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Review

Autophagy in tumour suppression and promotion

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ABSTRACT

Autophagy, a well-described cellular mechanism for lysosomal degradation of cytoplasmic content, has emerged as a tumour suppression pathway. Recent evidence indicates that the tumour suppressor function of autophagy is mediated by scavenging of damaged oxidative organelles, thereby preventing accumulation of toxic oxygen radicals that would cause genome instability. Paradoxically, however, in some cases autophagy can also promote the survival of cancer cells once tumours have developed. This is attributed to the ability of autophagy to promote cell survival under conditions of poor nutrient supply, as often faced by solid tumours and metastasising cancer cells. In addition, autophagy is frequently upregulated in tumours as a response to therapy and may protect tumours against therapy-induced apoptosis. In this review we discuss the mechanisms that link autophagy to tumour suppression and promotion and provide examples of the dual functions of autophagy in cancer.

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1. Introduction

The ability of cancer cells to evade apoptotic cell death is a well-known key to their survival and aggressiveness (Hanahan and Weinberg, 2000). However, increasing evidence is emerging for the influence of another process that regulates the life and death of cancer cells – autophagy, an evolutionarily conserved process that involves cellular self-eating (the expression derives from Greek; “auto” – self and “phagia”

– eating). (Mizushima et al., 2008). The fact that autophagy can have both tumour suppressive and tumour promoting functions makes it an interesting topic in cancer research (Hoyer-Hansen and Jaattela, 2008; Maiuri et al., 2009).

Three different autophagic mechanisms can be distinguished according to their mode of cargo delivery to the lysosome, namely microautophagy, chaperone-mediated autophagy and macroautophagy. Microautophagy refers to direct delivery of cytosolic components through invagination

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of the lysosomal membrane, whereas chaperone-mediated autophagy depends on chaperones that mediate the transfer of cytosolic constituents through lysosomal membranes (Dice, 2007). Macroautophagy is by far the best understood process and will be referred to as autophagy throughout this review.

Autophagy was originally characterized as an unspecific process for bulk degradation of cytosol and components therein. In this process cytosol is enwrapped by a double membrane structure termed the phagophore or isolation membrane (Seglen et al., 1990). Upon closure of this structure an autophagosome is formed, containing undegraded material that is enclosed by a double membrane. Autophagosomes are short-lived organelles that fuse either with endosomes, forming amphisomes, or with lysosomes forming autolysosomes. Within the lumen of amphisomes and (in particular) autolysosomes, the sequestered content is degraded by lysosomal enzymes, and amino acids and sugars are recycled into the cytosol.

The mechanisms that orchestrate the sequestration process have been characterized extensively using yeast genetics, which has led to the discovery of more than 30 so-called ATG genes (Suzuki and Ohsumi, 2007). The proteins encoded by these genes generate the autophagic sequestration membrane (called the phagophore) through a series of activation steps that include two ubiquitin-like conjugation systems. Initiation of this cascade starts with priming of Atg5, which is enriched in the preautophagosomal structure, and culminates with the insertion of lipidated Atg8 into the growing phagophore membrane, causing its elongation. Studies in higher organisms including, nematodes, flies and mammals, have indicated that both the Atg proteins and the overall mechanisms of autophagy are conserved through evolution.

In parallel with the molecular characterization of the autophagic machinery it has become apparent that autophagy not only sequesters cytosol in an unspecific manner, but also functions in a variety of specific disposal processes, targeting substrates such as peroxisomes (Yokota et al., 1993), ribosomes (Kraft et al., 2008), mitochondria (Narendra et al., 2008; Takano-Ohmuro et al., 2000), invading bacteria (Gutierrez et al., 2004; Nakagawa et al., 2004; Ogawa et al., 2005) and protein aggregates (Fortun et al., 2003; Ravikumar et al., 2002). The mechanism of recognition of these different substrates is still incompletely understood, although ubiquitination has been shown to be important in aggregate targeting (Bjorkoy et al., 2005) and specific proteins have been found to label peroxisomes, mature ribosomes and mitochondria for autophagic degradation (Farre et al., 2008; Kraft et al., 2008; Narendra et al., 2008; Schweers et al., 2007). Further recognition cues and adaptor proteins are likely to be identified as this interesting issue is investigated further.

Even though autophagy was originally recognized as a mechanism for supplying the cell with nutrients under unfavourable growth conditions, it is now clear that this process also plays a crucial role in development (Chen et al., 2008; Sandoval et al., 2008), and programmed cell death (Maiuri et al., 2009). A key question in this context is how autophagy on the one hand prevents normal cells from developing into

cancer cells, and on the other hand endows cancer cells with a mechanism for survival.

