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REVIEW

The multiple roles of autophagy in cancer

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Autophagy is an evolutionarily conserved, catabolic process that involves the entrapment of cytoplasmic components within characteristic vesicles for their delivery to and degradation within lysosomes. Autophagy is regulated via a group of genes called *AuTophaGy*-related genes and is executed at basal levels in virtually all cells as a homeostatic mechanism for maintaining cellular integrity. The levels and cargos of autophagy can be modulated in response to a variety of intra- and extracellular cues to bring about specific and selective events. Autophagy is a multifaceted process and alterations in autophagic signalling pathways are frequently found in cancer and many other diseases. During tumour development and in cancer therapy, autophagy has paradoxically been reported to have roles in promoting both cell survival and cell death. In addition, autophagy has been reported to control other processes relevant to the aetiology of malignant disease, including oxidative stress, inflammation and both innate and acquired immunity. It is the aim of this review to describe the molecular basis and the signalling events that control autophagy in mammalian cells and to summarize the cellular functions that contribute to tumourigenesis when autophagy is perturbed.

Introduction

In 1963, de Duve introduced the term autophagy, which is derived from the Greek words 'auto' and 'phagy' meaning 'self' and 'eating', to describe the occurrence of distinct intracellular, membranous vesicles that contained degraded cytoplasmic material (1). Since then, autophagy has been characterized as an adaptive, catabolic process that serves to deliver cytoplasmic proteins and organelles to lysosomes for digestion. Depending on the route of delivery to the lysosome, three different types of autophagy are defined: microautophagy, chaperone-mediated autophagy and macroautophagy (2). This review solely focuses on macroautophagy, which is hereafter simply termed autophagy.

Autophagy is orchestrated by a number of highly conserved *AuTophaGy*-related genes (*ATG*s), which were originally identified in yeast, with many of these genes having orthologues in mammalian cells (3,4). In mammalian cells, double-membrane-bound autophagosomes develop in a multi-step process from a precursor structure called the phagophore or isolation membrane. Autophagosomes subsequently fuse with lysosomes to form a degradative, single-membrane-bound vesicle called an autolysosome. In a process named autophagic lysosome reformation, lysosomes are then re-derived from autolysosomes (5).

The role of autophagy extends beyond the general homeostatic removal, degradation and recycling of damaged proteins and organelles to many specific physiological and pathological processes such as development, immunity, energy homeostasis, cell death, tumourigenesis and many more (2). The involvement of autophagy in tumour development is unquestioned but is at present incompletely understood. Deregulation of autophagy is known to affect many processes that can control the formation and existence of a cancer cell, but paradoxes still exist in what we currently understand about the relationship between autophagy and cancer (4,6). This review aims therefore to describe the molecular control of autophagy and to delineate how the deregulation of autophagy can contribute in context-specific ways to the development of cancer.

The molecular basis of autophagic vesicle formation

Autophagy is activated in response to a whole host of stimuli including nutrient depletion, hypoxia and activated oncogenes. The majority of pro-autophagic events converge on the serine/threonine protein kinase mTOR (MTOR, mammalian/mechanistic target of rapamycin) (7–9). Another important, nutrient-sensitive entry route to ATG signalling is the class III phosphatidylinositol 3-kinase complex (PI3K-III) consisting of hVps34 (PIK3C3, the orthologue of yeast Vps34), Beclin 1 (yeast Atg6) and p150/hVps35 (PIK3R4; yeast Vps15) (9,10). Many of the aforementioned ATGs are restricted to a certain type of autophagy (see above). The 'core' autophagic machinery encompasses only those ATGs that are necessary for autophagosome formation in all subtypes and these can be divided into several distinct groups: (i) the unc-51-like kinase 1/2 (ULK1/2) complex (ii) the multi-spanning membrane protein Atg9, (iii) the PI3K-III complex and (iv) the ubiquitin-like ATG12 and microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A) conjugation systems. The following sections detail how these proteins regulate the various stages of the autophagy process.

The autophagy machinery. Initiation and nucleation are terms used to describe the events that lead to the formation of the initial autophagic structure: the phagophore or isolation membrane. RAPTOR forms the catalytic subunit of two different protein complexes: mTORC1 and mTORC2. The former contains mTOR and RAPTOR (regulatory-associated protein of MTOR), whereas mTORC2 contains among others, mTOR and RICTOR (rapamycin-insensitive companion of MTOR) (11). In nutrient-rich states, mTORC1 but not mTORC2 forms a complex with ULK1/2 (orthologues of yeast Atg1), mAtg13, FIP200 (RB1CC1; mammalian orthologue of Atg17) and the newly identified ATG101, as a result of interaction between RAPTOR and ULK1 (8,12–14). mTOR phosphorylates ULK1 and Atg13 and thereby keeps the kinase activity of ULK1 in check. Upon treatment with rapamycin or in fasting conditions, mTORC1 breaks free from the ULK complex and the inhibitory phosphorylation of ULK1 is lost. ULK1 then autophosphorylates and activates Atg13 and FIP200. The activated ULK complex localizes to the developing phagophore. The relationship between mTOR

Abbreviations: ATG, AuTophaGy-related genes; CD, Crohn's disease; DRAM1, damage-regulated autophagy modulator 1; ER, endoplasmatic reticulum; LAMP 1/2, lysosomal-associated membrane protein 1/2; MAP1LC3A, microtubule-associated protein 1 light chain 3 alpha; MTOR, mammalian/mechanistic target of rapamycin; PI3K-III, class III phosphatidylinositol 3-kinase complex; PtdIns(3)P, phosphatidylinositol 3-phosphate; ROS, reactive oxygen species; ULK1/2, unc-51-like kinase 1/2; UVRAG, ultraviolet radiation resistance gene.

