arrest and autophagy was witnessed using curcumin nanocapsules as well as in free-curcumin treatments in rats bearing C6 gliomas. Curcumin loaded layered double hydroxide nanoparticles (Cur/LDH NPs) for autophagy induction in glioma was reported by Zhang *et al.*[203]. Autophagy was identified noticeably in A172 cells treated with NPs by the autophagic marker LC3A/B. Also, the expression levels of Atg5-Atg12 and LAMP-1 were augmented in the NPs treated groups, and autophagic vacuoles were witnessed via TEM. Other than autophagic cell death, apoptotic death induced by curcumin nanoparticles solely on their own as well as in combination with other drugs has been reported by various studies [221, 222]. A dual targeting system -T7-modified magnetic PLGA NP system having dual drugs - hydrophobic magnetic nanoparticles and drugs (i.e., paclitaxel and curcumin) was reported by Cui *et al.*[223]. The combined drugs generated synergistic effects on inhibition of tumor growth via the mechanisms of apoptosis induction and cell cycle arrest, exhibiting considerably amplified effect compared to single drugs alone. In another study, curcumin was able to induce apoptosis of brain cancer cells when co-delivered with pUNO1-hTRAILa in a tLyp-1-conjugated GSH-sensitive biodegradable micelles [224]. Maiti *et al.*[225]. investigated solid lipid curcumin nanoparticles for autophagy induction in cultured

glioblastoma cells. Inhibition of the PI3K-Akt/mTOR signaling pathway with an increase in autophagic markers was observed in C-6 glioma cells **(figure 3)**.

Resveratrol loaded NPs

Resveratrol has been shown to significantly modulate the process of autophagy. For GBM therapy, it was delivered using transferrin-targeted, resveratrol-loaded liposomes, wherein owing to cell cycle progression arrest and autophagy-triggered apoptosis induction by resveratrol, higher rate of apoptosis was seen with resveratrol nano formulation compared to the drug alone [195]. Resveratrol triggered autophagy and constrained the expression of AKT/mTOR signaling pathway-related proteins to improve cognitive dysfunction in rats having chronic cerebral hypoperfusion [196]. Apoptosis was induced by transferrin modified PEG-PLA-resveratrol conjugates in C-6 glioma bearing rats [197] while in another study resveratrol loaded solid lipid nanoparticles were used for brain delivery [198].

Crocetin loaded NPs

As described in the earlier section, crocetin prompts autophagy by inhibition of Akt/mTOR activity as well as by increasing Atg levels [226]. Mousavi *et al.*[216] first reported crocin nanoliposomes. Liposomal formulations encompassing crocin were superior cytotoxic compounds against cancer cells compared with free crocin, which was also confirmed by Rastgoo *et al.*[217], who evaluated the effect of crocin nanoliposomes against colon cancer cells. Since, crocetin has been shown to stimulate autophagic apoptosis by deterring Akt/mTOR activity and induce calpain-dependent programmed cell death in C6 glioma cells, a nanoformualtion of crocetin against glioblastoma may be benefical to induce autophagic cell death. Combinations with

other natural products such as curcumin, morusin, honokiol or other synthetic drugs may broaden the treatment efficiency.

Honokiol loaded NPs

Honokiol was stated to induce autophagy via instigation of the PI3K/Akt/mTOR and ERS/ROS/ERK1/2 signaling pathways in neuroblastoma as well as in combination with TMZ, it significantly enhanced cell death, brought greater caspase-3 activation, DNA fragmentation, cell apoptosis in U87-MG glioma cells[159]. Liu *et al.*[211] developed lactoferrin modified daunorubicin plus honokiol liposomes against glioma cells. Activation of apoptotic enzymes caspases and down regulation of PI3K, MMP-2, MMP-9, VE-Cadherin and FAK was observed with enhanced C6 cells inhibition. Honokiol was delivered in combination with doxorubicin to glioma cells employing biodegradable self-assembling micelles (MPEG-PCL) as a nanovector [212]. Honokiol and doxorubicin encapsulated in MPEG-PCL nanoparticles could proficiently subdue glioma cell proliferation and provoke cell apoptosis *in vitro*. Additional studies are necessitated for nano particulate delivery of honokiol for autophagy regulation in GBM with the findings of this review setting the groundwork in this regard.

Morusin loaded NPs

Morusin is isolated from chinese herbal medicine and has been stated to exhibit exceptional anti-cancer properties, however its full potential is yet to be explored by further research studies. The exact mechanism by which morusin induces autophagy is still unknown but a study proved that morusin exerts its autophagic effect via activation of AMPK and inhibition of mTOR activity as well as accumulation of LC3-II [122]. Agarwal *et al.*[204] studied use of PLGA-morusin NPs for autophagy induction in

glioblastoma. PLGA-MOR-CTX NPs were able to induce autophagy in glioma cells (U87 and GI-1) as substantiated by the research study **(Figure 3)**.

Paclitaxel Loaded NPs

Paclitaxel or taxol is the most widely used chemotherapeutic drug today and numerous nano formulations have been reported for its delivery. Being indicated as an autophagy modulator, studies are being carried out to decipher how autophagy affects paclitaxel activity. For glioma targeting, PLGA nanoparticles and magnetic nanoparticles were amalgamated in which superparamagnetic γ-Fe_2O_3 nanoparticles (MNPs) were used as a core, labelled with polyethylenimine (PEI)-conjugated fluorescein isothiocyanate (FITC), having PTX as a chemotherapeutic drug examined in human brain glioblastoma U251 cells [213]. Inhibition of cell proliferation and migration, and programmed cell death, through both apoptosis and autophagy with accretion of autophagosomes and LC3-II signals was identified in the treated glioblastoma U251 cells **(figure 3)**. Another group synthesized a polyethylene glycol-dipalmitoylphosphatidyle- thanoiamine (mPEG-DPPE) calcium phosphate nanoparticles (NPs) injectable thermoresponsive hydrogel (nanocomposite gel) for glioma therapy that might deliver a continual and local delivery of paclitaxel and TMZ and caused autophagic cell death [214]. The efficacy of the gel was also tested *in vivo* and it was concluded that C6 cell autophagy mediated cell death was enhanced *in vivo* by PTX:TMZ NPs and the nanocomposite gel. Xin *et al.*[215] investigated angiopep-conjugated PEG-PCL nanoparticles (ANG-PEG-NP) having paclitaxel as a drug and found enhanced anti-glioblastoma efficacy. Another study reported the impact of the penetrating peptide iRGD on the effect of paclitaxel-loaded MT1-AF7p-conjugated nanoparticles on glioma cells [227]. The anti-proliferative and apoptosis-

induction activity of PTX was drastically heightened upon its encapsulation in MT1-NP.

Artemisinin loaded NPs

Despite of escalating expression of autophagic markers like beclin-1, LC3-I/LC3-II, and caspase-3, artemisinin is still a new phytochemical and very few research studies have been reported by far concentrating on autophagic activity of artemisinin. For brain delivery, a transferrin-conjugated nanostructured lipid carrier (TF-NLCs) of artemisinin (ART) was reported by Emami *et al.*[205]. Heightened anticancer effect of the drug in human brain cancer cells compared to free artemisin was observed because of Tf receptor mediated endocytosis. No studies have been published regarding nano delivery of arteminsin for modulating autophagy. Hence, it will be of great interest to study this molecule and report novel delivery strategies.

Thymoquinone loaded NPs

Thymoquinone has been depicted to be a potent apoptosis inductor in glioma cells as stated by many studies [228, 229]. It was delivered to glioma cells using mesoporous silica NPs and was able to induce high cytotoxicity, enhanced caspase-3 activation, cytochrome c triggering as well as cell cycle arrest at G2/M compared to the drug alone[206]. Solid lipid nanoparticles were also used for thymoquinone delivery to glioma cells wherein high cytotoxicity was observed [207]. Nanoparticles having thymoquinone as a therapeutic payload for modulating autophagy is another area of interest as well to observe its potency in combination with other drugs.

Capsaicin loaded NPs

Capsaicin has lately been accepted as a natural tumor-blocking compound, which constrains tumor growth at different stages. It has a strong inhibitory role on glioma growth [208, 230]. Capsaicin was able to prompt apoptosis in glioma cells via down-regulation of Bcl-2 and p38 MAPK activation [208, 209]. Limited studies are available on capsaicin nanoparticle delivery to glioma cells. Jiang *et al.* [210] in one study reported capsaicin-loaded nanoparticles synthesized using methoxy polyethylene glycol-poly(caprolactone) (mPEG-PCL) amphiphilic block copolymer. Noteworthy inhibition on the growth of U251 cells was observed *in vitro.* Being a promising compound, it will be interesting to see whether nano-delivery of capsaicin can modulate autophagy in glioma cells using different mechanisms.

Ursolic acid loaded NPs

As discussed in the previous section, ursolic acid mostly displays anti-cancer and anti-inflammatory effect [231]. It was stated that the inhibitory effect of ursolic acid to C6 glioma cells was considerably linked to its concentration [173]. Multifunctional targeting ursolic acids liposomes had greater inhibitory effects to C6 glioma cells and C6 glioma stem cells in comparison to others as reported by one study [218]. As ursolic acid is known to induce autophagy in cancer cells via activation of LC3-II, p62 and inhibition of AKT/mTOR pathway, nanoparticles loaded with ursolic acid can be a favorable tool for autophagy induction in glioma cells.

5. Discussion

Despite of tremendous progress in cancer management, synthesizing targeted chemotherapeutics stays a key hurdle in contemporary medicine. Moreover, multi-

drug resistance is an additional setback in the clinical application of recent chemotherapeutic drugs. Therefore, autophagy modulators might aid as favorable anticancer agents to battle the unsettled difficulty of drug resistance and expand current cancer therapeutic strategies. Gliomas form the most challenging to treat brain tumors owing to their abnormal invasive capability, and activation of multi drug resistance mechanisms, which aid in tumorigenesis, as well as their inborn resistance to usual chemotherapy/radiotherapy approach. Physiologically, autophagy is a crucial affair for standard tissue homeostasis. In cancer cells, autophagy has been recognized as one of the key factors in certifying that cancer cells acclimatize proficiently to a severe tumor microenvironment and a deficiency of vital nutrients. Cancer cells can recover necessary amino acids by utilizing autophagic pathways and hence ensure their survival. In short, it is a competent way to control tumor cell growth via autophagy pathways. Autophagy modulators can aid as likely candidates for the cancer treatment and thus natural compounds can be contemplated as fundamental sources of drug development because of their capability to provoke effective apoptosis and autophagy. Being food compounds, they have high safety because of their intrinsically lower toxicity and through lessening of doses and side effects as equated with synthetic drugs. Additionally, autophagic potential of phytochemicals could be contemplated as a substitute for cell death in apoptosis resistant cells.

Presently, numerous studies have reported bioactive compounds with autophagy inducing abilities. Bioactive compounds can elicit autophagy pathways via formation of autophagosome and controlling other signal transduction pathways, like PI3K/Akt/mTOR pathway, to initiate autophagy or in some cases inhibit it. As autophagy is indispensible to equalize the cell survival and death, the likely effect of bioactive compounds, which target autophagy pathways, on cancer therapy will be

intense. They can be pondered as a suitable option for single therapy or in amalgamation with FDA-approved drugs for cancer treatment. But the efficacy of phytochemicals is limited due to restraints like poor bioavailability and multi-target properties that impedes their clinical applications. Nanotechnology has made delivery possible of the most difficult of drugs and hence it may enhance the efficacy of such molecules. NPs can form complexes with phenolic compounds via hydrogen bonds and hydrophobic interactions to capture phenolic compounds and enhance their aqueous solubility. Lastly, as NPs epitomize compelling autophagic activity by themselves, they can augment the autophagic potential of phytochemicals as therapeutic agents. In this review, we have summarized the autophagic process, and how natural substances can be exploited as autophagy modulators for GBM therapy using nanotechnology.