Many tumour suppressors and oncogenes have been identified as contributors to cancer development because of genetic or epigenetic alterations that change the expression or function of the encoded products. Furthermore, several common tumour suppressors and oncoproteins are components of receptor signaling pathways. Among the ~30 known components of the autophagy machinery only a few have so far been associated to specific malignancies, but more are likely to follow. Herein we discuss central autophagy proteins and their relation to cancer.

2. Regulation of autophagy

The autophagic machinery in higher organisms receives inputs from several signaling pathways that modulate autophagy according to specific physiological cues such as nutritional status, energy level and growth factor signaling (Figure 1). A master regulator of cell growth versus autophagy is mTOR (mammalian target of rapamycin), which engages into two separate complexes termed TORC1 (containing mTOR, GβL and raptor) and TORC2 (containing mTOR, GβL, rictor and SIN1), of which only the former is sensitive to rapamycin. TORC1 integrates inputs from growth factor signaling via class I phosphoinositide (PI) 3-kinase and AKT1/PKB and also senses energy level (Sarbasov et al., 2005) and nutrient status (Hall, 2008).

Importantly, TORC1 downregulates autophagy when activated in response to growth factor signaling, high nutrient levels or low energy levels (Amherdt et al., 1974; Grinde and Seglen, 1981; Plomp et al., 1987; Samari and Seglen, 1998). The key downstream targets of TORC1 in regulation of cell growth are the elongation factor 4EBP1, which promotes mRNA translation, and p70S6K, which also promotes translation when phosphorylated, acting through eEF-2 kinase. The known TORC1 targets in autophagy are the autophagic regulator kinase ULK1 and its accessory protein ATG13, whose TORC1-mediated phosphorylation causes suppression of autophagy (Chang and Neufeld, 2009; Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009).

Tumorigenesis is frequently initiated through oncogenic transformation that leads to increased signaling through the PI 3-kinase-AKT1 pathway, which acts upstream of mTOR. Class I PI 3-kinase, which converts PtdIns(4,5)P₂ into PtdIns(3,4,5)P₃ at the plasma membrane, resides at the core of this signaling cascade that transmits input from growth factor receptors and other receptor tyrosine kinases. Activated growth factor receptors can stimulate class I PI 3-kinase by binding of the regulatory p85/PIK3R1 subunit of PI 3-kinase to tyrosine phosphorylated receptors, either directly or via adaptors such as GAB2 and IRS1 (Domchek et al., 1992). The receptors can also activate PI 3-kinase indirectly through GRB2-SOS-mediated activation of the small GTPase RAS, whose GTP-bound form activates p110/PIK3C1, the catalytic subunit of class I PI 3-kinase, through a direct interaction (Cully et al., 2006; Pawson, 2004). TORC2 phosphorylates and activates AKT1, thereby participating in a positive feedback