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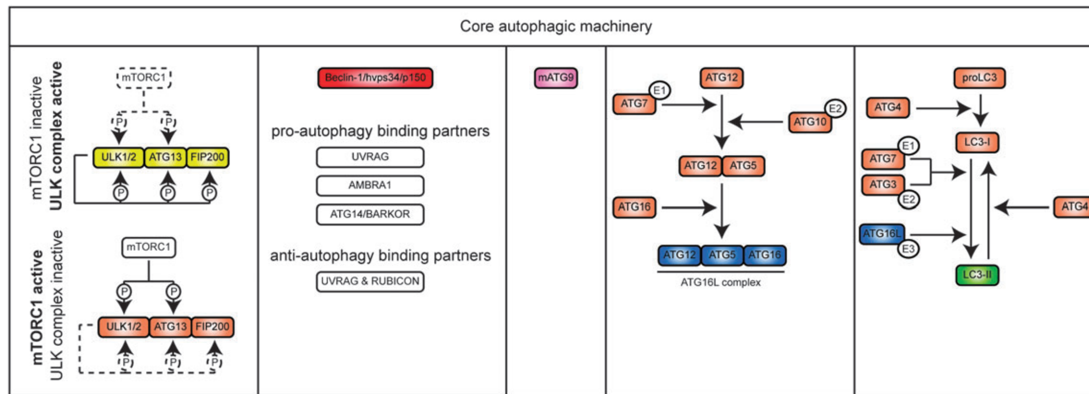

Fig. 1. Autophagic core machinery. The ULK kinase complex, the PI3K-III complex, mAtg9 and the two ubiquitination systems are indispensable for autophagy. Members of the core machinery are shown in coloured boxes. Modulators that are not part of the core machinery are shown in white boxes. For details, see text.

and activation of the ULK complex is shown in Figure 1. Startlingly, the inverse relationship between mTOR activity and autophagy is not universal, as autophagy induced by 6-thioguanine has been reported to require activation and not inhibition of mTOR (15).

There are two mammalian orthologues of Atg9: ATG9L1 (mAtg9) is ubiquitously expressed, whereas expression of ATG9L2 is restricted to the placenta and pituitary gland. The exact function of mAtg9 currently remains elusive but it is required for LC3 lipidation and knockout mice die after birth as do *Atg5*- and *Atg7*-knockout animals (16–18).

Phosphatidylinositol 3-phosphate (PtdIns(3)P) is a prerequisite for the nucleation process. It is produced by the PI3K-III-hVps34 complex when bound to its core partners Beclin 1 and p150/hVps35 (10). The complex is found on the phagophore and thought to facilitate recruitment of other ATGs to the developing vesicle. Importantly, autophagy can be positively and negatively modulated at the level of the Beclin 1–hVps34–p150/hVps35 complex depending on additional, regulatory binding partners of Beclin 1 (Figure 1) (4,10). ATG14/BARKOR (Beclin 1-associated autophagy-related key regulator), UVRAG (protein product of the ultraviolet radiation resistance gene) and activating molecule in Beclin 1-regulated autophagy are pro-autophagic regulators of the PI3K-III complex. Simultaneous binding of both UVRAG and RUBICON (RUN domain and cysteine-rich domain containing) inhibits the autophagy-promoting activity of the complex (19–23).

Elongation and closure describe the development of the characteristic double-membrane-bound autophagosome from its precursor structure and require two ubiquitin-like conjugation systems. The ubiquitin-like ATG12 is conjugated to ATG5 via the E1-like protein ATG7 and the E2-like ATG10. ATG16 then enters the complex and directs the large (L), newly formed ATG16L (ATG12-ATG5-ATG16) complex to the isolation membrane. The Atg16L complex is required for autophagosome formation, guides LC3 to the phagophore and promotes lipidation of LC3 (Figure 1) (24–26).

The ubiquitin-like yeast protein Atg8 has several orthologues in mammalian cells: MAP1LC3 (LC3), GABARAPL2 (GATE16), GABARAP and GABARAPL1 (ATG8L). LC3 is the most thoroughly investigated of these proteins and its modification during autophagy is exploited as a marker for autophagy (27,28). Newly synthesized LC3 is immediately cleaved at its C-terminal end by the protease Atg4 into the cytoplasmic form LC3-I. If autophagy is active, LC3-I is then conjugated to phosphatidylethanolamine via ATG7 and the E2-like ATG3 (29). In its conjugated form, LC3 is called LC3-II and is recruited via its lipid moiety to the inner and outer surfaces of the autophagosomal membrane, i.e. unlike LC3-I, LC3-II is not freely dispersed in the cytoplasm (Figure 1).

The exact order of Atg activation is not clear and there is intensive crosstalk between the different Atg systems. However, it is generally accepted that the ULK1 kinase complex and the PI3K-III complex act upstream of the ubiquitination systems (30).

The maturation process encompasses the fusion of autophagosomes with lysosomes to form autolysosomes. Autolysosomes are single-membrane-bound, acidic vesicles comprised of the outer membrane of autophagosomes and the lysosome that degrade the autophagosomal cargo via acidic hydrolases provided by the lysosome. The process is less well understood but involves the action of lysosomal proteins, such as lysosomal-associated membrane protein 1/2 (LAMP 1/2) and also again, Beclin 1 (3,4).