We comprehend that awareness in this field is still at a pilot stage and upcoming studies may help to broaden up to the existing database of NP delivery of natural products as autophagy modulators in GBM. Due to dual roles of autophagy in diverse pathophysiologic conditions, it can cause unlikable outcomes under some conditions, though autophagy inhibition might turn out to be promising therapeutic strategy. Hence, it is important to consider the therapeutic benefits of autophagy alongside its possible risks, and investigation into blends of autophagy-modulating natural compounds with other agents could aid in restricting the disagreeable side effects or therapy-resistance instigated by autophagy. It is still not completely elucidated how these natural compounds cooperate with autophagic targets to initiate or hinder autophagy. Hereafter, additional understanding of autophagy modulation by natural compounds, precisely the identification of subcellular targets can make a huge influence in accelerating an improved understanding of the molecular mechanisms of

natural compounds and accordingly drive the expansion of natural compounds in clinical usage. We are assertive that this review will deliver an outstanding platform for the documentation of natural compounds as well as their site-specific delivery employing nanotechnology for autophagy management in GBM.

References

M. Budke, A. Isla-Guerrero, C. Perez-Lopez, M. Perez-Alvarez, A. Garcia-Grande, M. J. Bello, et al., **[A comparative study of the treatment of high grade gliomas]**. *Rev Neurol*. 2003;37:912-6

D. N. Louis, H. Ohgaki, O. D. Wiestler, W. K. Cavenee, P. C. Burger, A. Jouvet, et al., **The 2007 WHO classification of tumours of the central nervous system**. *Acta Neuropathol*. 2007;114:97-109

R. Djedid, O. Tomasi, A. Haidara, M. Rynkowski and F. Lefranc, **[Glioblastoma treatment in 2010]**. *Rev Med Brux*. 2009;30:496-505

S. Deng, M. K. Shanmugam, A. P. Kumar, C. T. Yap, G. Sethi and A. Bishayee, **Targeting autophagy using natural compounds for cancer prevention and therapy**. *Cancer*. 2019;125:1228-1246

S. S. Singh, S. Vats, A. Y. Chia, T. Z. Tan, S. Deng, M. S. Ong, et al., **Dual role of autophagy in hallmarks of cancer**. *Oncogene*. 2018;37:1142-1158

A. Duffy, J. Le, E. Sausville and A. Emadi, **Autophagy modulation: a target for cancer treatment development**. *Cancer Chemother Pharmacol*. 2015;75:439-47

G. Manic, F. Obrist, G. Kroemer, I. Vitale and L. Galluzzi, **Chloroquine and hydroxychloroquine for cancer therapy**. *Mol Cell Oncol*. 2014;1:e29911

S. N. B. Selby-Pham, R. B. Miller, K. Howell, F. Dunshea and L. E. Bennett, **Physicochemical properties of dietary phytochemicals can predict their passive absorption in the human small intestine**. *Sci Rep*. 2017;7:1931

L. Mouhid, M. Corzo-Martinez, C. Torres, L. Vazquez, G. Reglero, T. Fornari, et al., **Improving In Vivo Efficacy of Bioactive Molecules: An Overview of Potentially Antitumor Phytochemicals and Currently Available Lipid-Based Delivery Systems**. *J Oncol*. 2017;2017:7351976

C. Giampietri, A. Paone and A. D'Alessio, **Cell death**. *Int J Cell Biol*. 2014;2014:864062

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

D. Tang, R. Kang, T. V. Berghe, P. Vandenabeele and G. Kroemer, **The molecular machinery of regulated cell death**. *Cell Res*. 2019;29:347-364

A. R. Monteiro, R. Hill, G. J. Pilkington and P. A. Madureira, **The Role of Hypoxia in Glioblastoma Invasion**. *Cells*. 2017;6

Y. Liu, Y. Fang, X. Chen, Z. Wang, X. Liang, T. Zhang, et al., **Gasdermin E-mediated target cell pyroptosis by CAR T cells triggers cytokine release syndrome**. *Sci Immunol*. 2020;5

Z. Zhang, Y. Zhang, S. Xia, Q. Kong, S. Li, X. Liu, et al., **Gasdermin E suppresses tumour growth by activating anti-tumour immunity**. *Nature*. 2020;579:415-420

R. Ojha, M. Ishaq and S. K. Singh, **Caspase-mediated crosstalk between autophagy and apoptosis: Mutual adjustment or matter of dominance**. *J Cancer Res Ther*. 2015;11:514-24

I. Dikic and Z. Elazar, **Mechanism and medical implications of mammalian autophagy**. *Nat Rev Mol Cell Biol*. 2018;19:349-364

Y. Kondo, T. Kanzawa, R. Sawaya and S. Kondo, **The role of autophagy in cancer development and response to therapy**. *Nat Rev Cancer*. 2005;5:726-34

H. Okada and T. W. Mak, **Pathways of apoptotic and non-apoptotic death in tumour cells**. *Nat Rev Cancer*. 2004;4:592-603

S. Tavakol, M. Ashrafizadeh, S. Deng, M. Azarian, A. Abdoli, M. Motavaf, et al., **Autophagy Modulators: Mechanistic Aspects and Drug Delivery Systems**. *Biomolecules*. 2019;9

I. Tanida, T. Ueno and E. Kominami, **LC3 conjugation system in mammalian autophagy**. *Int J Biochem Cell Biol*. 2004;36:2503-18

Q. W. Fan, C. Cheng, C. Hackett, M. Feldman, B. T. Houseman, T. Nicolaides, et al., **Akt and autophagy cooperate to promote survival of drug-resistant glioma**. *Sci Signal*. 2010;3:ra81

C. Trejo-Solis, N. Serrano-Garcia, A. Escamilla-Ramirez, R. A. Castillo-Rodriguez, D. Jimenez-Farfan, G. Palencia, et al., **Autophagic and Apoptotic Pathways as Targets for Chemotherapy in Glioblastoma**. *Int J Mol Sci*. 2018;19

F. Boutouja, C. M. Stiehm and H. W. Platta, **mTOR: A Cellular Regulator Interface in Health and Disease**. *Cells*. 2019;8

M. Laplante and D. M. Sabatini, **Regulation of mTORC1 and its impact on gene expression at a glance**. *J Cell Sci*. 2013;126:1713-9

F. Giampieri, S. Afrin, T. Y. Forbes-Hernandez, M. Gasparrini, D. Cianciosi, P. Reboredo-Rodriguez, et al., **Autophagy in Human Health and Disease: Novel Therapeutic Opportunities**. *Antioxid Redox Signal*. 2019;30:577-634

J. Kim, M. Kundu, B. Viollet and K. L. Guan, **AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1**. *Nat Cell Biol*. 2011;13:132-41

Y. Zhao, J. Yang, W. Liao, X. Liu, H. Zhang, S. Wang, et al., **Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity**. *Nat Cell Biol*. 2010;12:665-75

C. H. Jung, C. B. Jun, S. H. Ro, Y. M. Kim, N. M. Otto, J. Cao, et al., **ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery**. *Mol Biol Cell*. 2009;20:1992-2003

Y. Cao and D. J. Klionsky, **Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein**. *Cell Res*. 2007;17:839-49

R. C. Russell, Y. Tian, H. Yuan, H. W. Park, Y. Y. Chang, J. Kim, et al., **ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase**. *Nat Cell Biol*. 2013;15:741-50

P. Wild, D. G. McEwan and I. Dikic, **The LC3 interactome at a glance**. *J Cell Sci*. 2014;127:3-9

S. Kimura, T. Noda and T. Yoshimori, **Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes**. *Cell Struct Funct*. 2008;33:109-22

D. Metcalf and A. M. Isaacs, **The role of ESCRT proteins in fusion events involving lysosomes, endosomes and autophagosomes**. *Biochem Soc Trans*. 2010;38:1469-73

H. C. Hasselbalch, **Chronic inflammation as a promotor of mutagenesis in essential thrombocythemia, polycythemia vera and myelofibrosis. A human inflammation model for cancer development?** *Leuk Res*. 2013;37:214-20

N. Kaza, L. Kohli and K. A. Roth, **Autophagy in brain tumors: a new target for therapeutic intervention**. *Brain Pathol*. 2012;22:89-98

S. Roy and J. Debnath, **Autophagy and tumorigenesis**. *Semin Immunopathol*. 2010;32:383-96

X. H. Liang, S. Jackson, M. Seaman, K. Brown, B. Kempkes, H. Hibshoosh, et al., **Induction of autophagy and inhibition of tumorigenesis by beclin 1**. *Nature*. 1999;402:672-6

X. Qu, J. Yu, G. Bhagat, N. Furuya, H. Hibshoosh, A. Troxel, et al., **Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene**. *J Clin Invest*. 2003;112:1809-20

C. Miracco, E. Cosci, G. Oliveri, P. Luzi, L. Pacenti, I. Monciatti, et al., **Protein and mRNA expression of autophagy gene Beclin 1 in human brain tumours**. *Int J Oncol*. 2007;30:429-36

H. Aoki, Y. Kondo, K. Aldape, A. Yamamoto, E. Iwado, T. Yokoyama, et al., **Monitoring autophagy in glioblastoma with antibody against isoform B of human microtubule-associated protein 1 light chain 3**. *Autophagy*. 2008;4:467-75

Z. Weihua, R. Tsan, W. C. Huang, Q. Wu, C. H. Chiu, I. J. Fidler, et al., **Survival of cancer cells is maintained by EGFR independent of its kinase activity**. *Cancer Cell*. 2008;13:385-93

S. Arico, A. Petiot, C. Bauvy, P. F. Dubbelhuis, A. J. Meijer, P. Codogno, et al., **The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway**. *J Biol Chem*. 2001;276:35243-6

R. Mathew, S. Kongara, B. Beaudoin, C. M. Karp, K. Bray, K. Degenhardt, et al., **Autophagy suppresses tumor progression by limiting chromosomal instability**. *Genes Dev*. 2007;21:1367-81

D. Crighton, S. Wilkinson, J. O'Prey, N. Syed, P. Smith, P. R. Harrison, et al., **DRAM, a p53-induced modulator of autophagy, is critical for apoptosis**. *Cell*. 2006;126:121-34

E. Tasdemir, M. C. Maiuri, L. Galluzzi, I. Vitale, M. Djavaheri-Mergny, M. D'Amelio, et al., **Regulation of autophagy by cytoplasmic p53**. *Nat Cell Biol*. 2008;10:676-87

K. Degenhardt, R. Mathew, B. Beaudoin, K. Bray, D. Anderson, G. Chen, et al., **Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis**. *Cancer Cell*. 2006;10:51-64

A. R. Young, M. Narita, M. Ferreira, K. Kirschner, M. Sadaie, J. F. Darot, et al., **Autophagy mediates the mitotic senescence transition**. *Genes Dev*. 2009;23:798-803

C. Fung, R. Lock, S. Gao, E. Salas and J. Debnath, **Induction of autophagy during extracellular matrix detachment promotes cell survival**. *Mol Biol Cell*. 2008;19:797-806

