The role of autophagy in cancer, and therapies for cancer in relation to autophagy

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Abstract
Autophagy, or self-digestion, is the process in which a cell degradates its cellular content, what normally occurs when cells are in metabolic stress. Autophagy serves as a recycling mechanism, and is also responsible for removal of damaged proteins and organelles. Recently, tumor suppressor genes and oncogenes are identified to play an important role in autophagy, what suggest that autophagy is involved in cancer. This makes autophagy a potential new target in anti-cancer therapy. Whether autophagy plays a negative or positive role in cancer is still controversial. It is thought that autophagy has a dual role in cancer. When tumor cells are in metabolic stress, autophagy is induced what helps tumor cells to survive. On the other hand, suppression of autophagy kills tumor cells in combination with chemotherapy or radiotherapy. When tumor cells are more sensitive, the dose of therapy can be reduced causing less side effects. Inducing of autophagy may be used in patients who have an increased risk of developing cancer. Inhibiting autophagy may be used in patients who have already developed a tumor. This report will focus on the pathways that link autophagy with cancer, and how targeting autophagy may improve cancer therapy. Future research must focus on understanding autophagy and modulation of autophagy completely, so new therapies for cancer and prevention of cancer can be developed.

Introduction
Death of cells is associated with three different processes: apoptosis, autophagy and necrosis[1]. The term autophagy means literally eating of self. There are 3 types of autophagy: chaperone-mediated autophagy, microautophagy, and macroautophagy. This report will focus on macroautophagy (referred to from now as autophagy). Autophagy is the catabolic process in which a cell digests its cytoplasmic contents. Autophagy recycles energy and nutrients when stress and starvation occur in cells[2]. During starvation, autophagy increases and degrades proteins and organelles. As a result of this, cells can obtain macromolecules, such as fatty acids, nucleotides and amino acids which would be unavailable when autophagy did not occur. These results suggest that autophagy has a protective role in cells, especially when cells are in nutrient deprivation[4]. Autophagy is regulated by genes called autophagy regulators (Atg). In research it was found that Atg plays an important role in the initiation and progression of cancer. Activation of autophagy in cancer occurs in response to stress, such as hypoxia, starvation and unfolded protein response (endoplasmatic reticulum (ER) stress)[2]. Autophagy plays a major role as a survival mechanism in the response to stress. Studies showed that autophagy has an important homeostasis role in the balance between synthesis, degradation and recycling of cellular components[8].
Normally, autophagy function is clearing protein aggregates and damaged organelles. When autophagy fails, due to multiple causes, multiple pathological conditions can occur like cancer and neurodegenerative diseases[3]. The role of autophagy in cancer leads to discussion. The protective role of autophagy as mentioned above means that when tumor cells are starving as a result of limited angiogenesis, autophagy helps the tumor cells to survive. In contrast, there is evidence that autophagy has an anticancer role. The autophagy gene beclin1 (Atg 6 in yeast) is monoallelically lost in human breast and ovarian tumors[2].The tumor suppressor genes p53 and PTEN, which are often mutated in tumors, induces autophagy[5,6], which suggests that autophagy has an anticancer role. The mechanism of how autophagy has an anticancer role is unclear, but recent studies suggest that autophagy limits tumor cell growth, reduces mutagenesis and other damages caused by reactive oxygen species (ROS)[7].
The above mentioned data suggest that autophagy can stimulate and prevent cancer, depending on the context. In this report, the regulation and mechanism autophagy will be discussed. Further, the role of autophagy in cancer will be analysed, and possible anti-cancer therapies in relation to affecting autophagy will be discussed.

Molecular mechanism of autophagy

Autophagy is a lysosomal pathway involved in the turn over of organelles and long living proteins[9]. Autophagy starts with a phagophore, which is probably derived from a lipide bilayer made by the endoplasmatic reticulum (ER) and the trans-golgi system and endosomes[10]. This phagophore expends to take up cell compartments, such as protein aggregates, ribosomes and organelles (Fig. 1a). In this phagophore the cell compartments are taken up in a double membrane autophagosome[11] (Fig. 1b). The autophagosome containing cell compartments matures which causes the autophagosome to fuse with lysosomes (Fig. 1c). This process promotes the degradation of autophagosomal contents by lysosomal acid proteases (Fig. 1d). Products of degradation, such as permeases and transporter export amino acids, are released back into the cytoplasm, where these products can be used for building macromolecules and for metabolism. This mechanism shows that autophagy recycles cellular compartments, which also creates ATP and has a protective function in cells by removing damaged cell compartments[11].