Work by Yu *et al.* (5) has recently shed light on the ultimate fate of autolysosomes. During autophagy-initiation mTOR is inhibited but becomes reactivated at later stages as a result of the release of cellular constituents into the cytoplasm following the breakdown of macromolecules within autolysosomes. Increased mTOR activity then inhibits autophagy and leads to the formation of proto-lysosomal extensions (LAMP1+, LC3−) from autolysosomes (LAMP1+, LC3+) (30). Ultimately, these proto-lysosomal extensions detach from the autolysosome and mature into functional lysosomes. Inhibition of mTOR, or (auto-)lysosomal function, prevents autophagic lysosome reformation (Figure 2). Autophagy is therefore controlled by a negative feedback mechanism that is regulated by mTOR (5,30).

Until recently, it was believed that the two ubiquitination systems are indispensible for autophagy. However, Nishida *et al.* (31) introduced the term ‘alternative macroautophagy’ to describe a degradative process in response to starvation and etoposide treatment that involves autophagosome-like structures that are not decorated by LC3-II. Strikingly, this process is independent of both ATG5 and ATG7 but critically relies on ULK1 and Beclin 1. Double-membrane-bound vesicles that included cytoplasmic material were generated in a RAB9 (RAB9A, member RAS oncogene family)-dependent fashion by the fusion of isolation membranes and vesicles derived from the trans-Golgi and late endosomes (31). Since this process occurs without involvement of crucial regulators for ‘conventional’ or ‘canonical’ autophagy, it is debated whether this phenomenon is something altogether different from autophagy (32).

Origin of the phagophore/isolation membrane. The first detectable structure during autophagy in mammalian cells is the isolation membrane or phagophore. Considerable insight has been gained in the last 2 years in relation to its sites of origin. Current consensus favours that in mammalian cells, the isolation membrane develops from at least three different, preformed sources: the endoplasmic reticulum (ER), the plasma membrane and mitochondria (33–36). Axe *et al.* (33) proposed that the phagophore is derived from so-called omegasomes (cup-shaped protrusions from the ER). Moreover, it has recently been confirmed that isolation membranes are physically connected to the ER and are cradled by two ER membranes, which is reminiscent of the omegasome (37,38). Upon starvation, the hVps34 kinase is recruited via ATG14L to the ER, where it creates a local increase in PtdIns(3)P, that is essential for autophagosome development (39). Proteins that specifically recognize PtdIns(3)P

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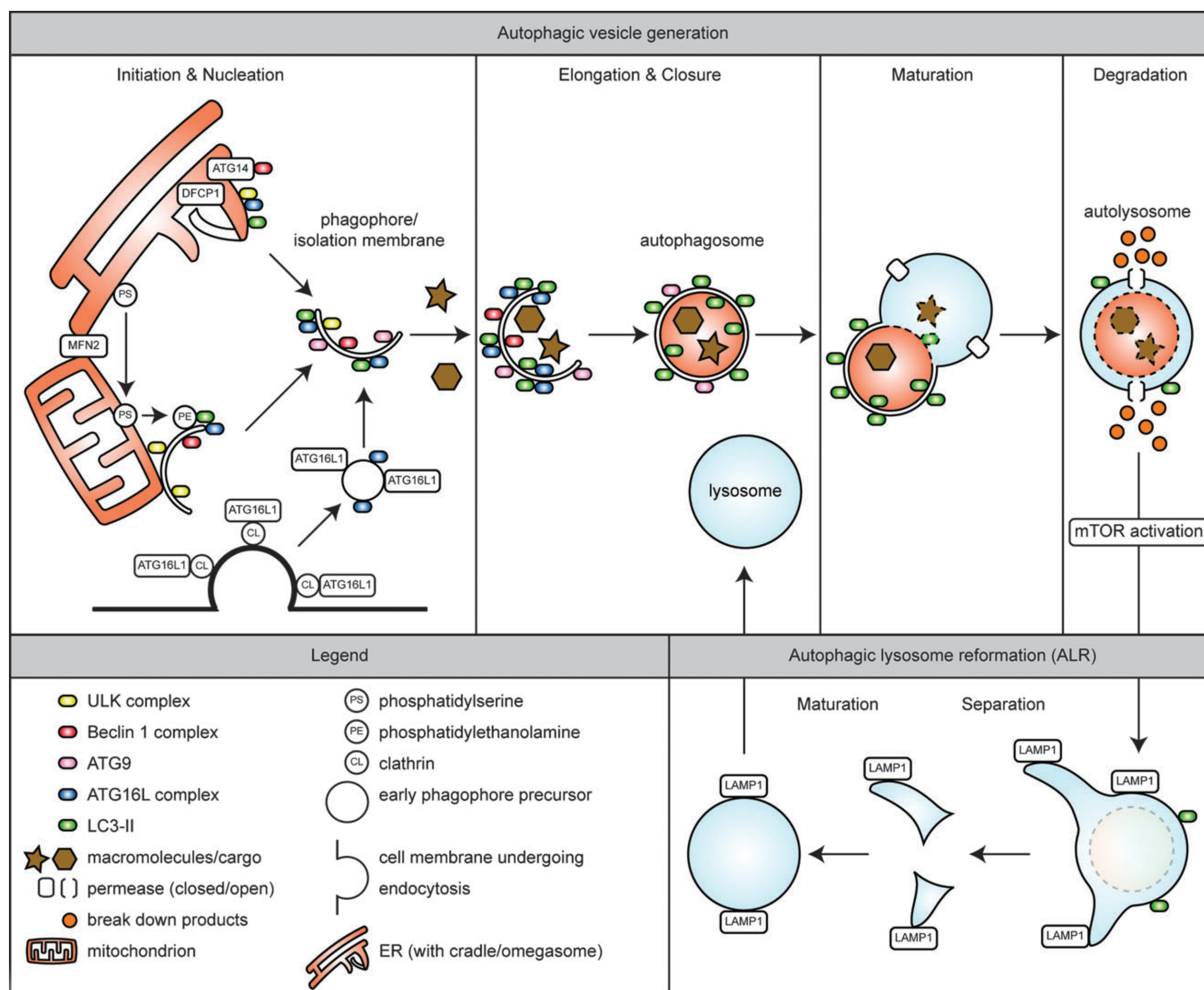