U. E. Martinez-Outschoorn, D. Whitaker-Menezes, S. Pavlides, B. Chiavarina, G. Bonuccelli, T. Casey, et al., **The autophagic tumor stroma model of cancer or "battery-operated tumor growth": A simple solution to the autophagy paradox**. *Cell Cycle*. 2010;9:4297-306

R. Lock, S. Roy, C. M. Kenific, J. S. Su, E. Salas, S. M. Ronen, et al., **Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation**. *Mol Biol Cell*. 2011;22:165-78

R. Ray, G. Chen, C. Vande Velde, J. Cizeau, J. H. Park, J. C. Reed, et al., **BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites**. *J Biol Chem*. 2000;275:1439-48

G. Bellot, R. Garcia-Medina, P. Gounon, J. Chiche, D. Roux, J. Pouyssegur, et al., **Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains**. *Mol Cell Biol*. 2009;29:2570-81

E. Pawlowska, J. Szczepanska, M. Szatkowska and J. Blasiak, **An Interplay between Senescence, Apoptosis and Autophagy in Glioblastoma Multiforme-Role in Pathogenesis and Therapeutic Perspective**. *Int J Mol Sci*. 2018;19

R. Mathew, V. Karantza-Wadsworth and E. White, **Role of autophagy in cancer**. *Nat Rev Cancer*. 2007;7:961-7

X. Huang, H. M. Bai, L. Chen, B. Li and Y. C. Lu, **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*. 2010;17:1515-9

S. Tamrakar, M. Yashiro, T. Kawashima, T. Uda, Y. Terakawa, Y. Kuwae, et al., **Clinicopathological Significance of Autophagy-related Proteins and its Association With Genetic Alterations in Gliomas**. *Anticancer Res*. 2019;39:1233-1242

S. Shukla, I. R. Patric, V. Patil, S. D. Shwetha, A. S. Hegde, B. A. Chandramouli, et al., **Methylation silencing of ULK2, an autophagy gene, is essential for astrocyte transformation and tumor growth**. *J Biol Chem*. 2014;289:22306-18

C. Kang and S. J. Elledge, **How autophagy both activates and inhibits cellular senescence**. *Autophagy*. 2016;12:898-9

J. S. Lee, E. Oh, J. Y. Yoo, K. S. Choi, M. J. Yoon and C. O. Yun, **Adenovirus expressing dual c-Met-specific shRNA exhibits potent antitumor effect through autophagic cell death accompanied by senescence-like phenotypes in glioblastoma cells**. *Oncotarget*. 2015;6:4051-65

A. V. Knizhnik, W. P. Roos, T. Nikolova, S. Quiros, K. H. Tomaszowski, M. Christmann, et al., **Survival and death strategies in glioma cells: autophagy, senescence and apoptosis triggered by a single type of temozolomide-induced DNA damage**. *PLoS One*. 2013;8:e55665

M. Catalano, G. D'Alessandro, F. Lepore, M. Corazzari, S. Caldarola, C. Valacca, et al., **Autophagy induction impairs migration and invasion by reversing EMT in glioblastoma cells**. *Mol Oncol*. 2015;9:1612-25

S. Palumbo, P. Tini, M. Toscano, G. Allavena, F. Angeletti, F. Manai, et al., **Combined EGFR and autophagy modulation impairs cell migration and enhances radiosensitivity in human glioblastoma cells**. *J Cell Physiol*. 2014;229:1863-73

C. Zhang, X. Zhang, R. Xu, B. Huang, A. J. Chen, C. Li, et al., **TGF-beta2 initiates autophagy via Smad and non-Smad pathway to promote glioma cells' invasion**. *J Exp Clin Cancer Res*. 2017;36:162

M. Strickland and E. A. Stoll, **Metabolic Reprogramming in Glioma**. *Front Cell Dev Biol*. 2017;5:43

Z. Guo, H. Guozhang, H. Wang, Z. Li and N. Liu, **Ampelopsin inhibits human glioma through inducing apoptosis and autophagy dependent on ROS generation and JNK pathway**. *Biomed Pharmacother*. 2019;116:108524

Y. L. Hu, M. DeLay, A. Jahangiri, A. M. Molinaro, S. D. Rose, W. S. Carbonell, et al., **Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma**. *Cancer Res*. 2012;72:1773-83

Y. Liu, Y. Fu, X. Hu, S. Chen, J. Miao, Y. Wang, et al., **Caveolin-1 knockdown increases the therapeutic sensitivity of lung cancer to cisplatin-induced apoptosis by repressing Parkin-related mitophagy and activating the ROCK1 pathway**. *J Cell Physiol*. 2020;235:1197-1208

E. E. Mowers, M. N. Sharifi and K. F. Macleod, **Autophagy in cancer metastasis**. *Oncogene*. 2017;36:1619-1630

S. P. C. Hsu, J. S. Kuo, H. C. Chiang, H. E. Wang, Y. S. Wang, C. C. Huang, et al., **Temozolomide, sirolimus and chloroquine is a new therapeutic combination that synergizes to disrupt lysosomal function and cholesterol homeostasis in GBM cells**. *Oncotarget*. 2018;9:6883-6896

X. Zhang, W. Li, C. Wang, X. Leng, S. Lian, J. Feng, et al., **Inhibition of autophagy enhances apoptosis induced by proteasome inhibitor bortezomib in human glioblastoma U87 and U251 cells**. *Mol Cell Biochem*. 2014;385:265-75

J. Shen, H. Zheng, J. Ruan, W. Fang, A. Li, G. Tian, et al., **Autophagy inhibition induces enhanced proapoptotic effects of ZD6474 in glioblastoma**. *Br J Cancer*. 2013;109:164-71

A. Giatromanolaki, E. Sivridis, A. Mitrakas, D. Kalamida, C. E. Zois, S. Haider, et al., **Autophagy and lysosomal related protein expression patterns in human glioblastoma**. *Cancer Biol Ther*. 2014;15:1468-78

X. Huang, Q. Qi, X. Hua, X. Li, W. Zhang, H. Sun, et al., **Beclin 1, an autophagy-related gene, augments apoptosis in U87 glioblastoma cells**. *Oncol Rep*. 2014;31:1761-7

D. J. Klionsky, K. Abdelmohsen, A. Abe, M. J. Abedin, H. Abeliovich, A. Acevedo Arozena, et al., **Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition)**. *Autophagy*. 2016;12:1-222

L. Jennewein, M. W. Ronellenfitsch, P. Antonietti, E. I. Ilina, J. Jung, D. Stadel, et al., **Diagnostic and clinical relevance of the autophago-lysosomal network in human gliomas**. *Oncotarget*. 2016;7:20016-32

S. Ghavami, S. Shojaei, B. Yeganeh, S. R. Ande, J. R. Jangamreddy, M. Mehrpour, et al., **Autophagy and apoptosis dysfunction in neurodegenerative disorders**. *Prog Neurobiol*. 2014;112:24-49

J. Bischof, M. A. Westhoff, J. E. Wagner, M. E. Halatsch, S. Trentmann, U. Knippschild, et al., **Cancer stem cells: The potential role of autophagy, proteolysis, and cathepsins in glioblastoma stem cells**. *Tumour Biol*. 2017;39:1010428317692227

Z. Min, Y. Ting, G. Mingtao, T. Xiaofei, Y. Dong, Z. Chenguang, et al., **Monitoring autophagic flux using p62/SQSTM1 based luciferase reporters in glioma cells**. *Exp Cell Res*. 2018;363:84-94

H. Wei, C. Wang, C. M. Croce and J. L. Guan, **p62/SQSTM1 synergizes with autophagy for tumor growth in vivo**. *Genes Dev*. 2014;28:1204-16

Y. Xu, C. Liao, R. Liu, J. Liu, Z. Chen, H. Zhao, et al., **IRGM promotes glioma M2 macrophage polarization through p62/TRAF6/NF-kappaB pathway mediated IL-8 production**. *Cell Biol Int*. 2019;43:125-135

R. Errafiy, C. Aguado, G. Ghislat, J. M. Esteve, A. Gil, M. Loutfi, et al., **PTEN increases autophagy and inhibits the ubiquitin-proteasome pathway in glioma cells independently of its lipid phosphatase activity**. *PLoS One*. 2013;8:e83318

S. Sarkar, **Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers**. *Biochem Soc Trans*. 2013;41:1103-30

S. V. Laddha, S. Ganesan, C. S. Chan and E. White, **Mutational landscape of the essential autophagy gene BECN1 in human cancers**. *Mol Cancer Res*. 2014;12:485-90

A. M. Choi, S. W. Ryter and B. Levine, **Autophagy in human health and disease**. *N Engl J Med*. 2013;368:651-62

Z. Zhang, H. Tian, Y. Miao, X. Feng, Y. Li, H. Wang, et al., **Upregulation of p72 Enhances Malignant Migration and Invasion of Glioma Cells by Repressing Beclin1 Expression**. *Biochemistry (Mosc)*. 2016;81:574-82

J. O. Pyo, M. H. Jang, Y. K. Kwon, H. J. Lee, J. I. Jun, H. N. Woo, et al., **Essential roles of Atg5 and FADD in autophagic cell death: dissection of autophagic cell death into vacuole formation and cell death**. *J Biol Chem*. 2005;280:20722-9

G. H. Jo, O. Bogler, Y. J. Chwae, H. Yoo, S. H. Lee, J. B. Park, et al., **Radiation-induced autophagy contributes to cell death and induces apoptosis partly in malignant glioma cells**. *Cancer Res Treat*. 2015;47:221-41

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

M. Russo, C. Spagnuolo, I. Tedesco and G. L. Russo, **Phytochemicals in cancer prevention and therapy: truth or dare?** *Toxins (Basel)*. 2010;2:517-51

M. A. Moosavi, A. Haghi, M. Rahmati, H. Taniguchi, A. Mocan, J. Echeverria, et al., **Phytochemicals as potent modulators of autophagy for cancer therapy**. *Cancer Lett*. 2018;424:46-69

S. Agarwal, P. Muniyandi, T. Maekawa and D. S. Kumar, **Vesicular systems employing natural substances as promising drug candidates for MMP inhibition in glioblastoma: A nanotechnological approach**. *Int J Pharm*. 2018;551:339-361

J. H. Ko, G. Sethi, J. Y. Um, M. K. Shanmugam, F. Arfuso, A. P. Kumar, et al., **The Role of Resveratrol in Cancer Therapy**. *Int J Mol Sci*. 2017;18

K. B. Harikumar, A. B. Kunnumakkara, G. Sethi, P. Diagaradjane, P. Anand, M. K. Pandey, et al., **Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine in vitro and in orthotopic mouse model of human pancreatic cancer**. *Int J Cancer*. 2010;127:257-68

D. Park, H. Jeong, M. N. Lee, A. Koh, O. Kwon, Y. R. Yang, et al., **Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition**. *Sci Rep*. 2016;6:21772

X. Kou and N. Chen, **Resveratrol as a Natural Autophagy Regulator for Prevention and Treatment of Alzheimer's Disease**. *Nutrients*. 2017;9

Y. Fan, J. F. Chiu, J. Liu, Y. Deng, C. Xu, J. Zhang, et al., **Resveratrol induces autophagy-dependent apoptosis in HL-60 cells**. *BMC Cancer*. 2018;18:581

