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ER Stress & Autophagy in Cancer: Contenders or Partners in Crime?

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Abstract

Tumour development and progression are associated to a variety of events responsible for normal cell transformation, resulting in uncontrolled cell proliferation, tumour core formation, vascularization and, finally, tumour cell dissemination. Although oncogenic transformation represents the first episode responsible for tumour initiation, secondary events are also required to sustain cancer cell growth and survival, and inhibit cell death. ER stress and autophagy are two physiological pathways activated by cells under stress conditions, primarily to cope with stress and ensure cell survival, although unresolved or acute stress may shift pro-survival into pro-deathsignalling of both pathways. Since autophagy may represent a barrier against cell transformation, established tumours cells induce both autophagy and ER stress in response to metabolic stress to promote survival. Autophagy induction is also stimulated by the unfolded protein response (UPR) under ER stress conditions and, on the other hand, the former counterbalances ER expansion during the UPR, indicating a close and intricate mutual cross-regulation, which is exploited by cancer cells to survive and inhibit death stimuli, such as those of chemotherapeutic drugs.

Keywords: endoplasmic reticulum stress; UPR; unfolded protein response; macroautophagy; tumour; chaperone; lysosome; cell death

ER stress: survive and let die

Endoplasmic Reticulum (ER) is anorganelle with several distinct functions ranging from lipid biosynthesis, carbohydrate metabolism, detoxification (smooth ER), protein synthesis and modification (rough ER), to calcium storage.

Secreted and plasmamembrane proteins together withthose destined to other organelles are all synthesized by ribosomes attached to the cytosolic face of ER and simultaneously transferredinto the ER lumen. Once there, nascent proteins are folded and N-glycosylated and only those who passed the quality control system are delivered to the Golgi apparatus to be subjected to further modifications (e.g. O-glycosylation) and delivered to their final destination(1, 2). The protein folding system consists of a finely regulated process in which ER resident chaperones work in tight coordination with PDIs (protein disulphide isomerases) in order to allow the nascent proteins to acquire the correct structure(3-5). Thus, the ER homeostasis, consisting of an adequate amount of chaperones and PDIs, is mandatory for a correct ER activity. Moreover, ER homeostasis also depends on a balanced intra-ER redox status, particularly important for PDI activity, that is strictly dependent and controlled by ER calcium concentration. Therefore, conditions affecting ER homeostasis result in an organelle stressed status called “ER stress”(6, 7). Protein misfolding, possibly caused by environmental factors, aging, or genetic mutations, is a common basis for ER stress induction, resulting in the activation of the unfolded protein response (UPR) pathway(8). UPR is stimulated when the amount of the main ER chaperone Grp78 is not sufficient to both bind nascent/misfolded proteins and interact with the three sensors: PERK, ATF6 and IRE-1. Thus, Grp78 dissociates from the luminal tails of the three proteins and at least three signalling pathways can be activated (Figure 1).

Indeed, in absence of ER stress, PERK, IRE-1 and ATF6 are maintained in a monomeric inactive state through the interaction with Grp78. Once the latter is released, both PERK and IRE-1 dimerize and undergo to trans-phosphorylation, thus resulting in their activation. On one hand, PERK activation results in eIF2α phosphorylation and cap-dependent protein translation inhibition, favouring the translation of specific mRNAs (with an IRES sequence), such as the transcription factor ATF4. The PERK/eIF2α/ATF4 branch controls the transcription of genes for i) chaperones, ii) amino acid metabolism, and iii) redox reactions. On the other hand, IRE-1 activation mediates the unconventional splicing of an mRNA coding for the transcription factor XBP1 that, in turn, stimulates the transcription of genes for i) protein degradation (ERAD), ii) chaperones and iii) PDIs. Finally, once ATF6 dissociates from Grp78, it translocates to the Golgi apparatus where is proteolitically cleaved with the release of a cytosolic fragment (active ATF6) that translocates to the nucleus to up-regulate the transcription of genes for i) chaperones, ii) XBP1 and iii) protein secretion(9) (Figure 1).