In yeast, the phagophore membrane formation is formed around the pre-autophagosomal structure (PAS), but no evidence is found that this happens also in mammels[12]. Probably the PAS in mammals is directly made in the ER in transaction with the trans-golgi and late endosomes[13]. For phagosome formation in yeast, activity of the Atg1 kinase in complex with the Atg13 and Atg17 is required, possible for regulating the protein Atg9 that may act by promoting lipid recruitment to the expanding phagophore [12]. This process is regulated by the energy sensing mammalian TOR (mTOR), which phosphorylates Atg13. This phosphorylation prevents that Atg13 interacts with Atg1, which leads to rendering initiation of autophagy[14]. ULK-1 and ULK-2 are mammalian homologues of the Atg1 in yeast. It is unknown which of these two is the human analogue promoting autophagy in mammals[15]. The step of autophagosome formation in mammals requires further investigation. Vesicular protein sorting 34 (Vsp34) and binding partner beclin-1 (Atg6) regulates phagophore formation in autophagy. in mammals. Vps34 is involved in membrane processing in the cell, but is only involved in autophagy when it is in complex with beclin-1[16]. The interaction of beclin-1 with Vsp34 increases level of PI3K, but how this is regulated when cells are in starvation is not clear yet.

Two ubiquitin like systems play a role in promoting autophagy: the Atg5-Atg12 conjugation and the microtubule associated protein light chain 3 (LC3) processing step. In the Atg5-Atg12 conjugation step, the Atg12 is activated by Atg7 and is transferred to Atg10. Conjugated Atg5-Atg12 complexes form pairs with Atg16L to form a complex that associated with the extending phagpophore[17]. When the autophagosome is formed, the complex dissociates from the membrane, which makes the conjugated Atg5-Atg12 a poor marker of autophagy. Genome wide association studies linked a mutation in Atg16L to Crohn's disease[18]. The other ubiquitin like system involved in the formation of the autophagosome is the processing of microtubule associated protein LC3B, which is the mammalian homologue of Atg8. LC3B is expressed as a cytosolic protein that is cleaved by Atg4 to generate LC3B-1. Activated LC3B-1 is transferred to Atg3 and then LC3B-2 is generated. Dependent on the Atg5-Atg12, recruitment and integration of LC3B-2 can occur. LC3B-2 is found on the external and internal surfaces of the autophagosome, and plays a role in hemifusion of membranes and selecting cell compartments for degradation[19]. During autophagy, the synthesis and processing of LC3 is increased, which makes it a good marker for autophagy levels. The role of LC3-related molecules is in not clear. It is thought that differences in protein interactions may determine which cell compartments are selected for uptake by the autophagosome[20].

Uptake of cell compartments by autophagosomes was thought as a random process, but evidence is growing that the membrane of phagophore can interact with protein aggregates and organelles. LC3B-2 may act as a receptor at the phagophore, which interacts with cell compartments that are targets for autophagy.

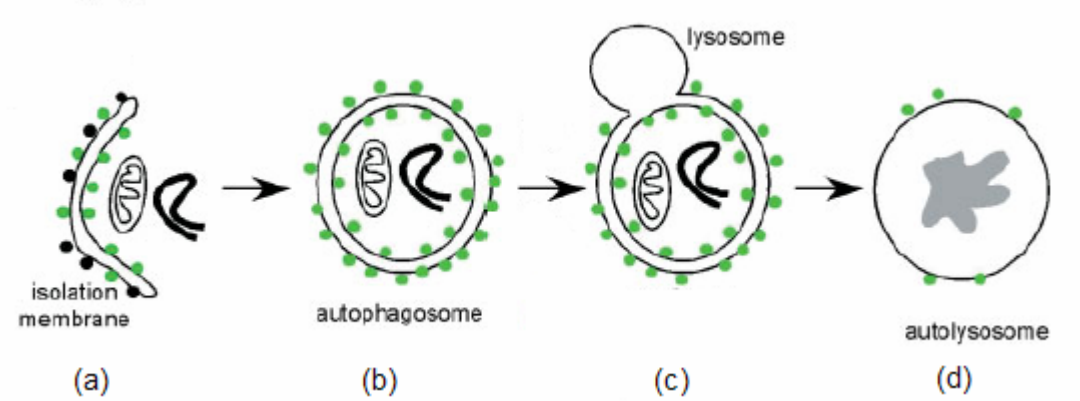

Figure 1. Mechanism of autophagy. (a) Capture of random or selective targets for degradation, (b) completion of the autophagosome, (c) fusion of the autophagosome with the lysosome (d) proteolic degradation by lysosomal proteases of engulfed molecules (taken from ref 40)

Major pathways and regulator molecules of autophagy

There are different pathways that leads to autophagy. The central player in these pathways is the mTOR kinase. The mTOR kinase plays a major role in sensing ATP and amino acids, and it can integrate hormonal stimuli with the PI3K/PKB pathway. The importants effector molecules of mTOR will be mentioned in this report.

In presence of amino acids, hVps34 is stimulated, which results in mTOR activation and autophagy inhibition[27]. The Ras/cAMP dependent protein kinase (PKA) pathway plays a major role in glucose sensing in mammals. When nutrients are available, small GTPases Ras1 and Ras2 are activated and this leads to elevation of cAMP generation. The cAMP binds to Bcy1, this inhibits PKA[28]. Activation of the Ras/PKA pathway suppresses autophagy. Amino acids are the final product of autophagy. Amino acids act as a negative feedback for autophagy. The amino acids TOR signalling pathway can be influenced by the AMP dependent protein kinase (AMPK). TSC is also a regulator in the autophagy pathway. TSC has a GTPase activating protein function, which hydrolyzes GTP, which regulates Rheb negatively thus inhibiting mTORC1 activity[54].