A. W. Opipari, Jr., L. Tan, A. E. Boitano, D. R. Sorenson, A. Aurora and J. R. Liu, **Resveratrol-induced autophagocytosis in ovarian cancer cells**. *Cancer Res*. 2004;64:696-703

M. Yamamoto, S. O. Suzuki and M. Himeno, **Resveratrol-induced autophagy in human U373 glioma cells**. *Oncol Lett*. 2010;1:489-493

C. Andreadi, R. G. Britton, K. R. Patel and K. Brown, **Resveratrol-sulfates provide an intracellular reservoir for generation of parent resveratrol, which induces autophagy in cancer cells**. *Autophagy*. 2014;10:524-5

S. P. Garcia-Zepeda, E. Garcia-Villa, J. Diaz-Chavez, R. Hernandez-Pando and P. Gariglio, **Resveratrol induces cell death in cervical cancer cells through apoptosis and autophagy**. *Eur J Cancer Prev*. 2013;22:577-84

S. Selvaraj, Y. Sun, P. Sukumaran and B. B. Singh, **Resveratrol activates autophagic cell death in prostate cancer cells via downregulation of STIM1 and the mTOR pathway**. *Mol Carcinog*. 2016;55:818-31

A. Alayev, S. M. Berger, M. Y. Kramer, N. S. Schwartz and M. K. Holz, **The combination of rapamycin and resveratrol blocks autophagy and induces apoptosis in breast cancer cells**. *J Cell Biochem*. 2015;116:450-7

N. F. Trincheri, C. Follo, G. Nicotra, C. Peracchio, R. Castino and C. Isidoro, **Resveratrol-induced apoptosis depends on the lipid kinase activity of Vps34 and on the formation of autophagolysosomes**. *Carcinogenesis*. 2008;29:381-9

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

J. H. Back, Y. Zhu, A. Calabro, C. Queenan, A. S. Kim, J. Arbesman, et al., **Resveratrol-mediated downregulation of Rictor attenuates autophagic process and suppresses UV-induced skin carcinogenesis**. *Photochem Photobiol*. 2012;88:1165-72

C. J. Lin, C. C. Lee, Y. L. Shih, T. Y. Lin, S. H. Wang, Y. F. Lin, et al., **Resveratrol enhances the therapeutic effect of temozolomide against malignant glioma in vitro and in vivo by inhibiting autophagy**. *Free Radic Biol Med*. 2012;52:377-91

L. Wang, L. Long, W. Wang and Z. Liang, **Resveratrol, a potential radiation sensitizer for glioma stem cells both in vitro and in vivo**. *J Pharmacol Sci*. 2015;129:216-25

M. K. Shanmugam, G. Rane, M. M. Kanchi, F. Arfuso, A. Chinnathambi, M. E. Zayed, et al., **The multifaceted role of curcumin in cancer prevention and treatment**. *Molecules*. 2015;20:2728-69

B. R. Duvoix A, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich M., **Chemopreventive and therapeutic effects of curcumin.** *Cancer Lett.* 2005;223:181-90

A. Agbarya, N. Ruimi, R. Epelbaum, E. Ben-Arye and J. Mahajna, **Natural products as potential cancer therapy enhancers: A preclinical update**. *SAGE Open Med*. 2014;2:2050312114546924

A. Shakeri, A. F. G. Cicero, Y. Panahi, M. Mohajeri and A. Sahebkar, **Curcumin: A naturally occurring autophagy modulator**. *J Cell Physiol*. 2019;234:5643-5654

I. V. Ulasov, G. Lenz and M. S. Lesniak, **Autophagy in glioma cells: An identity crisis with a clinical perspective**. *Cancer Lett*. 2018;428:139-146

T. Y. Aoki H, Kondo S, Sawaya R, Aggarwal BB, Kondo Y., **Evidence that curcumin suppresses the growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways**. *Mol Pharmacol*. 2007;72:29-39

W. Zhuang, L. Long, B. Zheng, W. Ji, N. Yang, Q. Zhang, et al., **Curcumin promotes differentiation of glioma-initiating cells by inducing autophagy**. *Cancer Sci*. 2012;103:684-90

J. J. Xiao K, Guan C, Dong C, Wang G, Bai L, Sun J, Hu C, Bai C., **Curcumin induces autophagy via activating the AMPK signaling pathway in lung adenocarcinoma cells**. *J Pharmacol Sci*. 2013;123:102-9

G. Zhao, X. Han, S. Zheng, Z. Li, Y. Sha, J. Ni, et al., **Curcumin induces autophagy, inhibits proliferation and invasion by downregulating AKT/mTOR signaling pathway in human melanoma cells**. *Oncol Rep*. 2016;35:1065-74

A. Zanotto-Filho, E. Braganhol, K. Klafke, F. Figueiro, S. R. Terra, F. J. Paludo, et al., **Autophagy inhibition improves the efficacy of curcumin/temozolomide combination therapy in glioblastomas**. *Cancer Lett*. 2015;358:220-231

J. Zhang, J. Wang, J. Xu, Y. Lu, J. Jiang, L. Wang, et al., **Curcumin targets the TFEB-lysosome pathway for induction of autophagy**. *Oncotarget*. 2016;7:75659-75671

Y. Yamauchi, Y. Izumi, K. Asakura, Y. Hayashi and H. Nomori, **Curcumin induces autophagy in ACC-MESO-1 cells**. *Phytother Res*. 2012;26:1779-83

H. H. Ko, S. M. Yu, F. N. Ko, C. M. Teng and C. N. Lin, **Bioactive constituents of Morus australis and Broussonetia papyrifera**. *J Nat Prod*. 1997;60:1008-11

N. W. Cho SW, Choi M, Kang SJ, Lee SG, Choi CY, **Autophagy inhibits cell death induced by the anti-cancer drug morusin**. *Am J Cancer Res.* 2017;7:518-530

S. H. K. Suk Hee Kim, and Bo Sun Kang, **Therapeutic effects of dihydroartemisinin and transferrin against glioblastoma**. *Nutr Res Pract.* 2016;10:393-397

W. Hu, S. S. Chen, J. L. Zhang, X. E. Lou and H. J. Zhou, **Dihydroartemisinin induces autophagy by suppressing NF-kappaB activation**. *Cancer Lett*. 2014;343:239-48

X. X. Du, Y. J. Li, C. L. Wu, J. H. Zhou, Y. Han, H. Sui, et al., **Initiation of apoptosis, cell cycle arrest and autophagy of esophageal cancer cells by dihydroartemisinin**. *Biomed Pharmacother*. 2013;67:417-24

N. Berte, S. Lokan, M. Eich, E. Kim and B. Kaina, **Artesunate enhances the therapeutic response of glioma cells to temozolomide by inhibition of homologous recombination and senescence**. *Oncotarget*. 2016;7:67235-67250

Z. S. Zhang, J. Wang, Y. B. Shen, C. C. Guo, K. E. Sai, F. R. Chen, et al., **Dihydroartemisinin increases temozolomide efficacy in glioma cells by inducing autophagy**. *Oncol Lett*. 2015;10:379-383

Z. C. Wang, Y. Liu, H. Wang, Q. K. Han and C. Lu, **Research on the relationship between artesunate and Raji cell autophagy and apoptosis of Burkitt's lymphoma and its mechanism**. *Eur Rev Med Pharmacol Sci*. 2017;21:2238-2243

S. Attoub, O. Sperandio, H. Raza, K. Arafat, S. Al-Salam, M. A. Al Sultan, et al., **Thymoquinone as an anticancer agent: evidence from inhibition of cancer cells**

viability and invasion in vitro and tumor growth in vivo. *Fundam Clin Pharmacol*. 2013;27:557-69

R. L. Gurung, S. N. Lim, A. K. Khaw, J. F. Soon, K. Shenoy, S. Mohamed Ali, et al., **Thymoquinone induces telomere shortening, DNA damage and apoptosis in human glioblastoma cells**. *PLoS One*. 2010;5:e12124

M. C. Chen, N. H. Lee, H. H. Hsu, T. J. Ho, C. C. Tu, D. J. Hsieh, et al., **Thymoquinone induces caspase-independent, autophagic cell death in CPT-11-resistant lovo colon cancer via mitochondrial dysfunction and activation of JNK and p38**. *J Agric Food Chem*. 2015;63:1540-6

I. O. Racoma, W. H. Meisen, Q. E. Wang, B. Kaur and A. A. Wani, **Thymoquinone inhibits autophagy and induces cathepsin-mediated, caspase-independent cell death in glioblastoma cells**. *PLoS One*. 2013;8:e72882

M. Pazhouhi, R. Sariri, A. Rabzia and M. Khazaei, **Thymoquinone synergistically potentiates temozolomide cytotoxicity through the inhibition of autophagy in U87MG cell line**. *Iran J Basic Med Sci*. 2016;19:890-898

A. A. A. Hanan A. Bashmail, Abdulwahab Noorwali, Gehan A. Hegazy, Ghada AJabnoor, Hani Choudhry & Ahmed M. Al-Abd **Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities**. *Nature Scientific Reports*. 2018

Y. T. Lin, H. C. Wang, Y. C. Hsu, C. L. Cho, M. Y. Yang and C. Y. Chien, **Capsaicin Induces Autophagy and Apoptosis in Human Nasopharyngeal Carcinoma Cells by Downregulating the PI3K/AKT/mTOR Pathway**. *Int J Mol Sci*. 2017;18

V. Fattori, M. S. Hohmann, A. C. Rossaneis, F. A. Pinho-Ribeiro and W. A. Verri, **Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses**. *Molecules*. 2016;21

I. Diaz-Laviada and N. Rodriguez-Henche, **The potential antitumor effects of capsaicin**. *Prog Drug Res*. 2014;68:181-208

C. C. Wu, J. P. Lin, J. S. Yang, S. T. Chou, S. C. Chen, Y. T. Lin, et al., **Capsaicin induced cell cycle arrest and apoptosis in human esophagus epidermoid carcinoma CE 81T/VGH cells through the elevation of intracellular reactive oxygen species and Ca2+ productions and caspase-3 activation**. *Mutat Res*. 2006;601:71-82

X. Chen, M. Tan, Z. Xie, B. Feng, Z. Zhao, K. Yang, et al., **Inhibiting ROS-STAT3-dependent autophagy enhanced capsaicin-induced apoptosis in human hepatocellular carcinoma cells**. *Free Radic Res*. 2016;50:744-55

K. M. Yang, J. O. Pyo, G. Y. Kim, R. Yu, I. S. Han, S. A. Ju, et al., **Capsaicin induces apoptosis by generating reactive oxygen species and disrupting mitochondrial transmembrane potential in human colon cancer cell lines**. *Cell Mol Biol Lett*. 2009;14:497-510

Z. F. Hong, W. X. Zhao, Z. Y. Yin, C. R. Xie, Y. P. Xu, X. Q. Chi, et al., **Capsaicin Enhances the Drug Sensitivity of Cholangiocarcinoma through the Inhibition of Chemotherapeutic-Induced Autophagy**. *PLoS One*. 2015;10:e0121538

C. Amantini, M. B. Morelli, M. Nabissi, C. Cardinali, M. Santoni, A. Gismondi, et al., **Capsaicin triggers autophagic cell survival which drives epithelial mesenchymal transition and chemoresistance in bladder cancer cells in an Hedgehog-dependent manner**. *Oncotarget*. 2016;7:50180-50194