Fig. 2. Autophagic vesicle generation and recycling. The first steps of autophagosome formation are initiation and nucleation. The earliest detectable autophagic structure is the double-membrane-bound phagophore/isolation membrane that evolves from the ER, mitochondria or the plasma membrane following activation of the ULK1 and Beclin 1 complexes (initiation/nucleation). Subsequently, the ATG16L complex, LC3-II and mAtg9 are recruited to the developing isolation membrane. The membraneous structure evolves (elongation) and encapsulates marcomolecules to become the closed hallmark structure of autophagy, the autophagosome. After fusion with a lysosome (maturation), the intra-vesicular constituents of the autophagosome get degraded and released into the cytosol, thereby creating a local rise in nutrient availability. This leads to reactivation of mTOR and regeneration of a mature lysosome from autolysosomes in a process called autophagic lysosome regeneration. Members of the core autophagic machinery that are involved in each step and can be found on the corresponding structure/vesicle are shown in coloured boxes. For details, see text.

are then recruited to the omegasome/cradle, WD repeat domain, phosphoinositide interacting 2 and ZFYVE1, zinc finger, FYVE domain containing 1, the latter of which can be used to pinpoint the location of the omegasome/cradle (40,41). The phagophore extends from the PtdIns(3)P-rich region and is cradled by two ER membranes (42). Small sections of ER are encapsulated within autophagosomes by this mechanism. The ULK complex and LC3 localize to the omegasome, as well as the ATG16L complex via ATG16 (42) (Figure 2).

ATG16L1 has also been reported to be associated with the plasma membrane (36). This association was mediated by an interaction between ATG16L1 and the heavy chain of clathrin and it is believed that this interaction is required for the formation of early autophagosome precursors (Figure 2). Inhibition of clathrin-mediated internalization reduces the formation of these pre-autophagosomal structures as well as mature autophagosomes (36). It was proposed by the authors of this study that due to the size of the plasma membrane, this source of autophagosomes may be particularly important during intense autophagic activity (36). A switch may therefore occur from sources of membrane utilized under basal conditions to the plasma membrane under stressed conditions in order to perhaps maintain intracellular organelle integrity.

Mitochondria have also recently been proposed as an alternative route of phagophore generation (34). Under starving conditions, ATG5 and LC3 localize to the outer membrane of mitochondria, which serves as a cornerstone for phagophore development. Mitofusin 2 connects mitochondria to the ER and thereby enables transfer of phosphatidylserine from the ER to mitochondria, which seems to be essential for autophagosome generation. In mitochondria, phosphatidylserine then gets processed to phosphatidylethanolamine, which becomes an essential component of the developing autophagosome as described previously (29). Figure 2 illustrates the development and recycling of autolysosomes from early precursors and the involvement of the core autophagic machinery in each step.

It seems appropriate to underline that the proposed models of autophagosome generation are not mutually exclusive and probably coexist. It is possible that depending on cellular context and activating triggers, one or all routes are initiated. However, in mammalian

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cells, each model points away from the assembly model (where phagophores develop *de novo*), unlike in yeast where the phagophore develops from a phagophore assembly site (43).

Autophagy and cancer

Autophagy is known to protect us against various forms of human disease (44). In some cases, for example neurodegenerative disease, it is clear that autophagy facilitates the removal of aggregate-prone proteins that lead to Huntington's and Parkinson's disease (45). The role of autophagy in cancer, however, is more complex. There is evidence that autophagy may be oncogenic in some contexts, whereas in others, it clearly contributes to tumour suppression (4,6,46,47). In the following sections, we detail the cellular functions, such as inflammation, cell death and immunology that may contribute to tumourigenesis when autophagy is impaired and we discuss situations where dichotomies and paradoxes exist in our understanding of the role of autophagy in both the genesis and treatment of human cancer. Numerous studies have described the effects of various specific oncogenes and tumour suppressor genes on the rate of autophagy, but due to the length of this review, these will not be discussed here. For summaries of these studies, we refer the reader to the following excellent and extensive recent reviews (4,48).

Links of autophagy to cancer: cell survival, quality control, inflammation and oxidative stress. Cancer is caused by the successive acquisition of mutations and epigenetic changes that override failsafe mechanisms such as cell death and cell cycle arrest, which normally prevent tumour development (49,50). The link between autophagy and cancer is broad based. Several studies using genetically engineered mice have established a link between autophagy and cancer development (51–53). For example, hemizygosity of *Becn1* (the gene encoding Beclin 1) or complete loss of the gene encoding the UVRAG-binding protein BIF-1 (Bax-interacting protein-1, also known as Endophilin B) results in tumour susceptibility in mice (51,52,54). In addition, the ectopic expression of Beclin 1 or UVRAG have both been shown to repress the growth of human cancer cell xenografts (23,55). In line with these studies, many autophagy genes have also been found to be inactivated in human cancers (55–59). Furthermore, both chemotherapy and radiation treatments for cancer have also been reported to modulate autophagy (60).