The mTOR complex 1 (mTORC1) is inhibited by rapamycin. When mTORC1 is inactivated, autophagy is stimulated in the presents of nutrients, which suggest that mTOR down regulates autophagy[25]. It is thought that mTORC1 is phosphorylated when nutrients are available[26], which inhibits autophagy. TOR signalling is influenced by different types of effector molecules (Fig. 2). Hormones may also play an important role in the regulation of autophagy. The hormones glucagon and ecdysone (in Drosophila) inhibit mTOR by downregulating PI3K, which results in an increase in autophagy. The hormone insulin has the opposite effect, and has an inhibitory effect on autophagy[29] The presence of ATP also inhibits autophagy[30]. If ATP is low in cells, the concentration of AMP will increase. The possibility exist that autophagy is controlled by the AMPK, which is sensitive to the cytosolic ATP/AMP ratio. AMPK is activated when there is an increase of the ratio AMP/ATP. Autophagy can be suppressed by AMP due to activation of AMPK. It is known that AMPK is involved in the mTOR signalling pathway[27].

The PI3K family enzyme, can trigger different pathways involved in differentiation, cell growth, apoptosis and cytoskeletal organization[60]. Two classes of the PI3K play a role in autophagy, notably PI3K 1 and 3. PI3K 1 has an inhibitory function on autophagy, and PI3K has an inducing function on autophagy. PI3K promotes cell growth, and constitutive activation is implicated in tumor development[61]. Ras and Akt are important regulators in this pathway that inhibits autophagy. Tumor suppressor genes like PTEN and TSC1 play a negative role in PI3K 1 signalling networks and stimulates autophagy. When the PI3K pathway is deregulated, this leads to tumorgenesis and resistance in therapy in prostate, pancreatic, ovarian and stomach cancers[62]. Class 3 PI3K has the opposite effect of class 1 PI3K, notably stimulating of autophagy[61].

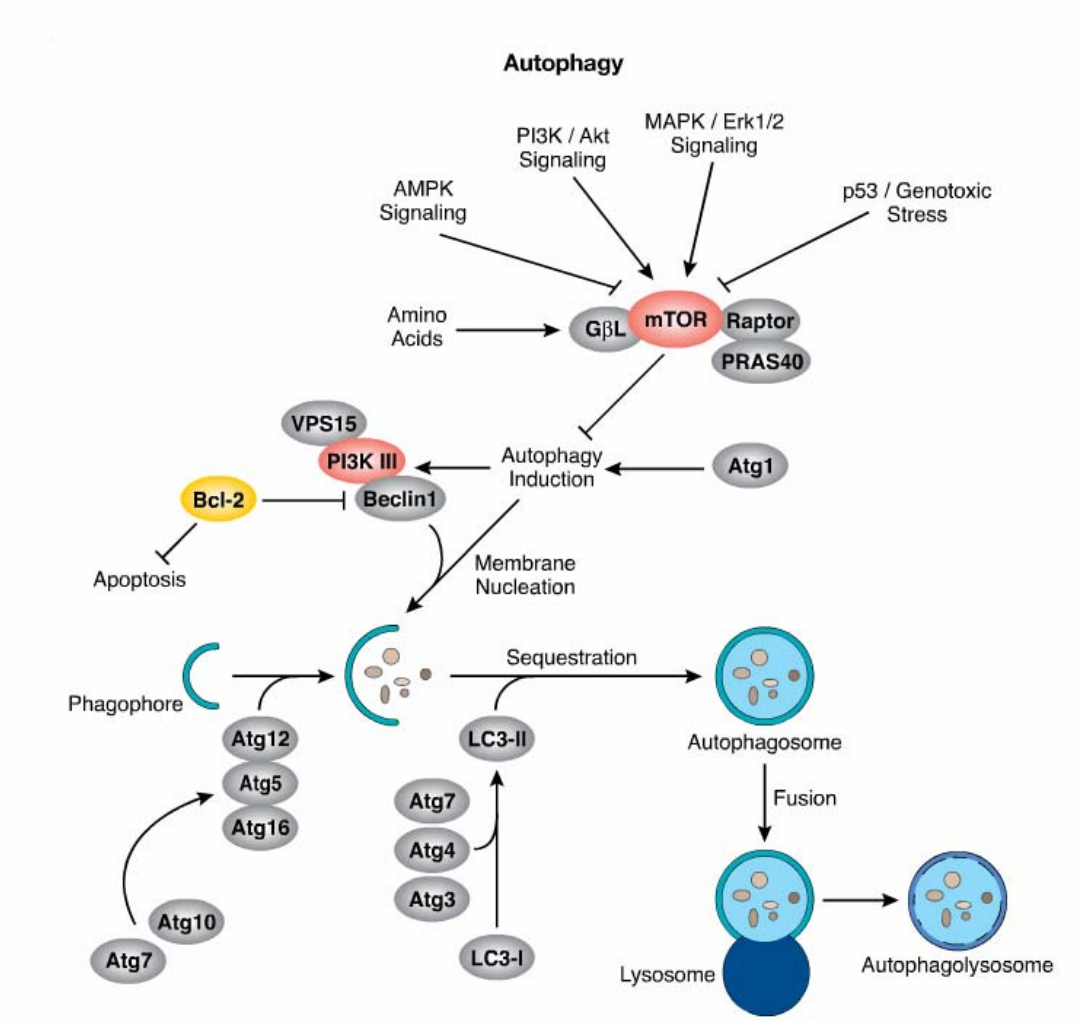

Figure 2. Pathways and molecules of autophagy. (taken from cellsignal.com)