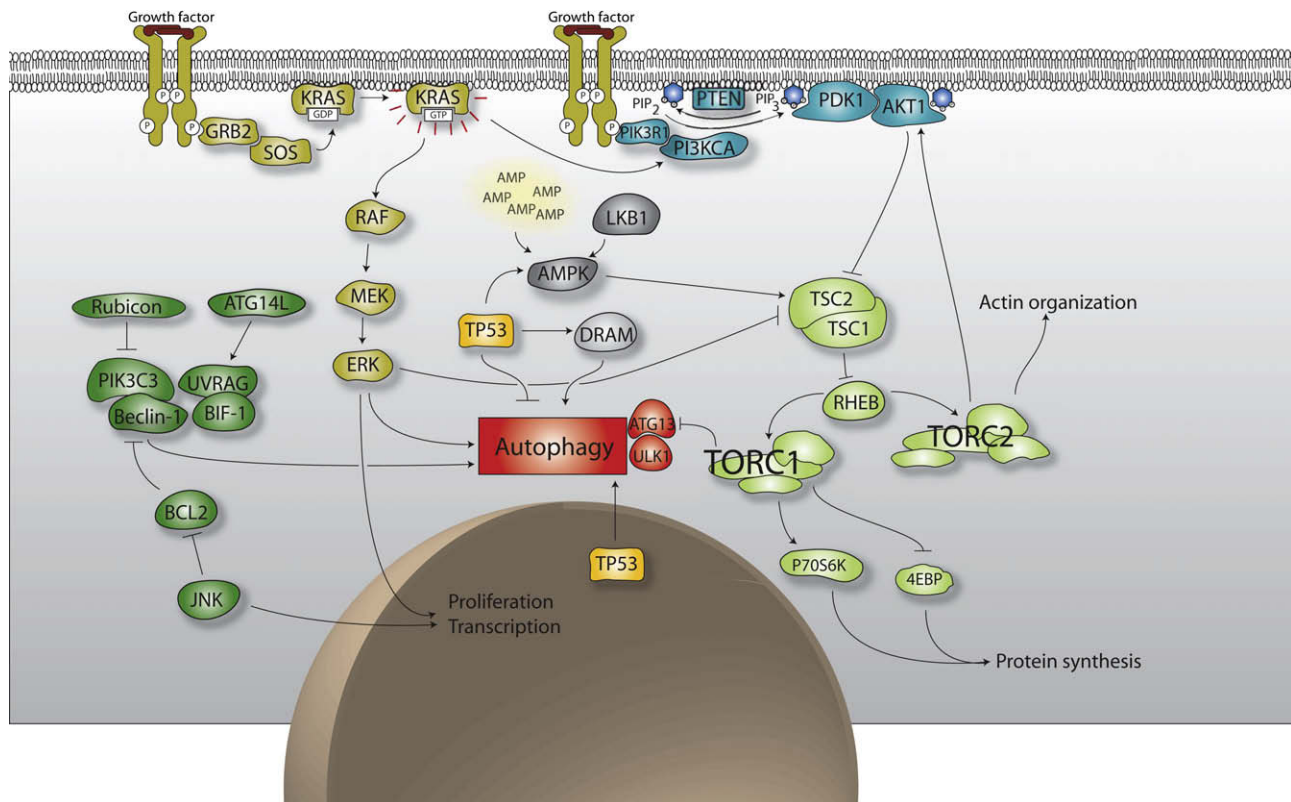


Figure 1 – Regulation of autophagy. Autophagy is regulated through a complex system of stimulatory and inhibitory inputs. Initiation of autophagy is dependent on class III PI 3-kinase (PI3KC3) activation through its accessory subunits UVRAG, Beclin 1 and Bif-1. This is negatively regulated through Beclin 1 interaction with Bcl-2, and through JNK. Activated JNK leads to Bcl-2 phosphorylation and Beclin 1 release and thereby promotes autophagy (Wei et al., 2008). Additional positive/negative regulation of the class III PI 3-kinase complex is achieved by Atg14L and Rubicon binding, respectively. An important regulatory input is mediated through activated growth factor receptors, which inhibit autophagy by activating the TORC1 complex via TSC2 inhibition. Similarly, the activated RAS-RAF pathway exerts negative control onto TSC2 via ERK. In contrast, at high AMP/ATP ratios resulting from energy depletion, the AMP-activated kinase (AMPK) is phosphorylated by LKB1, leading to inactivation of TORC1 via TSC2 phosphorylation and activation. AMPK can also be triggered by p53, either in a positive fashion from nuclear p53, or negatively from cytosolic p53.

loop for activation of this crucial regulator of metabolism and autophagy.

The catalytic product of class I PI 3-kinase, PtdIns(3,4,5)P₃, recruits the protein kinases PDK1 and AKT1 to the plasma membrane, followed by phosphorylation and activation of AKT1 by PDK1 and TORC1 (Sarbasov et al., 2005). Activated AKT1 inhibits heterodimerization of TSC2 and TSC1 by phosphorylation of TSC2, which inhibits the ability of this complex to stimulate the GTPase activity of Rheb. GTP-bound Rheb activates TORC1, which inhibits autophagy through phosphorylation of the ATG13-ULK1 complex. This signaling cascade is counterbalanced by the action of the tumour suppressor PTEN (phosphatase with tensin homology), which dephosphorylates PtdIns(3,4,5)P₃ into PtdIns(4,5)P₂.

Amino acid sensing is effected downstream of cellular uptake of L-glutamine and its subsequent rapid efflux in the presence of essential amino acids, which activates TORC1 (Nicklin et al., 2009). Recent studies have implicated the GTPase Rag, which interacts with TORC1 in its active, GTP-bound form and mediates sensing of amino acid levels (Kim et al., 2008a; Sancak et al., 2008). Energy levels are sensed via

the AMP-dependent kinase AMPK, which is activated by the upstream LKB1 kinase under low energy conditions that increase the AMP/ATP ratio. AMPK in turn phosphorylates TSC2 to stimulate its GAP activity, thereby turning off Rheb and TORC1 (Inoki et al., 2003). By this mechanism, tumours with limited blood supply would be expected to upregulate autophagy due to low ATP levels. However, recent studies have suggested that epidermal growth factor receptors, which are often overexpressed in tumours, serve to maintain intracellular glucose (and thereby ATP) levels through interaction and stabilisation of the sodium/glucose cotransporter SGLT1 (Weihua et al., 2008).