Autophagy has an evolutionarily conserved role in buffering metabolic stress caused by limited nutrients or oxygen. During tumour growth, periods of poor vascularization lead to constraints on nutrient availability and hypoxic regions within tumours (61). Since autophagy is known to be activated in these regions and could be utilized to keep these tumour cells alive, it seems paradoxical therefore how inactivation of a process that could keep tumour cells alive could benefit tumour development. The answer to this apparently conflicting issue simply lies in the basal function of autophagy: the alleviation of stress in order to preserve cellular integrity (46). Insufficient autophagy can no longer provide nutrients, prevent the accumulation of defective proteins and organelles, manage oxidative stress and limit inflammation. As a result intra- and extracellular events occur that favour malignant transformation.

Autophagy-dependent modulation of cell death and senescence is critical for tumourigenesis. Perhaps, the largest area in which dichotomies exist in relation to autophagy in cancer is in the control of cell death. For sometime, it has been heavily debated whether autophagy can actually be considered an independent form of programmed cell death, the so-called type II cell death (62). The Nomenclature Committee of Cell Death 2009 points out that the latter term 'may misleadingly suggest a form of death occurring through autophagy, as this process often promotes cell survival' (63). The committee took a morphological approach to more clearly define the observations of many laboratories and proposed the broader description of 'cell death occurring with autophagy' rather than 'autophagic cell death' (63). In fact, it is often the case as outlined above that autophagy is activated to keep cells alive. In many cases, this effect on cell survival would enhance tumour cell viability and would be tumour promoting, whereas in other cases, the death signals are maybe too strong for the activated autophagy to counter this effect and the tumour cell therefore dies—leading to tumour suppression. Critically therefore, despite being present in these cell death scenarios, autophagy does not constitute a positive contributing factor towards cellular demise.

In contrast to its role in cell survival, several studies in *Drosophila*, *Dictyostelium discoideum* and *Caenorhabditis elegans* have, however, implicated autophagy as an executor of cell death and not just a mere co-phenomenon (64–68). To the best of our knowledge, convincing *in vivo* evidence that autophagy alone can execute cell death in mammalian cells is lacking, even though this possibility is proposed in several cell culture-based systems (69,70). One view that supports a role for autophagy in promoting cell death dictates that autophagy is not an executor of cell death *per se* but that it is a required process in certain settings in combination with other pro-death signals. One important example that may be relevant to cancer is DRAM1 (damage-regulated autophagy modulator 1) (71,72). DRAM1 belongs to the recently described DRAM family and was identified due to the fact that it is activated by DNA damage and the tumour suppressor p53 (71–73). DRAM1 is a positive modulator of autophagy and is required for the full execution of p53-induced death. When expressed alone, however, DRAM1 can modulate autophagy but does not cause cell death, indicating that autophagy is required but is not sufficient to bring about this response (71,72).

In addition to the relationship between autophagy and apoptosis, it is important to note that in many scenarios, autophagy serves to protect against necrotic cell death with possible detrimental consequences as outlined in subsequent sections (46). Autophagy also prevents anoikis, cell death that occurs after cell detachment from the extracellular matrix (46,74). Consequently, autophagy may promote survival of cells that leave their physiological context and could therefore represent a mechanism that supports metastasis. Taken together, autophagy has conflicting roles in the regulation of cell death that may have both positive and negative effects on tumour development.

It is lastly also important to mention the role of autophagy in senescence. Although not a cell death *per se*, it is certainly an important end point in tumour suppression. Senescent cells are marked by sustained cell arrest and the expression and release of secretory cytokines that attract the immune system (75). Young *et al.* (76) showed that autophagy occurs during senescence and shapes the senescence-associated secretory phenotype. Impaired autophagy delayed cytokine production and thereby might modulate the clearance of senescent cells by the immune system.

Autophagy is important for protein and organelle quality control to prevent cell damage. Autophagy removes injured organelles, misfolded and aggregated proteins. If damaged proteins persist, they are akin to a non-inheritable mutation and are a source of increased oxidative stress, both of which can have detrimental effects on cells. Stressed organelles such as the ER also force the cell to execute adaptive programmes that allow for survival and ultimately promote tumour growth. Autophagy can therefore be considered to have dual roles with respect to the management of protein quality control. On the one hand, the removal of damaged protein and organelles may promote the survival of tumour cells, whereas on the other hand, the accumulation of damaged cellular constituents may result in the production of a hostile cellular environment that may ultimately be tumour promoting. In this regard, ER stress and the unfolded protein response are found in, and are required for, a large number of tumours (77). Impaired autophagy is unable to relieve ER stress and the resulting unfolded protein response. Therefore, altered signalling persists and potentially causes a reprogramming of the cell, including nuclear factor-kappaB activation. Kongara *et al.* (78) also recently found that defective autophagy itself induces ER stress in

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mammary cells, which might contribute to the development of breast cancer in a manner independent of genotoxic stress and genomic instability. They also underlined the impact of autophagy on the role of the chaperone protein Sequestome 1/p62 in cancer (see below) because autophagy-mediated keratin homeostasis is impaired when p62 levels are altered and keratin is found to be overexpressed in breast cancer (78).