A. Ramos-Torres, A. Bort, C. Morell, N. Rodriguez-Henche and I. Diaz-Laviada, **The pepper's natural ingredient capsaicin induces autophagy blockage in prostate cancer cells**. *Oncotarget*. 2016;7:1569-83

Y. P. Liu, F. X. Dong, X. Chai, S. Zhu, B. L. Zhang and D. S. Gao, **Role of Autophagy in Capsaicin-Induced Apoptosis in U251 Glioma Cells**. *Cell Mol Neurobiol*. 2016;36:737-43

C. Yao, B. B. Liu, X. D. Qian, L. Q. Li, H. B. Cao, Q. S. Guo, et al., **Crocin induces autophagic apoptosis in hepatocellular carcinoma by inhibiting Akt/mTOR activity**. *Onco Targets Ther*. 2018;11:2017-2028

M. Moradzadeh, H. R. Sadeghnia, A. Tabarraei and A. Sahebkar, **Anti-tumor effects of crocetin and related molecular targets**. *J Cell Physiol*. 2018;233:2170-2182

R. Hoshyar and H. Mollaei, **A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin**. *J Pharm Pharmacol*. 2017;69:1419-1427

S. Patel, M. Sarwat and T. H. Khan, **Mechanism behind the anti-tumour potential of saffron (Crocus sativus L.): The molecular perspective**. *Crit Rev Oncol Hematol*. 2017;115:27-35

A. Zhang and J. Li, **Crocetin shifts autophagic cell survival to death of breast cancer cells in chemotherapy**. *Tumour Biol*. 2017;39:1010428317694536

D. Giakoumettis, C. Pourzitaki, T. Vavilis, A. Tsingotjidou, A. Kyriakoudi, M. Tsimidou, et al., **Crocus sativus L. Causes a Non Apoptotic Calpain Dependent Death in C6 Rat Glioma Cells, Exhibiting a Synergistic Effect with Temozolomide**. *Nutr Cancer*. 2019;71:491-507

K. Huang, Y. Chen, R. Zhang, Y. Wu, Y. Ma, X. Fang, et al., **Honokiol induces apoptosis and autophagy via the ROS/ERK1/2 signaling pathway in human osteosarcoma cells in vitro and in vivo**. *Cell Death Dis*. 2018;9:157

H. C. Ou, F. P. Chou, T. M. Lin, C. H. Yang and W. H. Sheu, **Protective effects of honokiol against oxidized LDL-induced cytotoxicity and adhesion molecule expression in endothelial cells**. *Chem Biol Interact*. 2006;161:1-13

K. Y. Ho, C. C. Tsai, C. P. Chen, J. S. Huang and C. C. Lin, **Antimicrobial activity of honokiol and magnolol isolated from Magnolia officinalis**. *Phytother Res*. 2001;15:139-41

S. K. Tsai, C. H. Huang, S. S. Huang, L. M. Hung and C. Y. Hong, **Antiarrhythmic effect of magnolol and honokiol during acute phase of coronary occlusion in anesthetized rats: influence of L-NAME and aspirin**. *Pharmacology*. 1999;59:227-33

K. Ishitsuka, T. Hideshima, M. Hamasaki, N. Raje, S. Kumar, H. Hideshima, et al., **Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis**. *Blood*. 2005;106:1794-800

I. Wolf, J. O'Kelly, N. Wakimoto, A. Nguyen, F. Amblard, B. Y. Karlan, et al., **Honokiol, a natural biphenyl, inhibits in vitro and in vivo growth of breast cancer through induction of apoptosis and cell cycle arrest**. *Int J Oncol*. 2007;30:1529-37

M. L. Sheu, S. H. Liu and K. H. Lan, **Honokiol induces calpain-mediated glucose-regulated protein-94 cleavage and apoptosis in human gastric cancer cells and reduces tumor growth**. *PLoS One*. 2007;2:e1096

K. Shigemura, J. L. Arbiser, S. Y. Sun, M. Zayzafoon, P. A. Johnstone, M. Fujisawa, et al., **Honokiol, a natural plant product, inhibits the bone metastatic growth of human prostate cancer cells**. *Cancer*. 2007;109:1279-89

P. S. Yeh, W. Wang, Y. A. Chang, C. J. Lin, J. J. Wang and R. M. Chen, **Honokiol induces autophagy of neuroblastoma cells through activating the PI3K/Akt/mTOR and endoplasmic reticular stress/ERK1/2 signaling pathways and suppressing cell migration**. *Cancer Lett*. 2016;370:66-77

C. C. Chio, K. Y. Chen, C. K. Chang, J. Y. Chuang, C. C. Liu, S. H. Liu, et al., **Improved effects of honokiol on temozolomide-induced autophagy and apoptosis of drug-sensitive and -tolerant glioma cells**. *BMC Cancer*. 2018;18:379

G. J. Wu, C. J. Lin, Y. W. Lin and R. M. Chen, **Data analyses of honokiol-induced autophagy of human glioma cells in vitro and in vivo**. *Data Brief*. 2016;9:667-672

Y. C. Cheng, D. Y. Hueng, H. Y. Huang, J. Y. Chen and Y. Chen, **Magnolol and honokiol exert a synergistic anti-tumor effect through autophagy and apoptosis in human glioblastomas**. *Oncotarget*. 2016;7:29116-30

C. H. Lu, S. H. Chen, Y. S. Chang, Y. W. Liu, J. Y. Wu, Y. P. Lim, et al., **Honokiol, a potential therapeutic agent, induces cell cycle arrest and program cell death in vitro and in vivo in human thyroid cancer cells**. *Pharmacol Res*. 2017;115:288-298

E. K. Rowinsky and R. C. Donehower, **Paclitaxel (taxol)**. *N Engl J Med*. 1995;332:1004-14

A. Notte, N. Ninane, T. Arnould and C. Michiels, **Hypoxia counteracts taxol-induced apoptosis in MDA-MB-231 breast cancer cells: role of autophagy and JNK activation**. *Cell Death Dis*. 2013;4:e638

Y. Lee, J. Na, M. S. Lee, E. Y. Cha, J. Y. Sul, J. B. Park, et al., **Combination of pristimerin and paclitaxel additively induces autophagy in human breast cancer cells via ERK1/2 regulation**. *Mol Med Rep*. 2018;18:4281-4288

Y. F. Yu, P. C. Hu, Y. Wang, X. L. Xu, G. M. Rushworth, Z. Zhang, et al., **Paclitaxel induces autophagy in gastric cancer BGC823 cells**. *Ultrastruct Pathol*. 2017;41:284-290

G. Xi, X. Hu, B. Wu, H. Jiang, C. Y. Young, Y. Pang, et al., **Autophagy inhibition promotes paclitaxel-induced apoptosis in cancer cells**. *Cancer Lett*. 2011;307:141-8

S. H. Zou, X. Du, H. Lin, P. C. Wang and M. Li, **Paclitaxel inhibits the progression of cervical cancer by inhibiting autophagy via lncRNARP11-381N20.2**. *Eur Rev Med Pharmacol Sci*. 2018;22:3010-3017

J. Lin, Y. Chen, L. Wei, A. Shen, T. J. Sferra, Z. Hong, et al., **Ursolic acid promotes colorectal cancer cell apoptosis and inhibits cell proliferation via modulation of multiple signaling pathways**. *Int J Oncol*. 2013;43:1235-43

R. Yin, T. Li, J. X. Tian, P. Xi and R. H. Liu, **Ursolic acid, a potential anticancer compound for breast cancer therapy**. *Crit Rev Food Sci Nutr*. 2018;58:568-574

Y. Meng, Z. M. Lin, N. Ge, D. L. Zhang, J. Huang and F. Kong, **Ursolic Acid Induces Apoptosis of Prostate Cancer Cells via the PI3K/Akt/mTOR Pathway**. *Am J Chin Med*. 2015;43:1471-86

C. C. Wu, C. H. Cheng, Y. H. Lee, I. L. Chang, H. Y. Chen, C. P. Hsieh, et al., **Ursolic Acid Triggers Apoptosis in Human Osteosarcoma Cells via Caspase Activation and the ERK1/2 MAPK Pathway**. *J Agric Food Chem*. 2016;64:4220-6

C. W. Lin, H. K. Chin, S. L. Lee, C. F. Chiu, J. G. Chung, Z. Y. Lin, et al., **Ursolic acid induces apoptosis and autophagy in oral cancer cells**. *Environ Toxicol*. 2019;34:983-991

S. Leng, Y. Hao, D. Du, S. Xie, L. Hong, H. Gu, et al., **Ursolic acid promotes cancer cell death by inducing Atg5-dependent autophagy**. *Int J Cancer*. 2013;133:2781-90

S. Shen, Y. Zhang, R. Zhang, X. Tu and X. Gong, **Ursolic acid induces autophagy in U87MG cells via ROS-dependent endoplasmic reticulum stress**. *Chem Biol Interact*. 2014;218:28-41

C. C. Wu, Y. F. Huang, C. P. Hsieh, P. J. Chueh and Y. L. Chen, **Combined Use of Zoledronic Acid Augments Ursolic Acid-Induced Apoptosis in Human Osteosarcoma Cells through Enhanced Oxidative Stress and Autophagy**. *Molecules*. 2016;21

J. Luo, Y. L. Hu and H. Wang, **Ursolic acid inhibits breast cancer growth by inhibiting proliferation, inducing autophagy and apoptosis, and suppressing inflammatory responses via the PI3K/AKT and NF-kappaB signaling pathways in vitro**. *Exp Ther Med*. 2017;14:3623-3631

J. Wojton, J. Elder and B. Kaur, **How efficient are autophagy inhibitors as treatment for glioblastoma?** *CNS Oncol*. 2014;3:5-7

G. Kroemer and B. Levine, **Autophagic cell death: the story of a misnomer**. *Nat Rev Mol Cell Biol*. 2008;9:1004-10

F. Aqil, R. Munagala, J. Jeyabalan and M. V. Vadhanam, **Bioavailability of phytochemicals and its enhancement by drug delivery systems**. *Cancer Lett*. 2013;334:133-41

A. Shakeri and A. Sahebkar, **Opinion Paper: Nanotechnology: A Successful Approach to Improve Oral Bioavailability of Phytochemicals**. *Recent Pat Drug Deliv Formul*. 2016;10:4-6

R. Singh and J. W. Lillard, Jr., **Nanoparticle-based targeted drug delivery**. *Exp Mol Pathol*. 2009;86:215-23

H. Xin, X. Jiang, J. Gu, X. Sha, L. Chen, K. Law, et al., **Angiopep-conjugated poly(ethylene glycol)-co-poly(epsilon-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma**. *Biomaterials.* 2011;32:4293-305

X. Ling, J. Tu, J. Wang, A. Shajii, N. Kong, C. Feng, et al., **Glutathione-Responsive Prodrug Nanoparticles for Effective Drug Delivery and Cancer Therapy**. *ACS Nano*. 2019;13:357-370

T. Gunasekaran, T. Haile, T. Nigusse and M. D. Dhanaraju, **Nanotechnology: an effective tool for enhancing bioavailability and bioactivity of phytomedicine**. *Asian Pac J Trop Biomed*. 2014;4:S1-7

C. M. Lee, S. T. Huang, S. H. Huang, H. W. Lin, H. P. Tsai, J. Y. Wu, et al., **C60 fullerene-pentoxifylline dyad nanoparticles enhance autophagy to avoid cytotoxic effects caused by the beta-amyloid peptide**. *Nanomedicine*. 2011;7:107-14