3. Autophagy in tumour suppression

The first hint that autophagy may represent a tumour suppressor mechanism was the finding that this pathway is frequently downregulated in tumour cells (Gunn et al., 1977; Kisen et al., 1993), which is associated with tumour progression (Liang et al., 1999). One possible explanation to the

downregulation of autophagy observed in tumours is the upregulation of the class I PI 3-kinase pathway often manifested in cancer cells, which results in activation of TORC1 (Figure 1). Consistent with this, oncoproteins in the class I PI 3-kinase pathway, including PI 3-kinase itself and activated AKT1, are potent inhibitors of autophagy (Degenhardt et al., 2006; Rusten et al., 2004). Likewise, tumour suppressors in the same pathway, such as PTEN, are activators of autophagy (Rusten et al., 2004), as are tumour suppressors that regulate the TORC1 pathway, such as LKB1 (Liang et al., 2007). Later studies have shown that several other regulators of autophagy are bona fide tumour suppressors (see below), thereby confirming the concept that autophagy is a tumour suppressor pathway under normal physiological circumstances.

3.1. Mechanism of tumour suppression by autophagy

The fact that downregulation of autophagy promotes tumour growth is contradictory to the fact that this process enables cell survival under stress conditions and thereby should facilitate rather than restrict tumour growth. How can this paradox be explained? A plausible mechanism to explain the tumour suppression mediated by autophagy has emerged recently. Deficiency in autophagy caused by monoallelic loss of Beclin 1 (see below) or downregulation of Atg5 leads to accelerated DNA damage which may trigger accumulation of oncogenic events in the cells, indicative of a mutator phenotype (Abedin et al., 2007; Katayama et al., 2007). Autophagy-defective cells exhibit increased numbers of chromosomal gains or losses, suggesting that autophagy normally functions tumour suppressive by limiting chromosomal instability (Degenhardt et al., 2006; Karantza-Wadsworth et al., 2007). The most attractive explanation to the contribution of autophagy to suppress a chromosomal instability phenotype is the involvement of this process in disposal of damaged mitochondria and peroxisomes. These organelles produce reactive oxygen species as part of their functions in oxidative metabolism, and damaged mitochondria and peroxisomes thereby represent a potential source of DNA damaging compounds. Their elimination by autophagy is likely to play an important detoxifying role in normal cells, and tumour cells can have a selective advantage by downregulation of this mechanism since this favours genomic instability (Figure 2).

3.2. Beclin 1 as tumour suppressor

The first direct functional link between autophagy and cancer was established through the identification of Beclin 1 as a haploinsufficient tumour suppressor that is monoallelically deleted in a large proportion of sporadic breast and ovarian cancers (Liang et al., 1999). Beclin 1 is the human homologue of Atg6/Vps30, which is a key regulator of autophagy in yeast, and Beclin 1 has indeed been found crucial for autophagy induction in mammalian cells. Importantly, the autophagy-promoting activity of Beclin 1 in MCF7 breast carcinoma cells inhibits proliferation and clonogenicity *in vitro*, and it also inhibits tumorigenesis in nude mice. Interestingly, the expression of endogenous Beclin 1 is often low in breast epithelial carcinoma cell lines and tissues but is ubiquitously

high in normal breast epithelia. These findings indicate that Beclin 1 is a tumour suppressor in humans (Liang et al., 1999).

Beclin 1 contains a Bcl-2 homology-3 (BH3) domain that mediates its interaction with Bcl-2 and other anti-apoptotic multidomain proteins. Unlike other BH3-only proteins, Beclin 1 fails to stimulate apoptosis when overexpressed, and Bcl-2 does not lose its anti-apoptotic potential upon interacting with Beclin 1. On the other hand, interaction with Bcl-2 inhibits the pro-autophagic activity of Beclin 1 (Ciechomska et al., 2009). These findings indicate that Beclin 1 does not exert its tumour suppressor function through sequestration of Bcl-2, consistent with the notion that Beclin 1 functions tumour suppressive through its involvement in autophagy. They also raise the possibility that the tumour promoting potential of Bcl-2 might not only be related to its anti-apoptotic function but also its ability to sequester Beclin 1.