A-1 Antitrypsin deficiency causes chronic liver disease and illustrates how insufficient aggregate and organelle removal might lead to cancer (79). The disease is characterized by the occurrence of intra-hepatocytic globules that represent large aggregates of a mutant protein called Alpha-1-antitrypsin (AT) with substitution of lysine for glutamate at residue 342 within the ER. In affected livers, autophagy is significantly upregulated and responsible for the clearance of aggregates and damaged mitochondria that are injured from the stressed ER. Possibly, ER accumulation of Alpha-1-antitrypsin (AT) with substitution of lysine for glutamate at residue 342 induces the regulator of G signalling 16, which then inhibits the G protein Gαi3 and thereby alleviates Gαi3 inhibitory function on autophagy (79). It is believed that globule-containing cells transmit proliferative signals to globule-devoid cells that can then grow into adenomas and carcinomas upon chronic exposure. Furthermore, globule-containing cells are less proliferative and more resistant to apoptosis (79). Impaired autophagy would no longer be able to clear the aggregates and thus increase the transmission signals of globule-containing cells. This elevates oxidative damage resulting from injured mitochondria and increases the pressure to proliferate on globule-devoid cells.

Impaired autophagy shapes the inflammatory and immune responses to assist tumour development. Chronic inflammation is thought to be a risk factor for the development of cancer and many tumours present with an inflammatory component (80). It is believed that autophagy partly impacts on cancer development through its ability to shape the inflammatory reaction. Orderly removal of dying cells is a vital function of the organism to prevent undesirable oxidative stress and an inflammatory response that would be triggered from secondary necrosis. Impaired autophagy causes metabolically stressed and apoptosis-deficient cells to undergo necrotic cell death (46,61). The concomitant release of pro-inflammatory cytokines thereby creates a pro-proliferative environment for tumour cells (46,61).

Inflammatory bowel disease and pancreatitis are potentially examples how an underlying defect in autophagy initiates inflammation and thereby creates a pro-tumourigenic environment. Impaired autophagy is found in chronic inflammation of the pancreas and the intestine, both of which are risk factors for the development of cancer (81). Crohn's disease (CD) is an inflammatory bowel disease that usually causes transmural inflammation of the terminal ileum (small intestine) but can affect the whole gastrointestinal tract (82). Mice that are either hypomorphic for *Atg16l1* or deficient in either *Atg5* or *Atg7* in the intestine display severe cellular abnormalities that are confined to intestinal Paneth cells and resemble the changes seen in CD patients that carry the *ATG16L1* risk allele (83,84). Paneth cells have elevated levels of genes involved in peroxisome proliferator-activated receptor pathways and lipid metabolism (83–85). ATG16L1-deficient macrophages from chimeric mice (lethally irradiated mice with haematopoietic reconstitution from ATG16L1-deficient foetal liver cells) produce drastically elevated levels of the inflammatory cytokines interleukin-1β and-18 (86). It is tempting to speculate for a role of autophagy in the development of at least a subset of intestinal carcinomas because ulcerative colitis, another inflammatory bowel disease, is clearly recognized as a precancerous lesion, whereas in the case of CD, that relationship is probable but less clear (87–89).

Development of acute pancreatitis has also been attributed to retarded autophagy that results from a dysfunction of lysosomal cathepsins (90). Increased conversion of trypsinogen to trypsin by Cathepsin B coincides with a decreased action of Cathepsin L that degrades trypsinogen and trypsin. As a result, intra-acinar trypsin accumulates, digests surrounding tissue and thereby elicits an inflammatory response (90). Acute pancreatitis precedes chronic pancreatitis, which is a clear risk factor for the development of pancreatic carcinoma. CD and pancreatitis might therefore be examples of how hampered autophagy induces inflammation that does not largely result from necrotic cell death but still contributes to tumour development.

Autophagy modulates tumour immunology. Autophagy is important for the balanced execution of the innate and acquired immune response. Inhibition of autophagy via the PI3K-III inhibitor 3-methyladenine or knockdown of Beclin 1 or ATG12 drastically reduced the ability of HEK293T (human embryonic kidney cells) and melanoma cells for presentation of a model antigen or the endogenous tumour antigen gp100, whereas activation of autophagy had the opposite effect (91). Defective autophagy therefore potentially promotes tumourigenesis and inflammation by constraining immune recognition of tumour antigens.

T cells participate in inflammatory reactions and shape the response to tumour antigens (92). Autophagy-deficient T cells frequently undergo apoptosis and are less proliferative than autophagy-proficient cells (93,94). A causative explanation is that they cannot efficiently clear damaged mitochondria and thus have increased reactive oxygen species (ROS) levels and altered protein expression that favours pro-apoptotic genes (95,96).

Major histocompatibility complex class II molecules present antigenic, exogenous proteins on the cell surface of antigen-presenting cells and thereby activate CD4+ T-cells. Thymic epithelial cells require autophagy for proper selection of major histocompatibility complex-II-restricted antigen recognition i.e. to be able to separate host from foreign antigens. As a result, impaired autophagy leads to the production of T cells that recognize host proteins and illicit a strong inflammatory response (97). Epstein–Barr virus is found in a variety of human tumours, especially lymphoproliferative disorders. Autophagy-deficient antigen-presenting cells cannot process certain nuclear viral oncogenes (Epstein–Barr virus-coded nuclear antigen-1) via the major histocompatibility complex-II pathway, which are therefore not present on the cell surface and consequently evade an immune response (98–101).