Stress and autophagy

Various stressors can induce autophagy, what helps the cells to survive under stress circumstances. The endoplasmatic reticulum (ER) is an important compartment of the cell to synthesize proteins and initiates the pathway of proteins to organelles to the cell surface. The ER is in mammals a Ca2+ reservoir. ER stressors are over expression of aggregate prone proteins, glucose deprivation, Ca2+ efflux form the ER, oxidative stress and hypoxia. These types of stress lead to accumulation of unfolded proteins in the ER[31]. In mammals, knockdown of the UPR regulator inhibits autophagosome formation, but has no influence on LC3-2, which suggest that knockdown of the UPR regulator is essential for autophagy[33]. The UPR response is more complex in mammals then in yeast. In mammals, there are three pathways of UPR that leads to autophagy: the IRE1, PERK and the ATF6 pathway. These factors recognize misfolded proteins and activate target genes. If ER stress autophagy had a pro-survival role in cells, or causes cell death is not known yet[34].

Hypoxia is the condition in which low levels of oxygen are available for cells. Hypoxia exist in many pathological conditions, like tumors, cardiovascular ischemia and brain injuries. Recent data shows that autophagy is a result of hypoxia in mammal cells. However, insight in the relation between autophagy and hypoxia is in the beginning stage, but pathways for autophagy mediated by hypoxia seems to be different for types of cells[35]. The Hypoxia inducible factor 1 (HIF1) is transcribed under hypoxic conditions, and promotes transcription factors that encodes for erythropoieses and angiogenesis. In mouse embryonic fibroblasts, mitochondria are mitophaged, dependent of HIF1[36]. Autophagy caused by hypoxia is partially mTOR dependent, and ER caused by hypoxia might play a role in the autophagy induction.

Reactive oxygen species (ROS) are a common stress that leads to autophagy. The mitochondria are the major cell compartment in producing ROS, what will cause damage in cells. ROS producing molecules induce autophagic cell death in cancer cell lines. In healthy cells, ROS maintains at a stable level and cells can protect themselves for ROS, for example with superoxide dismutase (SOD). Over expression of SOD reduces autophagy[37]. Autophagy may also have a major role in eliminating pathogens when the body is invaded. Toll like receptors recognize LPS, ssRNA and zymoson that induce autophagy in the adaptive and the innate immune response, when bacteria and viruses are recognized[38,39].

Connection between autophagy and apoptosis

Recent studies showed that there is an interaction between autophagy and apoptosis. It might be that autophagy and apoptosis are interconnected , and regulated by the same triggers what results in different cellular outcomes. Cross talk of autophagy and apoptosis can happen in various ways. Autophagy might be essential by preceding and turning on apoptosis, and also might prevent or delay apoptosis[84]. There are also examples where apoptosis and autophagy might influence each other, where they act as back up for each other to cause irreversible cell death (Fig.3). Examples of cross talk are: Bcl2 protein is anti-apoptotic and anti-autophagic and Bcl-Xl protein is anti-apoptotic and is positively associated with autophagy.[84].