The involvement of Beclin 1 in autophagy is associated with its engagement in the class III PI 3-kinase complex, which converts PtdIns into PtdIns(3)P, a lipid required for formation of the phagophore (Obara and Ohsumi, 2008). The class III PI 3-kinase core complex consists of the catalytic subunit Vps34/PIK3C3, the regulatory subunit Vps15/PIK3R4, and several accessory proteins, including Beclin 1, UVRAG (see below), ATG14L and Rubicon (Matsunaga et al., 2009; Zhong et al., 2009). The two latter proteins bind to Beclin 1 in a mutually exclusive manner and appear to act antagonistically in regulation of PIK3C3 activity and autophagy: ATG14L stimulates PI 3-kinase activity and autophagy, whereas Rubicon inhibits both activities. This suggests that Beclin 1 forms at least two distinct complexes with differential roles in autophagy.

3.3. UVRAG as tumour suppressor

UVRAG was initially identified as a protein that confers resistance to UV sensitivity in xeroderma pigmentosum cells (Teitz et al., 1990). The link to cancer was suspected early because of the localization of the UVRAG gene on chromosome band 11q13, a region often found altered in human malignancies including breast and colon (Ormandy et al., 2003). However, the first report of cancer specific mutations in UVRAG was presented in a study that exploited nonsense-mediated decay to identify genes with premature stop-codons in microsatellite unstable colorectal tumours (Ionov et al., 2004). The (A)₁₀ repeat in exon 8 has been analyzed in microsatellite unstable colorectal cancer cell lines and identified monoallelic frameshift mutations. More recently, similar findings have been reported in gastric tumours (Kim et al., 2008c).

The link between UVRAG and autophagy was discovered recently when UVRAG was found to positively regulate Beclin 1 via direct interaction (Liang et al., 2008). A colon cancer cell line carrying a 1-bp deletion in the homopolymer tract (c.709delA) displays a marked reduction in endogenous UVRAG levels as well as impaired induction of autophagy compared to wild-type cells. Additionally, the effect of UVRAG-induced autophagy on proliferation, anchorage independent growth and *in vivo* tumorigenicity demonstrates that wild-type UVRAG suppresses the proliferation rate