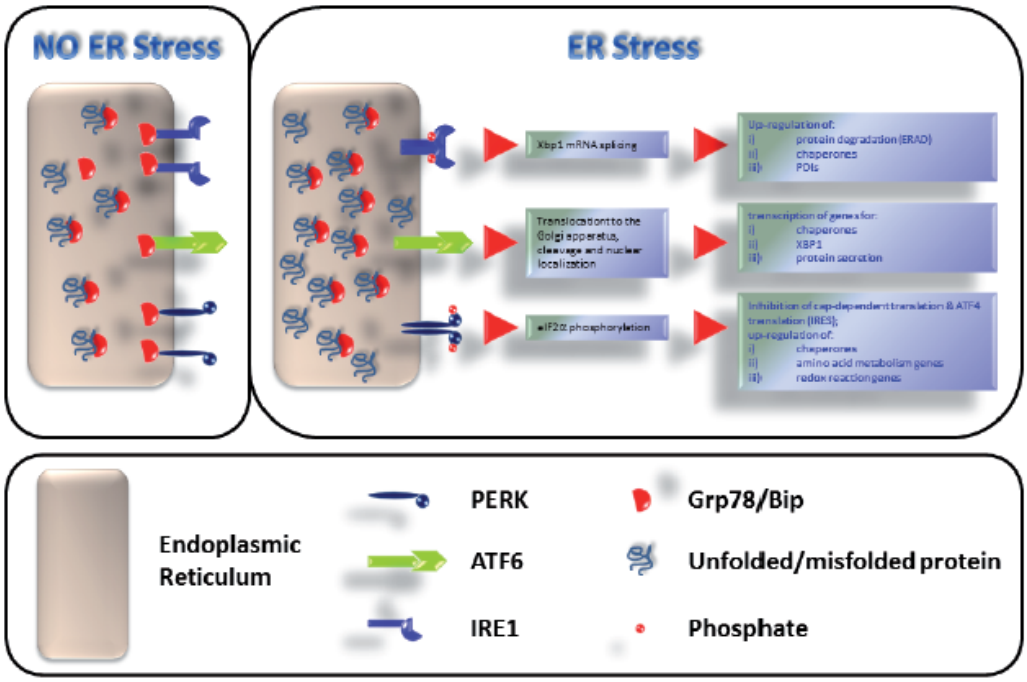

Figure 1. Cell signalling from the ER. During normal conditions, the main ER chaperone Grp78/Bip can bind both unfolded proteins and the luminal tails of the transmembrane proteins PERK, ATF6 and IRE1, the three ER stress 'sensors', inhibiting their activity. During ER stress conditions, unfolded/misfolded proteins build up 'saturating' free Grp78 and thus causing its release from ER stress sensors. PERK, ATF6 and IRE1 are then activated and initiate the UPR.

UPR activation, indeed, tend to limit the novo entry of proteins in the ER compartment and facilitates protein folding and/or degradation, representing, therefore, primarily a pro-survival process allowing the cells to adapt to stress stimuli. Ultimately, if UPR fails to restore ER homeostasis, a switch from pro-survival to apoptotic ER stress-mediated pathway takes place(10) (Figure 2).

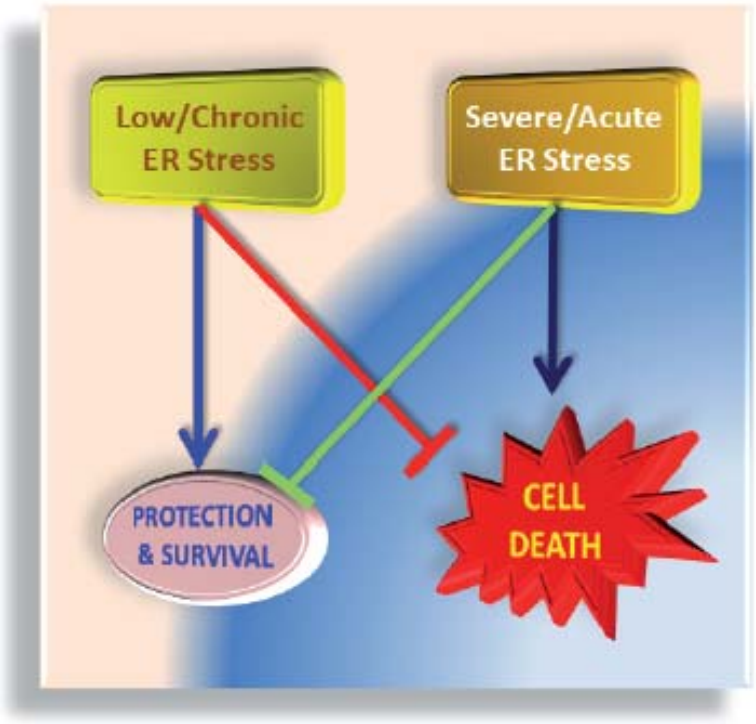

Figure 2. Differential ER stress response. During low or chronic ER stress, UPR acts as a cytoprotective mechanism inhibiting cell death and promoting cell survival. On the other hand, severe ER stress cannot cope with stress and initiates the cell death pathway.