P. Wei, L. Zhang, Y. Lu, N. Man and L. Wen, **C60(Nd) nanoparticles enhance chemotherapeutic susceptibility of cancer cells by modulation of autophagy**. *Nanotechnology*. 2010;21:495101

G. Y. Chen, C. L. Chen, H. Y. Tuan, P. X. Yuan, K. C. Li, H. J. Yang, et al., **Graphene oxide triggers toll-like receptors/autophagy responses in vitro and inhibits tumor growth in vivo**. *Adv Healthc Mater*. 2014;3:1486-95

X. Ji, B. Xu, M. Yao, Z. Mao, Y. Zhang, G. Xu, et al., **Graphene oxide quantum dots disrupt autophagic flux by inhibiting lysosome activity in GC-2 and TM4 cell lines**. *Toxicology*. 2016;374:10-17

L. Wu, Y. Zhang, C. Zhang, X. Cui, S. Zhai, Y. Liu, et al., **Tuning cell autophagy by diversifying carbon nanotube surface chemistry**. *ACS Nano*. 2014;8:2087-99

X. Xue, L. R. Wang, Y. Sato, Y. Jiang, M. Berg, D. S. Yang, et al., **Single-walled carbon nanotubes alleviate autophagic/lysosomal defects in primary glia from a mouse model of Alzheimer's disease**. *Nano Lett*. 2014;14:5110-7

F. Pistollato, S. Bremer-Hoffmann, G. Basso, S. S. Cano, I. Elio, M. M. Vergara, et al., **Targeting Glioblastoma with the Use of Phytocompounds and Nanoparticles**. *Target Oncol*. 2016;11:1-16

V. M. Pulgar, **Transcytosis to Cross the Blood Brain Barrier, New Advancements and Challenges**. *Front Neurosci*. 2018;12:1019

A. Jhaveri, P. Deshpande, B. Pattni and V. Torchilin, **Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma**. *J Control Release*. 2018;277:89-101

N. Wang, J. He, C. Pan, J. Wang, M. Ma, X. Shi, et al., **Resveratrol Activates Autophagy via the AKT/mTOR Signaling Pathway to Improve Cognitive Dysfunction in Rats With Chronic Cerebral Hypoperfusion**. *Front Neurosci*. 2019;13:859

W. Guo, A. Li, Z. Jia, Y. Yuan, H. Dai and H. Li, **Transferrin modified PEG-PLA-resveratrol conjugates: in vitro and in vivo studies for glioma**. *Eur J Pharmacol*. 2013;718:41-7

S. Jose, S. S. Anju, T. A. Cinu, N. A. Aleykutty, S. Thomas and E. B. Souto, **In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery**. *Int J Pharm*. 2014;474:6-13

S. Mukherjee, J. N. E. Baidoo, S. Sampat, A. Mancuso, L. David, L. S. Cohen, et al., **Liposomal TriCurin, A Synergistic Combination of Curcumin, Epicatechin Gallate and Resveratrol, Repolarizes Tumor-Associated Microglia/Macrophages, and Eliminates Glioblastoma (GBM) and GBM Stem Cells**. *Molecules*. 2018;23

K. A. Orunoğlu M, Pehlivan SB, Şahin S, Söylemezoğlu F, Oğuz KK, Mut M, **Effects of curcumin-loaded PLGA nanoparticles on the RG2 rat glioma model**. *Mater Sci Eng C Mater Biol Appl.* 2017;78:32-38

E. J. Cristina G.Venturini, Catiuscia P.Oliveira, Andressa Bernardi, Ana M.O. Battastini, Silvia S.Guterres, Adriana R.Pohlmann, **Formulation of lipid core nanocapsules**. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2011;375:200-208

A. Zanotto-Filho, K. Coradini, E. Braganhol, R. Schroder, C. M. de Oliveira, A. Simoes-Pires, et al., **Curcumin-loaded lipid-core nanocapsules as a strategy to improve pharmacological efficacy of curcumin in glioma treatment**. *Eur J Pharm Biopharm*. 2013;83:156-67

Z. Y. Zhang H, Sun X, He X, Wang M, Wang Z, Wang, Zhu R, Wang S., **Curcumin-Loaded Layered Double Hydroxide Nanoparticles-Induced Autophagy for Reducing Glioma Cell Migration and Invasion**. *J Biomed Nanotechnol*. 2016;12:2051-62

M. S. M. Srishti Agarwal, Toru Mizuki, Toru Maekawa, D. Sakthi Kumar, **Chlorotoxin modified morusin–PLGA nanoparticles for targeted glioblastoma therapy**. *Journal of Materials Chemistry B*. 2019;7:5896-5919

J. Emami, H. Yousefian and H. Sadeghi, **Targeted Nanostructured Lipid Carrier for Brain Delivery of Artemisinin: Design, Preparation, Characterization, Optimization and Cell Toxicity**. *J Pharm Pharm Sci*. 2018;21:225s-241s

S. A. Shahein, A. M. Aboul-Enein, I. M. Higazy, F. Abou-Elella, W. Lojkowski, E. R. Ahmed, et al., **Targeted anticancer potential against glioma cells of thymoquinone delivered by mesoporous silica core-shell nanoformulations with pH-dependent release**. *Int J Nanomedicine*. 2019;14:5503-5526

S. T. Surekha R, **An Efficient Encapsulation of Thymoquinone Using Solid Lipid Nanoparticle for Brain Targeted Drug Delivery: Physicochemical Characterization, Pharmacokinetics and Bio-Distribution Studies**. *International Journal of Pharmaceutical and Clinical Research*. 2016;8:1616-1624

C. Amantini, M. Mosca, M. Nabissi, R. Lucciarini, S. Caprodossi, A. Arcella, et al., **Capsaicin-induced apoptosis of glioma cells is mediated by TRPV1 vanilloid receptor and requires p38 MAPK activation**. *J Neurochem*. 2007;102:977-90

Y. G. Gil and M. K. Kang, **Capsaicin induces apoptosis and terminal differentiation in human glioma A172 cells**. *Life Sci*. 2008;82:997-1003

Z. Jiang, X. Wang, Y. Zhang, P. Zhao, Z. Luo and J. Li, **Effect of Capsaicin-Loading Nanoparticles on Gliomas**. *J Nanosci Nanotechnol*. 2015;15:9834-9

S. Liu, S. M. Zhang, R. J. Ju, Y. Xiao, X. Wang, X. L. Song, et al., **Antitumor efficacy of Lf modified daunorubicin plus honokiol liposomes in treatment of brain glioma**. *Eur J Pharm Sci*. 2017;106:185-197

X. Gao, T. Yu, G. Xu, G. Guo, X. Liu, X. Hu, et al., **Enhancing the anti-glioma therapy of doxorubicin by honokiol with biodegradable self-assembling micelles through multiple evaluations**. *Sci Rep*. 2017;7:43501

X. Wang, L. Yang, H. Zhang, B. Tian, R. Li, X. Hou, et al., **Fluorescent magnetic PEI-PLGA nanoparticles loaded with paclitaxel for concurrent cell imaging, enhanced apoptosis and autophagy in human brain cancer**. *Colloids Surf B Biointerfaces*. 2018;172:708-717

L. Ding, Q. Wang, M. Shen, Y. Sun, X. Zhang, C. Huang, et al., **Thermoresponsive nanocomposite gel for local drug delivery to suppress the growth of glioma by inducing autophagy**. *Autophagy*. 2017;13:1176-1190

H. Xin, X. Sha, X. Jiang, W. Zhang, L. Chen and X. Fang, **Anti-glioblastoma efficacy and safety of paclitaxel-loading Angiopep-conjugated dual targeting PEG-PCL nanoparticles**. *Biomaterials*. 2012;33:8167-76

S. H. Mousavi, S. A. Moallem, S. Mehri, S. Shahsavand, H. Nassirli and B. Malaekeh-Nikouei, **Improvement of cytotoxic and apoptogenic properties of crocin in cancer cell lines by its nanoliposomal form**. *Pharm Biol*. 2011;49:1039-45

M. Rastgoo, H. Hosseinzadeh, H. Alavizadeh, A. Abbasi, Z. Ayati and M. R. Jaafari, **Antitumor activity of PEGylated nanoliposomes containing crocin in mice bearing C26 colon carcinoma**. *Planta Med*. 2013;79:447-51

X. Ying, Y. Wang, H. Xu, X. Li, H. Yan, H. Tang, et al., **The construction of the multifunctional targeting ursolic acids liposomes and its apoptosis effects to C6 glioma stem cells**. *Oncotarget*. 2017;8:64129-64142

J. Hu, J. Wang, G. Wang, Z. Yao and X. Dang, **Pharmacokinetics and antitumor efficacy of DSPE-PEG2000 polymeric liposomes loaded with quercetin and temozolomide: Analysis of their effectiveness in enhancing the chemosensitization of drug-resistant glioma cells**. *Int J Mol Med*. 2016;37:690-702

Y. Wang, X. Ying, H. Xu, H. Yan, X. Li and H. Tang, **The functional curcumin liposomes induce apoptosis in C6 glioblastoma cells and C6 glioblastoma stem cells in vitro and in animals**. *Int J Nanomedicine*. 2017;12:1369-1384

J. Shao, D. Zheng, Z. Jiang, H. Xu, Y. Hu, X. Li, et al., **Curcumin delivery by methoxy polyethylene glycol-poly(caprolactone) nanoparticles inhibits the growth of C6 glioma cells**. *Acta Biochim Biophys Sin (Shanghai)*. 2011;43:267-74

F. Dilnawaz and S. K. Sahoo, **Enhanced accumulation of curcumin and temozolomide loaded magnetic nanoparticles executes profound cytotoxic effect in glioblastoma spheroid model**. *Eur J Pharm Biopharm*. 2013;85:452-62

Y. Cui, M. Zhang, F. Zeng, H. Jin, Q. Xu and Y. Huang, **Dual-Targeting Magnetic PLGA Nanoparticles for Codelivery of Paclitaxel and Curcumin for Brain Tumor Therapy**. *ACS Appl Mater Interfaces*. 2016;8:32159-32169

X. D. Yang Xiang, Longbao Feng, Shuqi Jiang, Li Deng, Jun Shen, Yijun Yang, Rui Guo, **tLyp-1-conjugated GSH-sensitive biodegradable micelles mediate enhanced pUNO1-hTRAILa/curcumin co-delivery to gliomas**. *Chemical Engineering Journal*. 2019;374:392-404

P. Maiti, J. Scott, D. Sengupta, A. Al-Gharaibeh and G. L. Dunbar, **Curcumin and Solid Lipid Curcumin Particles Induce Autophagy, but Inhibit Mitophagy and the PI3K-Akt/mTOR Pathway in Cultured Glioblastoma Cells**. *Int J Mol Sci*. 2019;20

C. Zeng, H. Li, Z. Fan, L. Zhong, Z. Guo, Y. Guo, et al., **Crocin-Elicited Autophagy Rescues Myocardial Ischemia/Reperfusion Injury via Paradoxical Mechanisms**. *Am J Chin Med*. 2016;44:515-30

G. Gu, X. Gao, Q. Hu, T. Kang, Z. Liu, M. Jiang, et al., **The influence of the penetrating peptide iRGD on the effect of paclitaxel-loaded MT1-AF7p-conjugated nanoparticles on glioma cells**. *Biomaterials*. 2013;34:5138-48