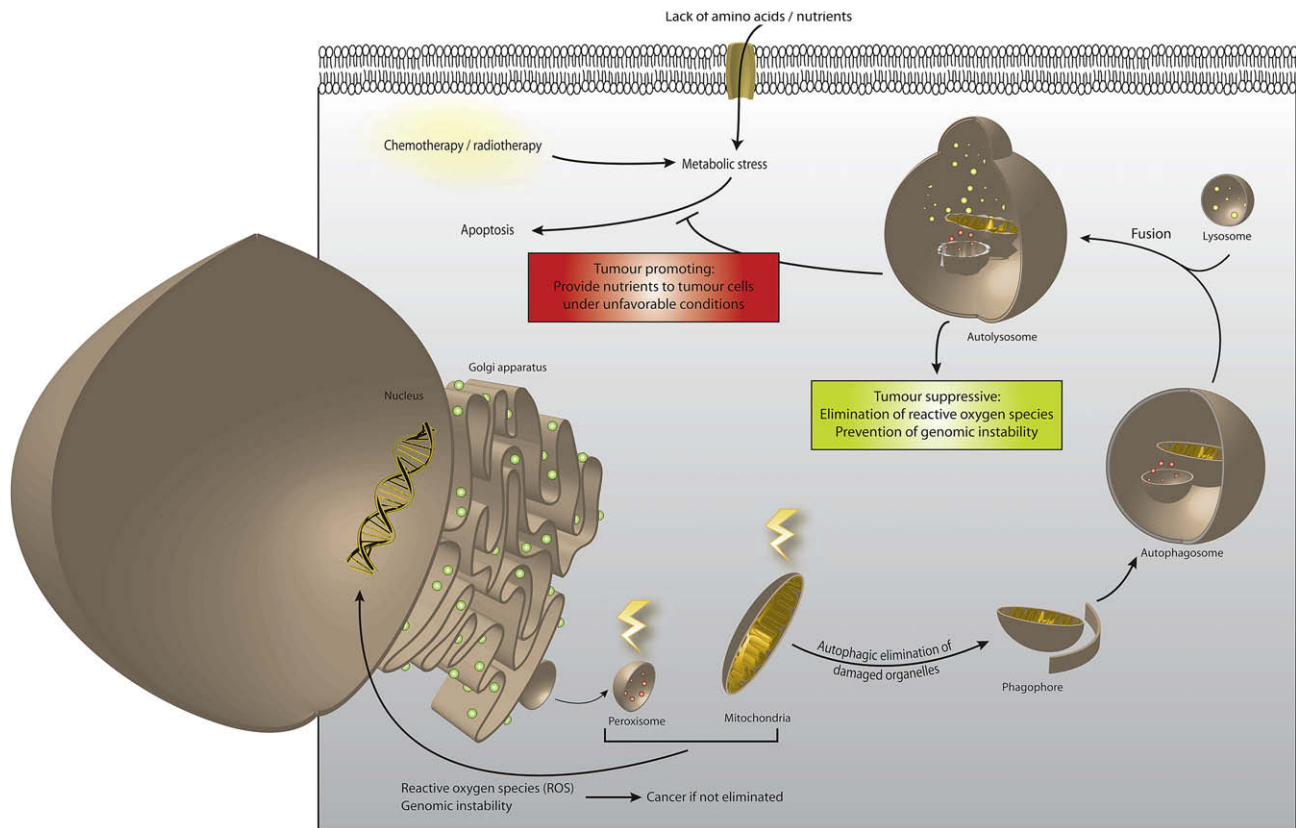


Figure 2 – Autophagy in tumour suppression and promotion. Autophagy involves sequestration of cytoplasmic content by a double membrane, the phagophore, resulting in formation of an autophagosome. The sequestered material becomes degraded by lysosomal enzymes when the autophagosome fuses with a lysosome to yield an autolysosome. The lower part of the figure illustrates the tumour suppressive function of autophagy: If not eliminated by autophagy, damaged peroxisomes and mitochondria will cause cytosolic accumulation of reactive oxygen species (ROS), which may enter the nucleus and cause mutagenesis and genomic instability. As illustrated in the upper part of the figure, autophagy can alternatively be usurped by tumour cells to sustain survival under conditions of low nutrient supply or cytotoxic therapy.

compared to the truncated protein. These results underline UVRAGs role as a tumour suppressor.

3.4. Bif-1 as tumour suppressor

A third protein associated with the class III PI 3-kinase complex, Bif-1/Endophilin B1, has recently been established as a tumour suppressor. Bif-1 interacts with Beclin 1 through UVRAG to stimulate the activity of the class III PI 3-kinase complex (Takahashi et al., 2007) although it does not appear to be a constitutive subunit of this complex (Matsunaga et al., 2009). Consistently, loss of Bif-1 suppresses autophagosome formation (Takahashi et al., 2007). During nutrient deprivation, Bif-1 accumulates in cytoplasmic puncta that contain the autophagic effectors LC3/ATG8, ATG5 and ATG9. Importantly, knockout of Bif-1 enhances the development of spontaneous tumours in mice. Even though the exact mechanisms that explain the tumour suppressor function of Bif-1 are not clarified, it is reasonable to speculate that it, similarly to Beclin 1 and UVRAG, suppresses tumours through its involvement in autophagy. However, it is worth noting that ablation of Bif-1 prolongs cell survival under starvation conditions. While this could possibly be explained by increased genome

instability, an alternative explanation might be that apoptosis is downregulated. It may be relevant in this context that Bif-1 was originally identified as an interactor of the pro-apoptotic effector Bax, and therefore might represent a regulator of apoptosis in addition to autophagy (Cuddeback et al., 2001).

3.5. An ATG protein as tumour suppressor

The identification of the tumour suppressors Beclin 1, UVRAG and Bif-1 as specific regulators of autophagy supports the concept that autophagy has a tumour suppressor function (Liang et al., 2006, 1999; Takahashi et al., 2007). However, all these proteins are subunits of the class III PI 3-kinase complex, which not only controls autophagy but also regulates other processes such as endosome biogenesis and sorting (Lindmo and Stenmark, 2006). Moreover, Beclin 1 and Bif-1 have been identified as interactors of the apoptotic regulators Bcl-2 and Bax, respectively (Cuddeback et al., 2001; Liang et al., 1998; Takahashi et al., 2005). These findings raise the possibility that Beclin 1, UVRAG and Bif-1 might function as tumour suppressors by mechanisms that are independent of autophagy, and a definitive conclusion that autophagy is a tumour suppressor mechanism would require the demonstration that