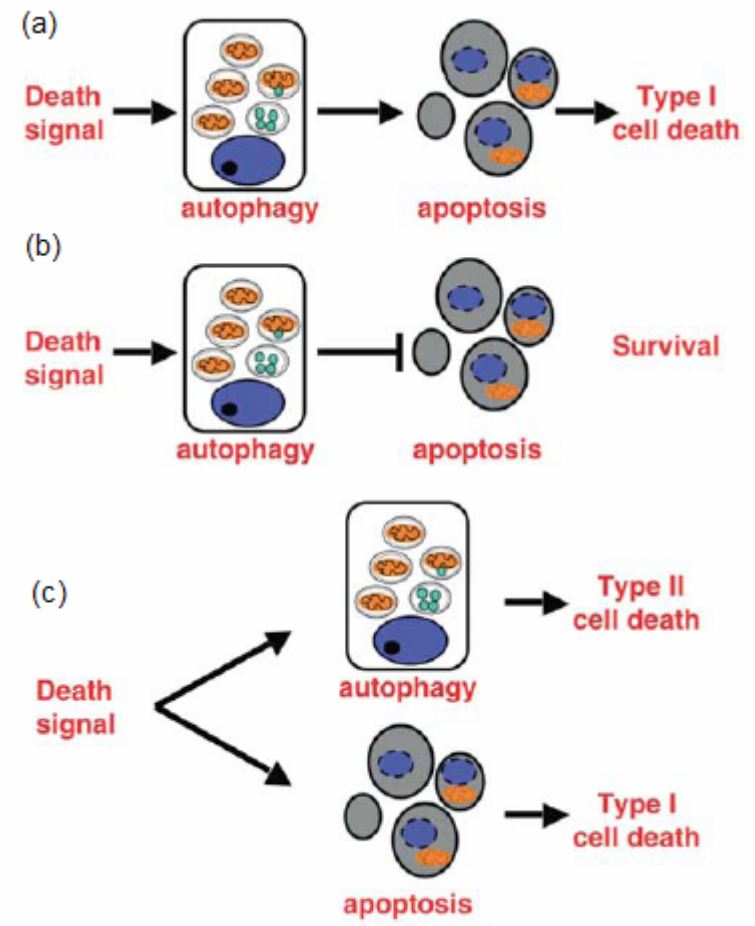

Figure 3. Apoptosis and autophagy connection in programmed cell death. (a) Autophagy may be essential for programmed cell death. (b) Autophagy may antagonize apoptosis. (c) Apoptosis and autophagy may occur independent of each other (taken from ref. 84)

Loss of Autophagy and Cancer

The role of autophagy in tumors was first discovered by a study of the beclin1 gene, which was monoallelically deleted in a high percentage in prostate, ovarian and breast cancers. Expression of beclin1 is reduced in breast carcinomas[43]. In mice lacking one copy of beclin1 allele, there was an high incidence of tumors, like B cell lymphoma and lung carcinomes[44]. This data suggest that beclin1 functions as a tumor suppressor, and that autophagy has a role in tumor suppression. Next to beclin1, mutations in other genes that play a role in autophagy, are associated with the incidence of different types of cancer.

In autophagy defective cells more DNA double strand breaks are seen when metabolic stress occurs. However, this study was done with an inactivated p53, a tumor suppressor gene which normally maintains genome stability[45]. Cells with a disrupted autophagy mechanism might enhance DNA damage that results in an increase of mutations in cells, which will lead to tumorigenesis. Autophagy-defective cells have defects in protein turnover, which leads to accumulation of scaffold protein p62, what is a major contributor to tumorgenesis[46].

A mechanism of how autophagy-defective cells will lead to tumor suppression is that autophagy defected cells causes necrosis during metabolic stress, while cells which can induce normal autophagy, are more resistant to cell death when metabolic stress occurs[47].

Ras is an oncogene that also regulates autophagy, and is found to be mutated in lung , pancreas and colon cancer[55]. Ras had a double effect on autophagy; Ras can activate the PI3K pathway, which inhibits autophagy. Ras can also induce autophagy, by activating the MAPK pathway[56]. The PI3K pathway inhibits autophagy but activates cancer growth, and the AMPK pathway promotes both autophagy and cancer growth. These data suggest that autophagy mediated by activation of AMPK protects cancer cells from death, and that inhibition of autophagy by the PI3K pathway inhibits growth, and induces death of cancer cells.

The most important regulator of autophagy, mTORC1 is deregulated in most human cancer[50]. Genes that regulates mTORC1 are mostly identified as oncogenes and tumor suppressor genes. Another example of a gene that regulates autophagy is Rheb, which is over expressed in prostate cancer. Loss of TSC causes high Rheb activity, which induces autophagy by activating mTOR. PI3K and Akt, are activators of mTORC1. In cancer, PI3K activity is increased, which should inhibit autophagy. In colon cancer the products of PI3K suppresses autophagy[51]. The regulator of the PI3K-Akt pathway is the tumor suppressor gene PTEN, and when PTEN is over expressed this leads to inducing autophagy[52]. PTEN is a tumor suppressor gene that regulates cell cycle, cell growth and cell survival. PTEN regulates autophagy by inhibiting the PI3K pathway. PTEN mutations, which leads to constitutive Akt activation, are associated with glioma, prostate and breast cancer[57,58]. Mice with mutations of PTEN develop all kinds of tumors[59.] Another tumor suppressor gene, ARHI, which function is lost in ovarian cancers, also regulates the PI3K pathway, which induces autophagy[53]. To summarize, loss of autophagy is associated with tumorgenesis and tumor growth (Fig 4).

Autophagy and tumor cell survival

Tumor cells obtain their necessary nutrients from the blood flow .To proliferate, tumor cells have a high demand for oxygen and nutrients. This is why tumors, which often are poorly vascularised, encounter hypoxia and metabolic stress. Tumor cells promote angiogenesis to increase the blood flow. Cells on the inside of the tumor have a higher incidence of autophagy than cells on the edge of the tumor. This protects the cells from apoptosis and necrosis[49]. Autophagy had an important role in providing tumors cells from nutrients in periods during metabolic stress when the apoptosis pathway is disrupted[41]. Due to autophagy, tumors can survive for weeks in vitro and in vivo in periods of metabolic stress[42]. Autophagy has a pro survival effect on tumors (Fig 4), and might also has an influence on metastasis, which is the leading cause of mortality in cancer patients. Autophagy restricts necrosis and inflammation, and this may limit invasion and dissemination of tumor cells from a primary site. This restricts metastasis at an early step. It is also thought that autophagy promotes metastasis at a later stage, by protecting stressed tumor cells when tumor cells want to colonize at other places. So, autophagy can have a pro-metastatic role and a anti-metastatic role in cancer, depending on the context[65].