M. Asaduzzaman Khan, M. Tania, S. Fu and J. Fu, **Thymoquinone, as an anticancer molecule: from basic research to clinical investigation**. *Oncotarget*. 2017;8:51907-51919

F. A. Chowdhury, M. K. Hossain, A. G. M. Mostofa, M. M. Akbor and M. S. Bin Sayeed, **Therapeutic Potential of Thymoquinone in Glioblastoma Treatment:**

Targeting Major Gliomagenesis Signaling Pathways. *Biomed Res Int.* 2018;2018:4010629

J. Y. Kim, E. H. Kim, S. U. Kim, T. K. Kwon and K. S. Choi, **Capsaicin sensitizes malignant glioma cells to TRAIL-mediated apoptosis via DR5 upregulation and survivin downregulation**. *Carcinogenesis.* 2010;31:367-75

Z. Zhu, S. Du, F. Ding, S. Guo, G. Ying and Z. Yan, **Ursolic acid attenuates temozolomide resistance in glioblastoma cells by downregulating O(6)-methylguanine-DNA methyltransferase (MGMT) expression**. *Am J Transl Res.* 2016;8:3299-308

Table legend:

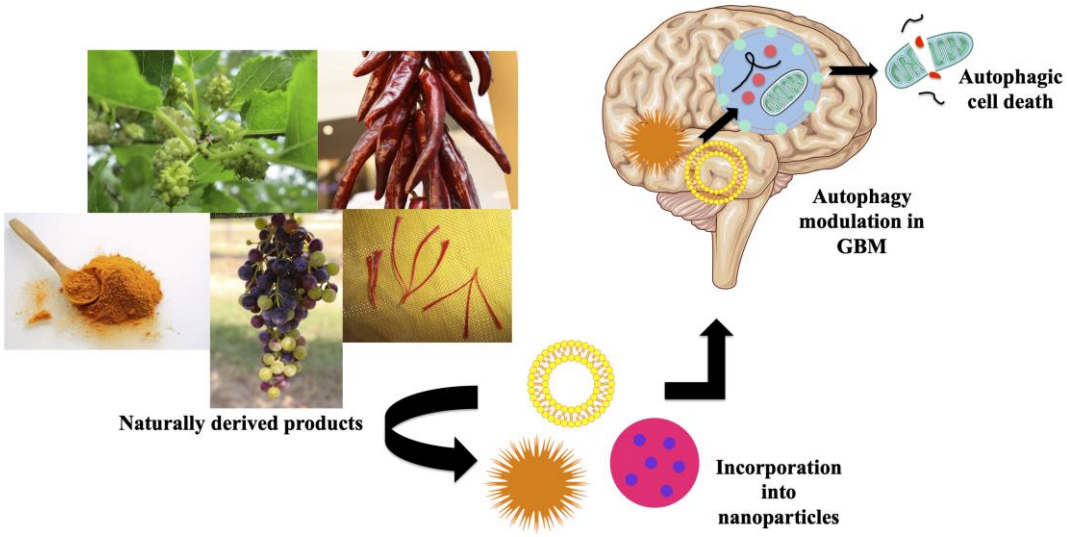
Table 1. Drug delivery of phytochemicals for GBM therapy. The molecular targets involved in autophagy whose expression is induced by phytochemicals are denoted by ↑ while the molecules whose expression levels are inhibited are depicted by ↓.The corresponding nanotechnologies are also highlighted in the table for delivery of natural substances for GBM therapy.

Figure Legends:

Figure 1. The autophagic process. Autophagy consists of 5 steps: initiation, nucleation, elongation, fusion and degradation each step assisted by a complex network of proteins.

Figure 2. Autophagic pathways and characteristic autophagy moderating natural compounds. Natural compounds control autophagy in tumor cells by inhibition or stimulation of major autophagy pathways. Natural compounds activating (denoted as green) and inhibiting (denoted as red) autophagy via contact with the main autophagy controllers have displayed their potential in the management of multiple cancer types.

Figure 3. Autophagic activity depicted by natural substances loaded NPs in GBM: A) Enhanced expression of autophagic proteins atg5, atg7, beclin-1, LC3A/B and p62 was observed in GBM cells after treatment with curcumin and curcumin loaded solid lipid nanoparticles in western blots and immunocytochemistry experiments. Reproduced with permission copyright © 2019 Maiti *et al.*[225] **B)** Morusin and Morusin loaded PLGA NPs were able to induce enhanced autophagosomes formation and autophagic flux in glioma cells compared to normal neuronal cells. Reproduced with permission from the royal society of chemistry copyright 2019 Agarwal *et al.*[204] **C)** Enhanced cytoplasmic LC3 signal, which is a trademark of autophagy was observed in brain glioblastoma U251 cells treated with paclitaxel loaded NPs. Reproduced with permission from Elsevier copyright 2018 Wang *et al.*[213].



Graphical abstract

Nano delivery of natural substances as prospective autophagy modulators in glioblastoma

Srishti Agarwal , Toru Maekawa

Bio-Nano Electronics Research Center, Graduate School of Interdisciplinary New Science, Toyo University, Kawagoe, Saitama, 350 - 8585, Japan,

Graphical abstract: Text

Autophagic modulation in glioblastoma using nano delivery of naturally derived products. Dietary food products are a vast source of phytochemicals, which possess potent cytotoxic and autophagic activity. Using nanotechnology, these phytochemicals can be delivered to the Glioma tumor site where they can induce autophagic cell death thereby curbing GBM.

Table 1:

Naturally Derived Substance	Molecular Target Involved in Autophagy	References	Nano-mediated delivery of natural substances for GBM therapy	References
Resveratrol	LC3-II, Beclin-1, Atg5, lysosomal permeability ↑ AKT/mTOR ,Bcl-2/Bcl-xL, Rictor ↓	54, 95-97, 100-106	Transferrin-targeted resveratrol loaded liposomes, transferrin modified PEG-PLA-resveratrol conjugates	195-198
Curcumin	ERK1/2, AMPK, LC3II, Beclin-1, Atg5 ↑ AKT/mTOR Bcl-2/Bcl-xL ↓	111, 112, 114 115-118	Liposomal TriCurin (TrLp), Curcumin loaded PLGA NPs, curcumin loaded lipid core nanocapsules, Curcumin loaded layered double hydroxide nanoparticles (Cur/LDH NPs) , solid lipid curcumin NPs	199-203
Morusin	AMPK ↑ mTOR ↓	122	Chlorotoxin targeted morusin loaded PLGA NPs	204
Artemisinin	Beclin-1, LC3-II, caspase-3 ↑ NF-κB, p62 ↓	124, 125, 128	Transferrin-conjugated nanostructured lipid carrier (TF-NLCs) of artemisinin (ART)	205
Thymoquinone	LC3-II, p62, JNK, p38 ↑	131-134	Mesoporous silica NPs	206, 207
Capsaicin	LC3-II, p62, p38 ↑ PI3K/AKT/ mTOR, Bcl-2/Bcl-xL ↓	135, 143	Capsaicin loaded Methoxy polyethylene glycol-poly(caprolactone) (mPEG-PCL) amphiphilic block copolymer	208-210

Honokiol	ROS/ERK/MAP, Atg7, PI3K/AKT/mTOR, LC3-II ↑	151, 159, 163	lactoferrin modified daunorubicin plus honokiol liposomes, honokiol and doxorubicin self-assembling micelles (MPEG-PCL)	211, 212
Paclitaxel	ERK1/2, Atg5, Beclin 1, LC3-II ↑	165-169	PLGA and magnetic NPs marked with polyethylenimine (PEI)-conjugated fluorescein isothiocyanate (FITC), polyethylene glycol-dipalmitoylphosphatidyle- thanoiamine (mPEG-DPPE) calcium phosphate nanoparticles (NPs) injectable thermoresponsive hydrogel, angiopep-conjugated PEG-PCL nanoparticles (ANG-PEG-NP) having paclitaxel	213-215
Crocetin/Crocin	Atg1 ↑ Beclin 1, AKT/mTOR ↓	145, 147, 149	Crocin nanoliposomes	216, 217
Ursolic Acid	LC3-II, p62, Atg5 ↑ AKT/mTOR/ NFκB /ERK/p38 ↓	174-177	Ursolic acid loaded liposomes	218

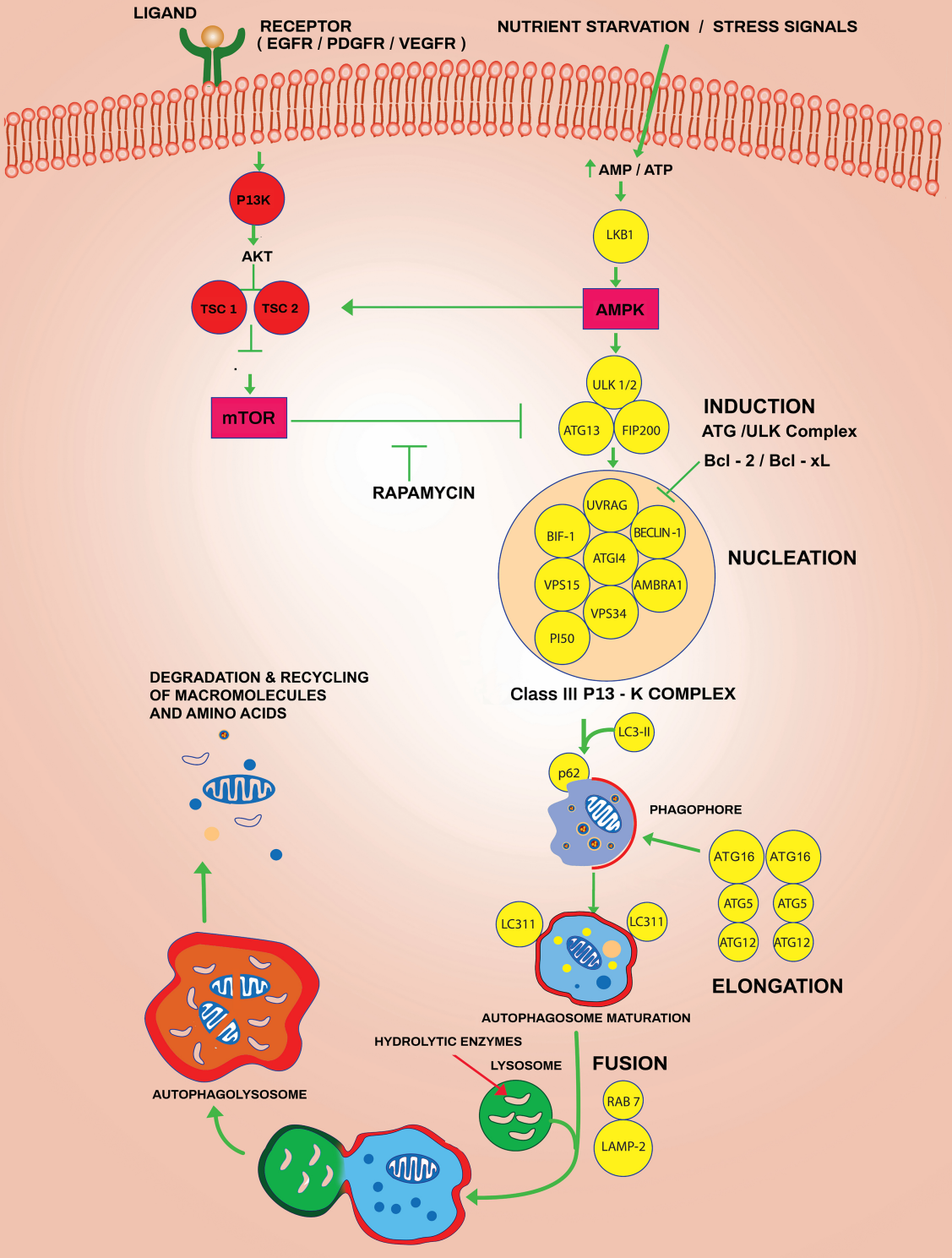

LIGAND
RECEPTOR
(EGFR / PDGFR / VEGFR)
NUTRIENT STARVATION / STRESS SIGNALS
AMP / ATP
P13K
AKT
TSC 1
TSC 2
LKB1
AMPK
mTOR
RAPAMYCIN
ULK 1/2
ATG13
FIP200
INDUCTION
ATG /ULK Complex
Bcl - 2 / Bcl - xL
UVRAG
BIF-1
BECLIN-1
ATGI4
VPS15
AMBRA1
VPS34
PI50
NUCLEATION
Class III P13 - K COMPLEX
DEGRADATION & RECYCLING
OF MACROMOLECULES
AND AMINO ACIDS
LC3-II
p62
PHAGOPHORE
ATG16
ATG16
ATG5
ATG5
ATG12
ATG12
LC311
LC311
ELONGATION
AUTOPHAGOSOME MATURATION
HYDROLYTIC ENZYMES
LYSOSOME
FUSION
RAB 7
LAMP-2
AUTOPHAGOLYSOSOME