Oxidative stress is a crucial component of tumourigenesis in stressed autophagy-deficient cells. The one common denominator of impaired autophagy in nearly all situations is the creation of oxidative stress. Oxidative stress results from a disparity between production and elimination of free radicals and reactive metabolites, so-called ROS. The mitochondrial respiratory chain is the main source of intracellular ROS. ROS impact on all stages of tumour formation: initiation, promotion and progression (81). Notably, physiological levels of ROS are required for many cell signalling events. Physiological concentrations of ROS are also critical for autophagy. H_2O_2 oxidizes and thereby inhibits Atg4, which leads to increased availability of lipidated LC3 during starvation (102). Cells also use a selective form of autophagy, mitophagy, to remove damaged mitochondria as a source of aberrant ROS production. Two molecular routes of mitophagy have been suggested. phosphatase and tensin homolog-induced kinase 1 recruits the E3 ubiquitin-like ligase PARKIN to compromised mitochondria, which results in ubiquitination of voltage-dependent anion channel 1 on the mitochondrial membrane and recruitment of p62 (103–105). P62 may through its LC3-interacting region steer damaged mitochondria to autophagosomes. Alternatively, mitochondrial BNIP3L (BCL2/adenovirus E1B 19kDa interacting protein 3-like (aliases: Nix, BNIP3a)) can via its LC3-interacting region interact with Atg8 homologues and deliver mitochondria to autophagosomes (106,107). Furthermore, p62 has also been implicated in the delivery of oxidized proteins for autophagic degradation. Work from Eileen White's group has underlined that management of oxidative stress is probably the key element how autophagy deficiency promotes cancer (46,61,108–110). It convincingly explains how the loss of a survival mechanism benefits cancer or in other words it explains why

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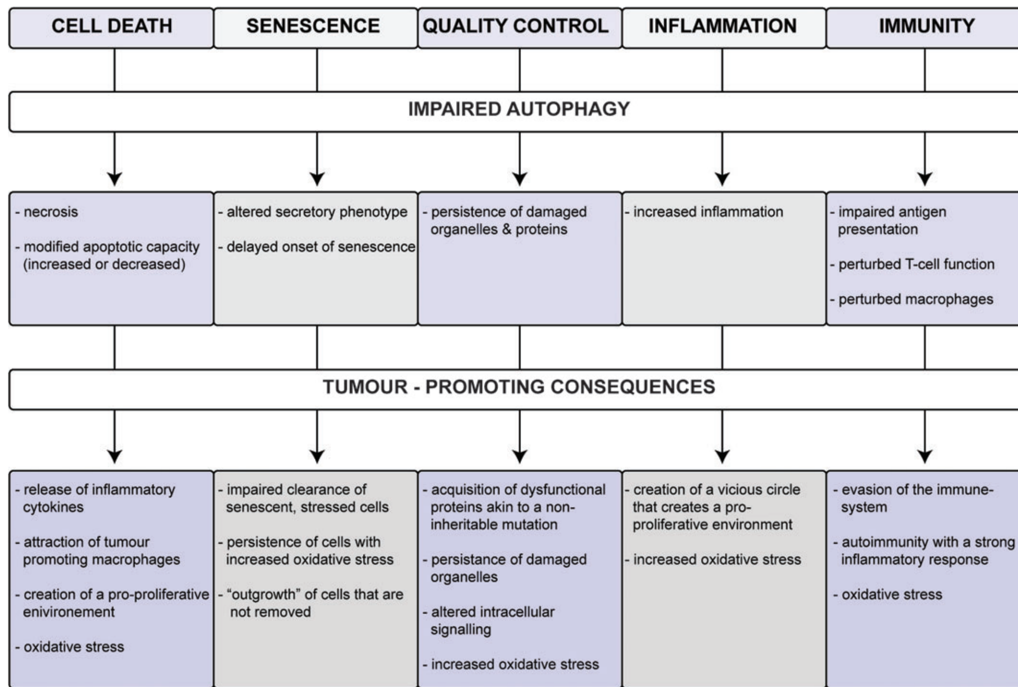

Fig. 3. Cellular and organismal functions that contribute to tumour development when autophagy is impaired. Shown is the impact of autophagy impairment on crucial cellular processes and how their alteration contributes to tumourigenesis. Importantly, oxidative stress is a recurring phenomenon in nearly all autophagy-impaired settings. For details, see text.