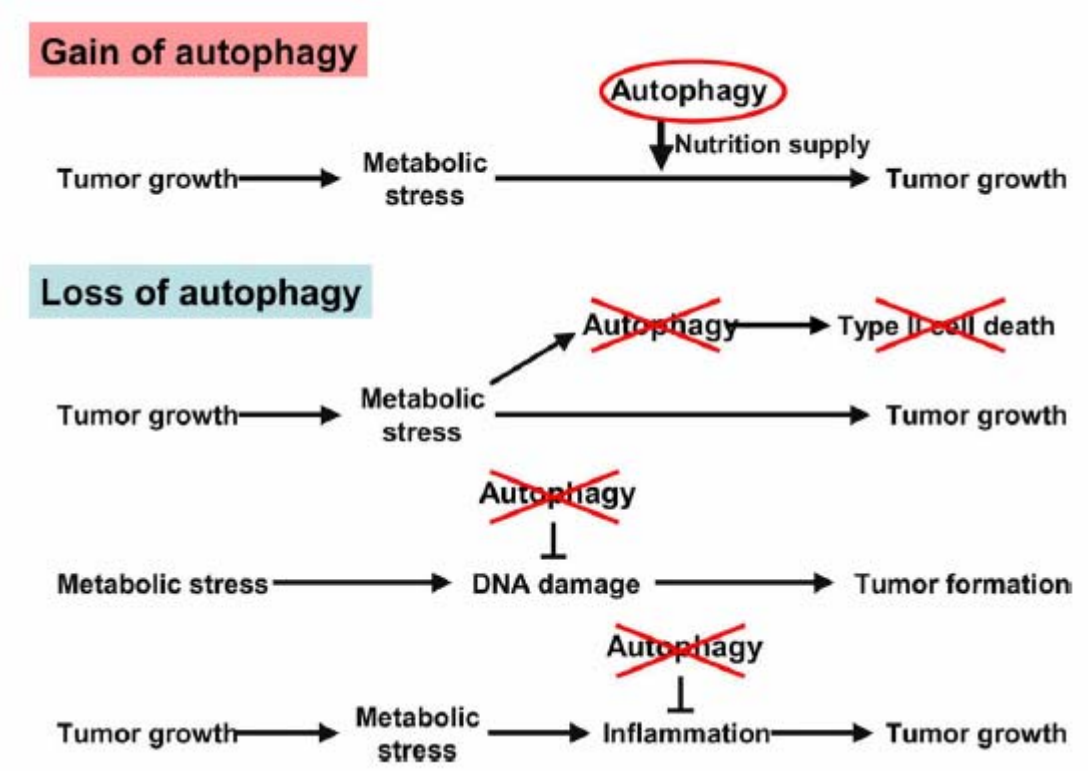

Figure 4. Gain of autophagy and loss of autophagy can lead to tumor growth and tumorgenesis. Activation of autophagy can help tumor cells to survive when metabolic stress occurs. Loss of autophagy can lead to tumor growth and tumor formation. (taken from ref. 92)

Autophagy as target for cancer therapy

As reported, autophagy play a major role in cancer, what autophagy an interesting target makes for therapy in the treatment of cancer. Increased autophagy is seen often in tumor cells in response to chemotherapies and radiotherapy. Recent studies showed that inhibition of autophagy sensitizes tumor cells for many cancer therapies. However, other studies showed that treatment of tumor cells need an intact autophagy mechanism, and autophagy protects cancer against chemotherapies. The goal of therapy is to kill cancer cells quickly and effectively. In many tumors, apoptosis mechanism is defected, which increases the resistance against chemotherapy and radiotherapy. Recent studies showed that chemotherapeutic drugs induces cell death, even when cells are resistant to apoptosis. Therapy of cancer in relation to autophagy may be achieved by several mechanisms, such as autophagy inhibition to promote chemotherapeutic efficacy, inducing ER stress and apoptosis inhibition to promote autophagy.?

Autophagy inducing to promote chemotherapeutic efficacy

Rapamycin is the main mTOR inhibitor and has significant chemotherapeutic antitumor activity[66]. Inhibition of the mTOR, in combination with radiation decreases tumor vascular density, and sensitive vascular endothelium in mouses[67]. Examples of mTOR inhibitors are CCI-779, RAD001, Temsirolimus and Everolimus. RAD001 increases radio sensitivity in breast cancer and in prostate cancer[69]. Inhibitors of mTOR are more effective in tumor cells then in normal cells, because tumor cells have increased activation of the mTOR pathway[68]. When the pro-apoptotic proteins Bak and Bax are absent, radio sensitization of lung and breast cancers increased. This was the results of increased autophagy, which was up regulated by RAD001[70]. Inhibiting mTOR to induce autophagy, kills tumor cells efficient in malignant gliomas[89].