Although the precise molecular mechanisms linking the ER stress response to the apoptotic pathway are not yet fully outlined, it is now generally accepted that a pivotal role is played by both the three UPR branches and calcium(10). The transcription factor CHOP/Gadd153, which expression is directly regulated by the PERK/ATF4 and ATF6 axes, promotes both the transcriptional repression of the pro-survival Bcl-2 factor and contributes to the up-regulation of pro-apoptotic BH3-only proteins, together with ATF4 and p53(9).

The IRE1 axis, on the other hand, stimulates the TRAF2/ASK1/JNK and the caspase apoptotic pathways.

Finally, calcium released from the ER, in response to ER stress, is promptly picked up by mitochondria that stimulates mitochondrial membrane fission and caspase activation by an uncertain mechanism, and also activates calpains which, in turn, initiate the apoptotic pathway(11).

Since ER stress can result in both cell survival by adaptation and cell death by apoptosis induction, it is highlyattractive to understand how UPR sensors are able to shift their signalling output to determine the cell fate. Two mainfascinatinghypothesis have been formulated so far to answer this question. One is based on the endpoint activation of the three UPR branches due to specific negative feedback loops.

According to this hypothesis, under ER stress, cell survival is ensured by the sequential inactivation of the IRE1, ATF6 and PERK axes, respectively, with the termination of the IRE1 signalling representing the crucial factor in allowing cell death induction after UPR activation(12). On the other hand, a second hypothesis assumes that upon UPR activation both pro-survival and pro-apoptotic genes are positively regulated to produce mRNA and proteins. However, the relative stability of both mRNA and proteins of survival/cell death factors determines the cell fate. According to this hypothesis, pro-survival factors are characterized by stable mRNA and relatively high protein half-life while pro-apoptotic factors have unstable mRNA and short protein half-life. Thus, a transient exposure should result in persistent up-regulation of pro-survival factors that facilitate adaptation without the accumulation of pro-apoptotic factors, resulting in cell survival. On the contrary, with prolonged stimulus, the initial pro-survival outcome, shared with the short exposure condition, will be shutdown and apoptosis will take place due to the gradual accumulation of pro-apoptotic factors(13).

ER stress and cancer

Cancer development is a multistep process consisting in genetic alterations driving the progressive cell transformation impairing the senescence and growth arrest control systems, stimulating uncontrolled cell proliferation and, very often, suppressing pro-apoptotic signals(14, 15). The rapidovergrowth of transformed cells generates a tumour microenvironment devoid of vascularization and, thus, characterized by hypoxia and nutrient shortage, resulting in an hostile habitat. Moreover, rapid proliferation requires new cellular structures, proteins and lipids, to be promptly produced. Overall these conditions result in a general cell stress status affecting several signalling pathways, that typically also induces an ER stress response(16). Indeed, ER stress induction has been well documented during the early stages development of several tumour types, since UPR allow cells to cope with stress, stimulating angiogenesis, enhancing protein folding and secretion, and inhibiting apoptosis induction(16). Accordingly, crucial components of the UPR apparatus, e.g. ATF4, Grp78 and Xbp1, have been found to be over-expressed in several tumours such as breast cancer, multiple myeloma, melanoma and hepatocarcinoma, and frequently associates with unfavourable prognosis, indicating a pivotal role played by ER stress in tumour development and progression(17, 18).

The notion that UPR is required for and sustains tumorigenesis is also corroborated by animalstudies demonstrating that Xbp1 is required for tumour growth in vivo since its over-expression has been associated to spontaneous multiple myeloma development, in a transgenic mouse model(19). Patients with breast cancer associated to high levels of Xbp1 showed a poor prognosis(20) and, moreover, Xbp1 also confers both estrogen independence and antiestrogen resistance in breast cancer cell lines(21). High level of Grp78 generally correlates with the occurrence of prostate, colon, breast, and liver cancers, and frequently also correlates with poor patient survival(22-24). Although the exact molecular mechanism(s) linking Grp78 to cell survival is still not completely clear, several reports demonstrate that Grp78

enhanced expression in cancer cells is responsible for: i) inhibition of apoptosis by binding and inactivating pro-apoptotic proteins such as BIK and Caspase 7, localized onto the ER membranes(25); ii) inhibition of Gadd153/CHOPexpression, the main pro-apoptotic member of the UPR(26); iii) neutralization of extracellular pro-apoptotic signals once translocated on the plasmamembrane, or secreted(27); and iv) tumour vascularization, behaving as a receptor for angiogenic peptides via a mechanism independent of the VEGF receptor(28, 29). The role of PERK in cell proliferation is still controversial with the protein exhibiting both pro- and anti-tumour properties, depending on cell and tissue environment. However, several reports indicate that PERK promotes tumour growth and angiogenesis under ER stress conditions caused by hypoxia and nutrient shortage, possibly due the activity of ATF4 and increased levels of P-eIF2α(30-32).