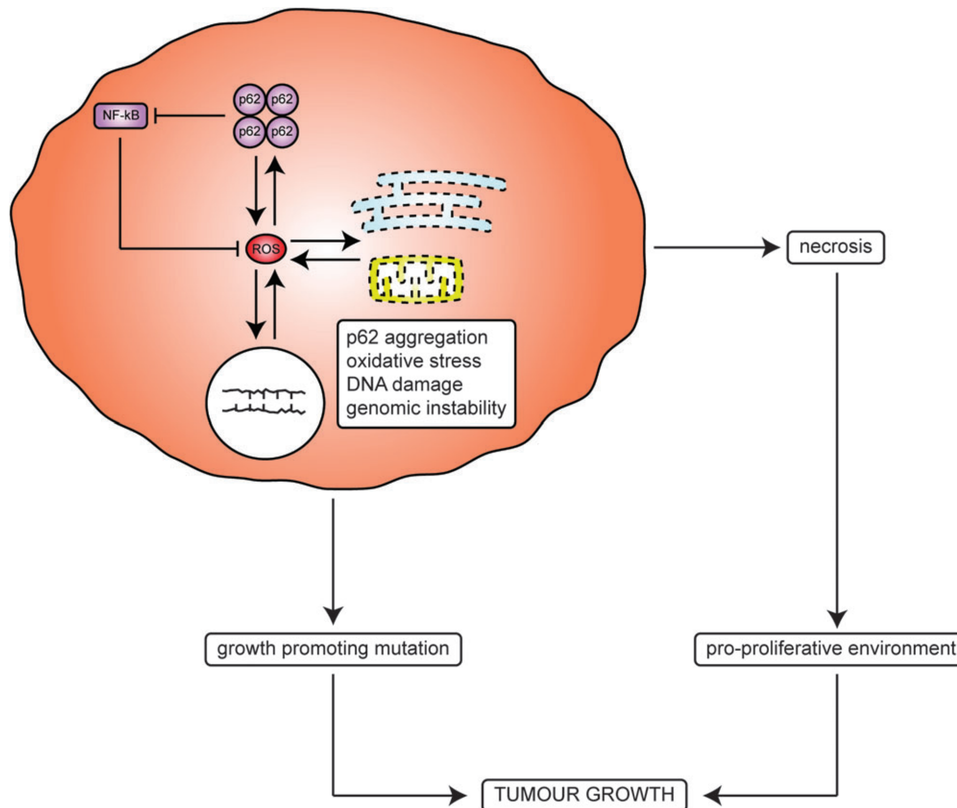

Fig. 4. Consequences of impaired management of oxidative stress in autophagy-deficient cells. Impaired autophagy results in reduced clearance of p62 and damaged organelles, both of which can fuel ROS levels and thereby aggravate oxidative stress. Furthermore, p62 aggregation alters Nf-κB signalling to favour pro-survival signalling and therefore tumourigenic events. This vicious circle of increased ROS production and decreased clearance of ROS producers and p62 aggregates leads to genetic instability and an increased propensity to mutate DNA. As a result of overburdened ROS levels and DNA damage, the cells either die by necrosis and produce a pro-tumourigenic inflammatory response or a subset of cells acquire growth-promoting mutations. For details, see text.

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autophagy-impaired tumour cells are both more susceptible to cell death while at the same time having enhanced tumourigenicity. Figure 3 summarizes how different cellular functions contribute to tumour formation/progression when autophagy is impaired.

P62 critically links impaired autophagy, oxidative stress and tumourigenesis. Degenhardt *et al.* (61) showed that autophagy is upregulated in hypoxic tumour regions and that autophagy deficiency indeed promotes cell demise but also necrotic cell death. The resulting, uncontrolled cytokine release creates an inflammatory response and attracts tumour-promoting macrophages. Genomic stress that leads to DNA damage and an increased propensity to acquire growth-promoting mutations are cell intrinsic consequences of insufficient autophagy (109,110). P62 is overexpressed in a large number of tumours (111). Sequestome-1/p62 is a multi-domain adapter protein that has emerged as a key player for autophagy-dependent quality control mechanisms (112). Mice that are deficient for *Atg7* accumulate poly-ubiquitinated aggregates in the liver and brain that co-localize with p62 and cause cellular damage (113–115). Mice lacking both p62 and Atg7 are free of aggregates, indicating that p62 is required for aggregate formation in autophagy-deficient states and that autophagy is responsible for the clearance of these aggregates (111,116). In a landmark study, Mathew *et al.* (108) showed that p62 is critically required for tumourigenesis, and depletion of p62 by autophagy suppressed tumour development. Autophagy-deficient cells are unable to clear p62 aggregates that arise as a result of metabolic stress. Increased p62 then fuels a detrimental positive feedback loop by which p62 itself leads to ROS production, enhanced induction of the protein folding machinery in the ER and a DNA damage response (108). Suppressing ROS or p62 accumulation alleviated cellular damage resulting from impaired autophagy. Strikingly, sustained expression of p62 also altered nuclear factor-kappaB signalling and thereby initiated additional molecular pathways that favour tumour formation (108). These data convincingly imply that the consequences of oxidative DNA damage and increased tumourigenesis of autophagy-deficient cells are related to insufficient clearance of p62 (Figure 4).

Summary and therapeutic perspectives

Since it is clear that autophagy has roles in tumour development, it is natural to speculate if targeting autophagy is a realistic prospect forcancer therapy. As evident from this review, one immediate problem relates to whether we can selectively target the oncogenic role played by autophagy in keeping stressed tumour cells alive, while not inhibiting its role in tumour suppression. It may well be the case that autophagy is oncogenic at certain stages of tumour development and tumour suppressive at other stages and analysis of the role played by autophagy at the different stages of cancer in mouse models will certainly provide insight into this issue. The issue, however, may be even more complicated by the fact that the oncogenic and tumour-suppressive effects of autophagy may coexist not just within one patient but even within any individual tumour. It must not be forgotten too that autophagy has many beneficial roles in our normal tissues and in an ideal world it would be best if autophagy in these contexts was not affected by a systemic therapy aimed at targeting malignant disease. We consider therefore that the identification of cellular signalling pathways, which selectively regulate autophagy in response to specific stimuli, may well hold the key to the selective targeting of autophagy in human disease. Work in our own laboratory has sought to address this point with respect to cancer by searching for signalling pathways that selectively regulate autophagy in response to hypoxia—a state common in many solid tumours, but for the most part absent in normal tissue. We found that autocrine platelet-derived growth factor family signalling—an event common in cancer—was critical for cytoprotective autophagy induced by hypoxia but was, in contrast, seemingly dispensable for autophagy induced by other stimuli (117,118). We feel that our findings, therefore, act as a proof of principle paradigm that disease-associated autophagy could be selectively targeted for therapeutic gain. Ultimately, due to the intense interest in the role of autophagy in human cancer, it is reasonable to be optimistic that new studies will lead to important insights into the significance of autophagy in tumour development that will also make targeting autophagy for cancer therapy a realistic and realizable goal.

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