the core autophagic machinery has such a function. An interesting candidate in this context is ATG4C, a protease that mediates autophagosome formation (Scherz-Shouval et al., 2007) through processing of LC3/ATG8. ATG4 proteins appear as likely ROS sensors in autophagy due to ROS-mediated oxidation of specific cysteine residues in the active site of these proteases (Scherz-Shouval et al., 2007). ATG4C *-/-* mice not only show reduced starvation-induced autophagy but also display increased susceptibility to develop fibrosarcomas induced by chemical carcinogens (Marino et al., 2007). The relatively weak tumour suppressor effect of ATG4C in these studies might be attributed to other ATG4 isoforms that might compensate for the loss of ATG4C. Further studies are therefore still required to firmly establish the role of the core autophagy machinery in tumour suppression.

3.6. Dual regulation of autophagy by the tumour suppressor p53

p53 is a tumour suppressor protein that is found mutated in almost 50% of all cancers (Tasdemir et al., 2008). It can function as a positive or negative regulator of autophagy depending on the type of stimuli received by the cells, and on the subcellular localization of p53. Genotoxic stress leads to autophagy induction via AMPK activation by p53, which in turn activates TSC1/TSC2 (Feng et al., 2005), leading to mTOR inhibition. In addition, nuclear p53 can also induce autophagy via transcriptional upregulation of DRAM (damage-regulated modulator of autophagy) (Crichton et al., 2006). On the other hand, loss of p53 function in the cytoplasm also propagates autophagy (Tasdemir et al., 2008). How can p53 function as both an inhibitor and a stimulator of autophagy? Part of the answer is that p53 differentially regulates autophagy according to whether it is localized in the cytosol or in the nucleus. p53^{-/-} cells transfected with a p53 mutant protein that is restricted to cytosol due to lack of a nuclear localization signal fail to restore an autophagic response, whereas strictly nuclear p53 fails to block autophagy (Tasdemir et al., 2008). In conclusion, while p53 can act as an autophagy-inducing transcription factor, cytoplasmic p53 functions as a tonic inhibitor of autophagy, and its degradation is required for the induction of autophagy. Even though control of autophagy is not the key mechanism of p53-mediated tumour suppression, it is conceivable that the transcriptional regulation of pro-autophagic genes mediated by p53 does play a role in tumour suppression.

4. Autophagy in tumour promotion

Even though autophagy is frequently downregulated in tumours, (Gunn et al., 1977; Kisen et al., 1993) this is not always the case, and upregulation of autophagy can give tumour cells a selective advantage, manifested as increased aggressiveness and therapy resistance (Kondo et al., 2005). The upregulation of autophagy in cancers after use of rapamycin analogues is expected due to inhibition of TOR (Kim et al., 2008b). More surprisingly, accumulation of autophagosomes is frequently observed in cancer cells after exposure to various other chemotherapeutics such as the DNA alkylating agent temozolomide (Kanzawa et al., 2004), the estrogen receptor

antagonist tamoxifen (Bursch et al., 1996), resveratrol (Opipari et al., 2004), vitamin D3 (Wang et al., 2008) and anthocyanins (Longo et al., 2008). Likewise, hypoxia, hyperthermia and radiotherapy also cause upregulation of autophagy (Komata et al., 2004; Papandreou et al., 2008). Indeed, autophagy has been proposed to account for the ability of apoptosis-defective tumour cells to survive under conditions of metabolic stress induced by chemo- or radiotherapy (Kondo et al., 2005). Metabolic stress is also prevalent in untreated primary tumours with poor blood supply or in cancer cells that metastasize to organs that provide limited nutrient supply (Witz, 2008). Although poorly characterized in this context, it is conceivable that autophagy, because of its ability to supply nutrients from breakdown of cytoplasm, helps tumour cell to survive under such harsh conditions (Figure 2). The fact that autophagy is upregulated as response to cellular detachment from the extracellular matrix supports the view that autophagy could represent a survival mechanism for metastasising cells (Lock and Debnath, 2008).