Figure 1

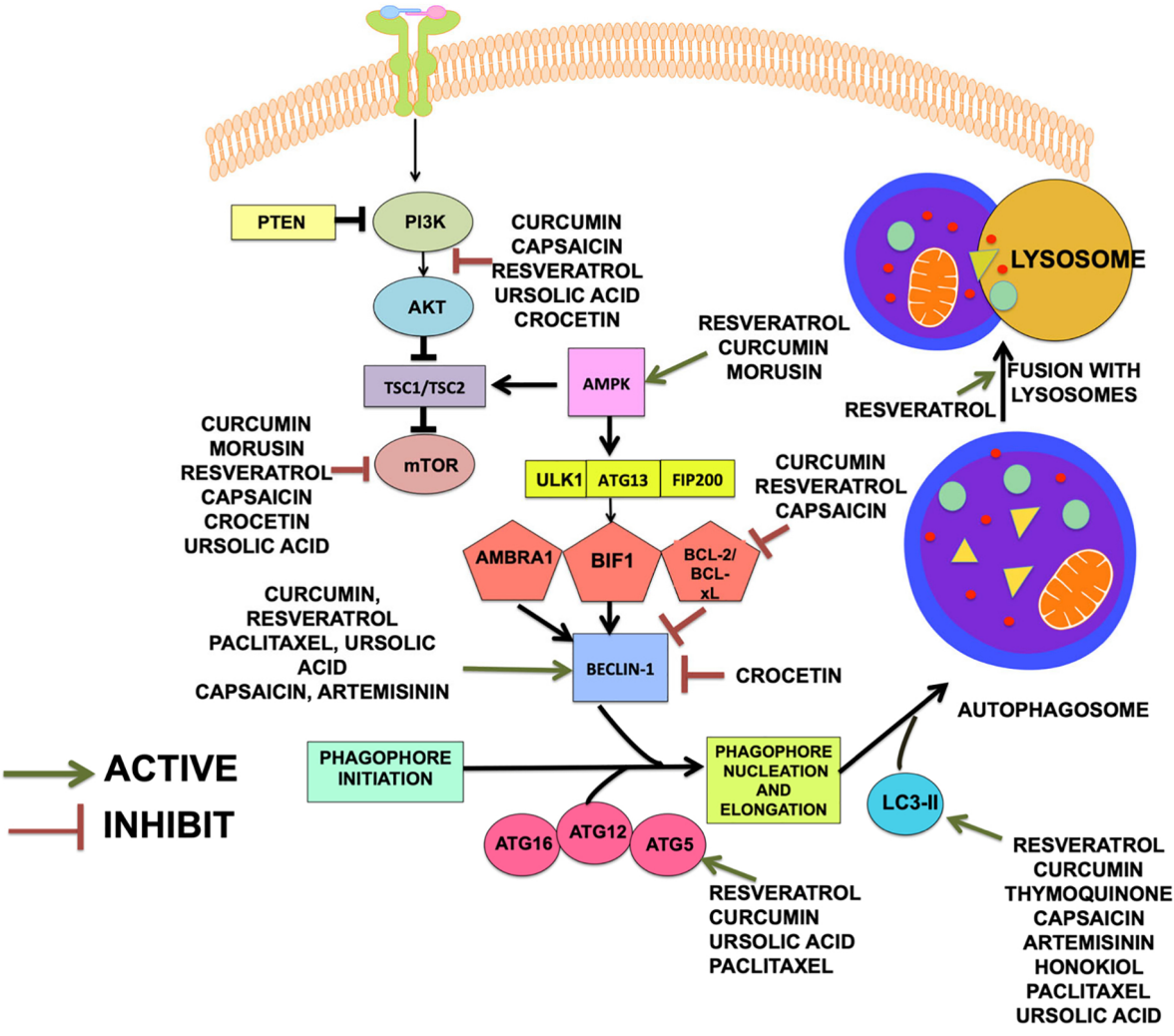

PTEN
PI3K
CURCUMIN
CAPSAICIN
RESVERATROL
URSOLIC ACID
CROCETIN
AKT
TSC1/TSC2
AMPK
RESVERATROL
CURCUMIN
MORUSIN
CURCUMIN
MORUSIN
RESVERATROL
CAPSAICIN
CROCETIN
URSOLIC ACID
mTOR
ULK1
ATG13
FIP200
CURCUMIN
RESVERATROL
CAPSAICIN
AMBRA1
BIF1
BCL-2/
BCL-
xL
CURCUMIN,
RESVERATROL
PACLITAXEL, URSOLIC
ACID
CAPSAICIN, ARTEMISININ
BECLIN-1
CROCETIN
LYSOSOME
FUSION WITH
LYSOSOMES
RESVERATROL
AUTOPHAGOSOME
ACTIVE
INHIBIT
PHAGOPHORE
INITIATION
PHAGOPHORE
NUCLEATION
AND
ELONGATION
LC3-II
ATG16
ATG12
ATG5
RESVERATROL
CURCUMIN
URSOLIC ACID
PACLITAXEL
RESVERATROL
CURCUMIN
THYMOQUINONE
CAPSAICIN
ARTEMISININ
HONOKIOL
PACLITAXEL
URSOLIC ACID

Figure 2

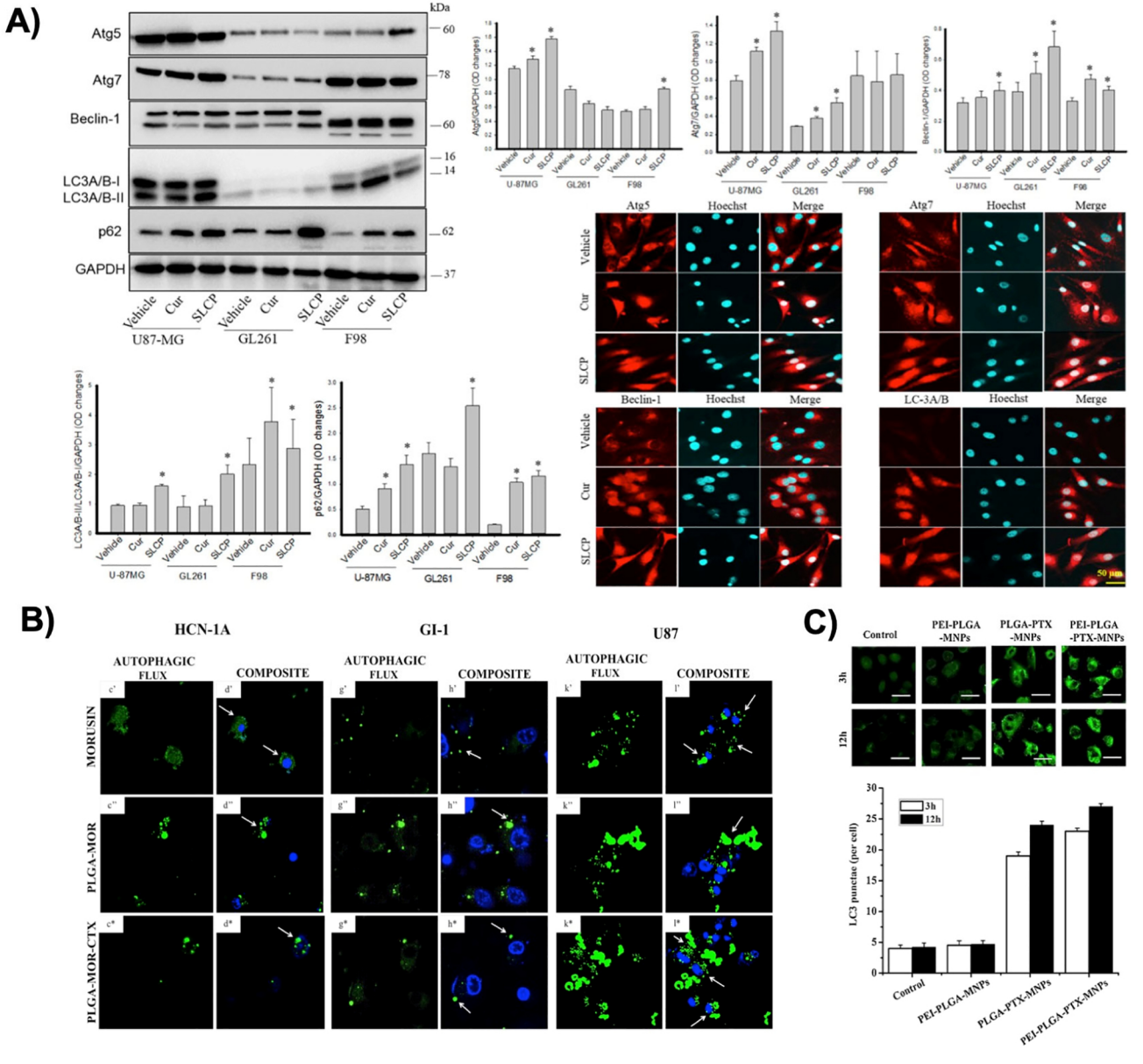

A)
Atg5
Atg7
Beclin-1
LC3A/B-I
LC3A/B-II
p62
GAPDH
kDa
60
78
60
16
14
62
37
Vehicle
Cur
SLCP
U87-MG
GL261
F98
Atg5/GAPDH (OD changes)
Atg7/GAPDH (OD changes)
Beclin-1/GAPDH (OD changes)
LC3AB-II/LC3AB-I/GAPDH (OD changes)
p62/GAPDH (OD changes)
Hoechst
Merge
LC-3A/B
50 μm
B)
HCN-1A
GI-1
U87
AUTOPHAGIC FLUX
COMPOSITE
MORUSIN
PLGA-MOR
PLGA-MOR-CTX
C)
Control
PEI-PLGA -MNPs
PLGA-PTX -MNPs
PEI-PLGA -PTX-MNPs
3h
12h
LC3 punctae (per cell)

Figure 3