Clinical trials with mTOR inhibitors only showed modest results till now. Only in the treatment of renal cell carcinoma positive results are found[96]. This might be explained by the negative feedback loop downstream of mTOR, and also that rapamycine is specific for mTORC1[95]. New treatments should focus on inhibiting mTORC1 and mTORC2, and on treatments that combines mTOR inhibitors with inhibitors of other pathways that inhibit autophagy, like the PI3K pathway. With more knowledge about mTOR, mTOR inhibition in combination with PI3K inhibitors may be a potential autophagy target to improve therapy for cancer (Fig 5a).
3 methykadenine (3MA) is an inhibitor of Vps34, a class 3 PI3K[51]. As reported earlier, Vsp34 is involved in membrane modelling events, like autophagosome isolation and membrane formation. 3MA had influence on autophagy by inhibiting autophagy at an early stage[85]. 3MA also regulates other cellular activities, like endocytosis, lysosomal biogenesis and intracellular trafficking. Another inhibitor of PI3K is PX-866, and PX-866 is used in clinical trials now. PX-866 is an irreversible PI3K inhibitor, and has as advantage that the enzymatic and cellular activities in in vitro and in vivo experiments are closely related[90]. PI3K inhibitors in combination with other chemotherapeutic agents or radiation may kill tumor cells, by facilitating apoptosis[91].
Imatinib is an inducer of autophagy, and is used in the treatment of chronic myelogenous leukemia (CML) and other tumors[86]. In rat C6 cells, the combination of imatinib with anaframil, induced apoptosis and autophagy, which resulted in growth inhibition and cell death[88]. When Kaposi's carcinoma cells were treated with imatinib, autophagy was induced and reduced survival of tumor cells[87] (Fig 5b). Recent clinical trials reveals that imatinib was effective in the treatment of glioblastomas, CML and multi-resistant Kaposi's sarcoma
cells.

Inducing ER stress
As reported, ER stress can also induce autophagy. Examples of agents that induce ER stress are bortezomib, selenium and fatty acid synthase inhibitor. Type of ER stressor, dose, stress duration, stress intensity and presence of apoptotic proteins, will influence if cells will undergo apoptosis or autophagy. To improve the efficacy of cancer therapy, ER stress inducers must be combined with chemotherapy and radiotherapy.
Bortezomib is a reversible and selective proteasome inhibitor, and is used in clinical trials for its antitumor effect (multiple myeloma). Bortezomib raises p53 level, which may leads to pro-apoptotic signallig. When proteasomes are inhibited, transformed cells will undergo apoptosis, while normal cell are not affected[71]. Bortezomib blocks proteasomal degradation and inhibits p-ERK activity, which results in ubiquitin-conjugated protein aggregation and non stop protein synthesis. It may be that bortezomib combined with other therapies, increases tumor cytotoxity. However, this combination is toxic for neural tissue.
Selenium is an element, which also induces ER stress and thereby causes apoptosis[73]. Selenium is being tested no for prostate cancer chemoprevention. One hour after selenium exposure, ER stress occurs by increasing p-ERK dependent phosphorylation. High doses of selenium are needed to cause apoptosis. H2SE is a common metabolite of inorganic and organic selenium compounds. H2SE is metabolized in selenium compounds which may have chemopreventive effects[73]. Selenium sensitizes tumor cells for other anticancer drugs, like paclitaxel, irinotecan, fluorouracil, doxorubicin and cisplatin, while normal cells are not affected[74]. Selenium might have a selective role in inducing ER stress what causes apoptosis. The combination of selenium with DNA damaging agents like radiation, to take advantage of both modalities as autophagy related ER stressors, is not yet studied[75].
Fatty acids synthase (FAS) is a enzyme which synthesis fatty acids in humans. FAS is over expressed in many carcinomas, like breast and prostate cancer. Over expression of FAS is associated with a poor prognosis for cancer patients[76]. FAS inhibitors, like cerulenin and C75, can induce selective apoptosis in human cancer cells[77], and are an effective agent for inducing apoptosis in p53 deficient cells, while normal cells are not affected.[78] FAS induced apoptosis was studies in ovarian cancer cells and showed an association between FAS and Akt expression. In tumor cells, FAS inhibitors induce ER stress, while in normal cells FAS inhibitors do not have any effect on ER stress[79]. FAS inhibitors, also in combination with other cancer therapies, are a potential way to improve cancer treatment.

Apoptosis inhibition to promote autophagy

Recent studies shows that a defective apoptosis pathway, often found in cancer, might lead to autophagy, which can be a new target of cancer therapy. By blocking apoptosis, latency and regeneration of tumor cells should be decreased.