The upregulation of autophagy in response to therapy in some cancers begs the question whether autophagy inhibition might represent a potential adjuvant for cancer therapy. At least in experimental models, this strategy seems to be viable. For instance, in mice with c-Myc-induced lymphomas, chloroquine, which inhibits autophagy by neutralizing the autolysosome, enhances the efficacy of either p53 or a DNA alkylating agent to induce tumour cell death and tumour regression (Amaravadi et al., 2007). Likewise, siRNA-mediated depletion of Atg proteins sensitizes cancer cells to radio- and chemotherapy (Apel et al., 2008; Tiwari et al., 2008), and the autophagy inhibitors 3-methyladenine and bafilomycin A1 cause radiosensitization of malignant glioma cells (Ito et al., 2005). A major obstacle to the use of autophagy inhibitors as cancer therapeutics is the fact that autophagy is beneficial to normal cells. As outlined above, autophagy contributes to tumour suppression, and this process also has the advantage of preventing the accumulation of aggregate proteins, which may cause neuronal death. Therefore, the ideal drug to target the autophagic pathway in cancer would be one that inhibits autophagy in tumour cells without affecting normal cells.

5. Autophagy and therapy-induced cell death

Programmed cell death by way of apoptosis plays an important role in tumour suppression (Hanahan and Weinberg, 2000), and selective activation of apoptosis in tumour cells is an attractive strategy in cancer therapy (Takeda et al., 2007). Programmed cell death can also be triggered by autophagy during certain developmental processes (Berry and Baehrecke, 2008; Mohseni et al., 2009; Nezis et al., 2009), although the contribution of autophagy to programmed cell death in general remains controversial (Kroemer and Levine, 2008)). As discussed above, autophagy is frequently upregulated in tumours in response to chemo- or radiotherapy, and can attenuate the efficacy of therapy (Apel et al., 2008). Conversely, however, there are also several examples that autophagy induction appears to facilitate successful therapy-induced killing of tumour cells (Cao et al., 2006; Fujiwara et al., 2007; Kim et al., 2007; Lefranc et al., 2007; Peng et al., 2008). For instance,

Table 1 – Beneficial and harmful functions of autophagy in cancer.

Beneficial functions of autophagy in cancer	Harmful functions of autophagy in cancer
Maintains genome stability by scavenging damaged peroxisomes and mitochondria	Provides a survival mechanism for solid tumours with restricted blood supply, and for metastasising cells
May contribute to tumour cell death induced by some therapies	Prevents radio- and chemotherapy-induced apoptosis in tumours

activation of autophagy by mTOR inhibition has been found to radiosensitize PTEN null prostate cancer cells (Cao et al., 2006), and pro-autophagic drugs such as temozolomide are promising candidates for selective killing of apoptosis-resistant glioblastomas (Lefranc et al., 2007). Even though the prospect of activating autophagy for cancer destruction is an appealing one, it has to be kept in mind that therapies that induce autophagy also affect other cellular pathways, and it is often difficult to ascertain whether cancer cells die by autophagy or with autophagy (Kroemer and Levine, 2008).

6. Conclusions and perspectives

Substantial progress has been made in recent years in the understanding of autophagic mechanisms and how autophagy is regulated. At the same time, oncogenes and tumour suppressor have been identified that are implicated in autophagy regulation, thereby linking autophagy directly to cancer development. It will be of great importance to investigate how the combined input of these regulatory circuits influences cellular decisions for or against autophagy induction. A better understanding of this complex regulation may aid in the development of novel cancer therapies.

It will be necessary to develop models that should allow us to study how autophagic responses are turned on and off throughout cancer development. This will give us a deeper insight into what cancer stages could be most responsive for treatment with drugs affecting the autophagic pathway. The fact that autophagy protects some cancers against chemotherapy yet appears to sensitize others to chemotherapy-induced cell death (Table 1) illustrates the need for a further clarification of the dual roles of autophagy in survival and programmed cell death. It is important to stress that therapy-induced accumulation of autophagosomes is by itself not sufficient to draw any conclusions whether autophagy has a cytotoxic or -protective function. Moreover, such accumulation does not necessarily reflect increased autophagy but could equally well represent inhibited turnover of autophagosomes. This calls for extensive control experiments in studies of autophagy in cancer, including experimental models for blocking autophagy and for measurements of autophagic flux in tumour cells.

An emerging area of cancer related autophagy research involves the identification of novel substrates that are turned over by autophagy, and elucidation of how these are recognized by the autophagic machinery. A recent and very

interesting example is the autophagy substrate p62, whose accumulation in the absence of autophagy causes carcinogenesis through dysregulated NF- κ B signalling (Mathew et al., 2009). Progress in this research could reveal many important clues to a better understanding of the relationship between cancer and autophagy. The dual role of autophagy in both tumour suppression and promotion represents a major challenge when targeting this pathway in cancer therapy. Therefore, unraveling the molecular mechanisms related to autophagy in cancer will guide the potential use of autophagy inducers or inhibitors in future treatment of advanced cancers.

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