Zinc is an element, which inhibits the apoptotic proteins Bak/Bax, what inhibits apoptosis. Deficiency of the Bak/Bax induces autophagy, which promotes radiation sensitivity[81]. Radiation induced apoptosis caused by Bak/Bax is believed to be responsible for only 20% of cell death[82]. Bak/Bax inhibition by zinc, might improve radiation induced cell death, mainly by induction of autophagy. In addition, zinc can have a negative effect on vascular endothelium after ionizing radiation. This results suggest that Bak/Bax inhibitors like zinc, which induces autophagy, may be used as an effective death pathway when cancer cells are treated with ionizing radiation[83].

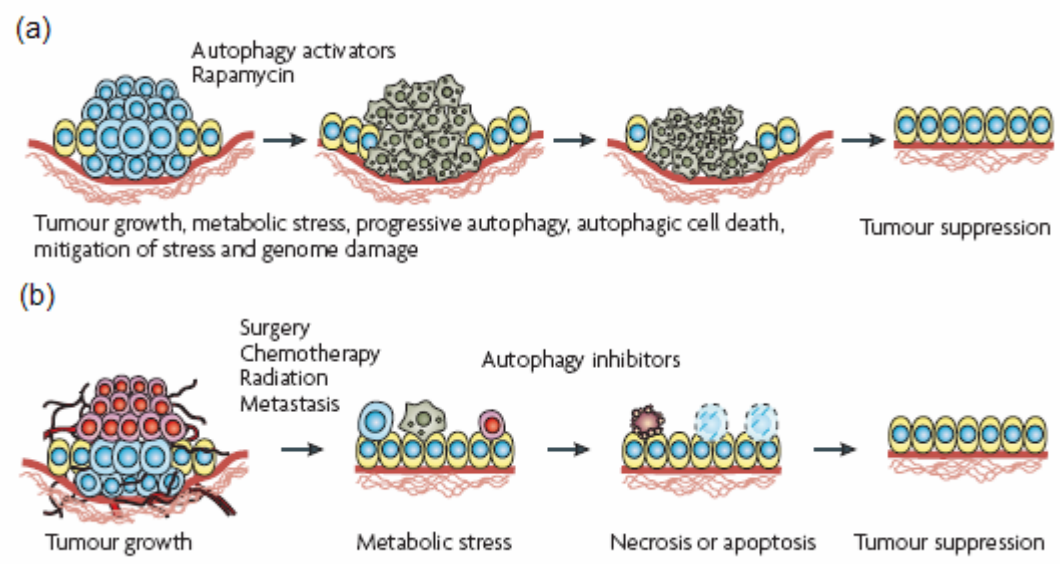

Figure 5. Autophagy modulation in cancer therapy. (a) Activator of autophagy Rapamycin. (b) Inhibition of autophagy in combination with radiotherapy or chemotherapy. (taken from ref. 94)

Conclusions and perspectives

Autophagy is a complex network which is involved in the response to metabolic stress. The relation between autophagy and cancer can not be understood by a simple principle. Defects in autophagy seem to be a common feature in cancer. However, autophagy is activated when tumor cells encounter metabolic stress, which helps tumor cells to survive. Detection of autophagy in tumor cells appeared to be a difficult task, because of the lack of markers for autophagy. Fortunately, anti LC3 antibodies for immunostaining became available, which makes it easier to detect autophagy[93].

In the past years, many studies revealed the autophagic mechanism and how autophagy is regulated. However, parts of the mechanisms and regulation of autophagy are still unclear. Tumor suppressor genes and oncogenes are identified to play a role in the regulation of autophagy, which links autophagy to the development of cancer. The dual role of autophagy in tumor suppression and promotion is an interesting part in the future research to cancer therapy. Additional studies have to be done to understand how autophagy is regulated is cancer, thus how autophagy is turned on and off in cancer cells. This may give us better inside in cancer stages in relation to autophagy, and with that information a better treatment can be achieved. The dual role of autophagy in cancer must be clarified to better understand when autophagy protects cancer cells against chemotherapy and when autophagy sensitizes cancer cells to chemotherapy. By better understanding the mechanism of autophagy, new therapies to threat cancer may be developed in the future. When suppression of autophagy combined with other chemotherapeutic agents makes tumor cells more sensitive, the dose of the chemotherapeutic agents can be reduced, what will lead to less side effects of chemotherapy. Perhaps, inducing of autophagy must be realised to prevent tumor formation in individuals who have a high risk of developing cancer, while autophagy should be inhibited in patients with already established tumors. Inducing of autophagy, can be achieved for example by inhibiting mTOR or PI3K. Inhibition of

autophagy can be achieved for example by a combination of metformin and 2-deoxyglucose, or with hydroxychloroquine**.** Because the contribution of autophagy in cancer is not yet clarified, it must be carefully considered if autophagy inhibitors and/or activators must be used for therapeutic use. Moreover, inhibitors and inducers of autophagy may lead to disturbance of autophagy in all body cells, and can lead to several side effects. Additionally, the drugs used in trials to inhibit or induce autophagy have more functions than regulating autophagy alone. In further studies, the mechanism of autophagy in relation to cancer development and metastasis needs to be studied better. When this mechanism is cleared, it will help to understand if either autophagy inducers or inhibitor must be used in the different stages of cancer, and this will open a new field of cancer biology and therapy.

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