Overall, these data underline that in case of tumorigenesis and cancer progression, the UPR pathway is promptly stimulated to sustain cancer cell survival and proliferation.

Furthermore, ER stress associatedtumour response usually correlates to chemotherapy resistance, that is not surprisingly since UPR is fundamentally a cytoprotective response. Indeed, several reports show that ER stress induction protects various types of cancer against apoptosis induced by chemotherapeutic agents(17, 24, 33, 34) with the activation of the p38 MAPK pathway playing a pivotal role in cell survival and proliferation under drug treatment(35, 36).

Autophagy: eating the excess

Autophagy is a self-degradative process involved both in basal turnover of cellular components and in response to nutrient starvation or organelle damage in a wide range of eukaryotes(37). Autophagy represents a fundamental cellular homeostatic process that enables cells to clean up, in a finely regulated manner, portion of their own cytoplasm and degrade their constituent by lysosomal digestion(38). Low levels of basal autophagy ensure cellular homeostasis, whereas stressful conditions, including nutrient deprivation, hypoxia and low energy, lead to a rapid increase in autophagy, which allows the removal of damaged, unwanted or unnecessary constituents and their recycling in order to maintain macromolecular synthesis and energy homeostasis(39). Autophagic pathways differ in the way that cytosolic components and organelles are delivered to the lysosome, therefore, three types of autophagy have been described: micro-, macro- and chaperone-mediated autophagy, all involving the lysosomal breakdown of cytoplasmic material. Macroautophagy (hereafter autophagy) starts with the formation of a double membrane around a target region of the cell, separating the contents from the rest of the cytoplasm, to form a vesicle. Once formed, these vesicles (autophagosomes) then move along the cytoskeletal network, to fuse with the lysosomes where the sequestered material is degraded and the bulk products are released into the cytosol to be recycled(40). Although autophagy was originally described as an unspecific process for bulk degradation of cytosolic materials, the molecular mechanisms of selectivity are now starting to emerge with the discovery of a complex class of autophagy receptors that

bind to the inner sheath of autophagosomes(41). Microautophagy on the other hand differs in that cytosolic contents sequestration is facilitated by direct invagination, or projection, of the lysosomal membrane creating small intralysosomal vesicles from where the contained cytosolic material is released and broken down(42). Chaperone-mediated autophagy (CMA) differs from both macro- and micro-autophagy in that i) it is only described in mammals, ii) is involved in direct substrate delivery across the lysosomal membrane, and iii) only soluble proteins but not organelles can be degraded through CMA(43). Once recognized by cytosolic and lysosomal chaperones, specific cytosolic proteins are targeted to the lysosome by binding to lysosomal membrane receptor LAMP-2A.

Autophagy is a multistep process involving its induction, the development of an isolation membrane, the completion and maturation of an 'autophagosome' and the ultimate fusion with a lysosome (forming the autophagolysosome) for degradation by lysosomal enzymes(44) (Figure 3).

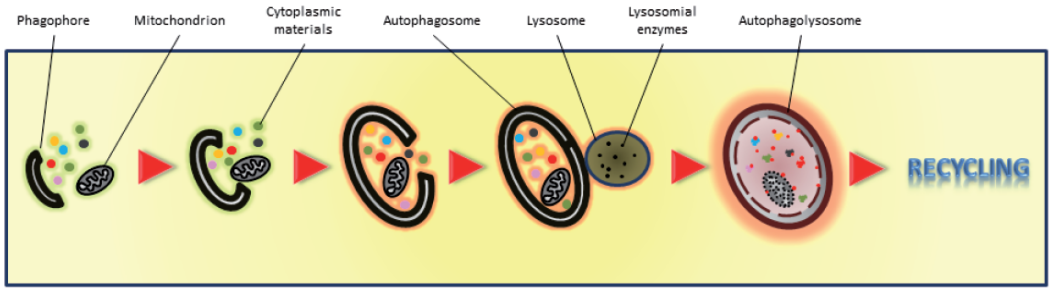

Figure 3. Autophagy program. Mammalian autophagy proceeds through a series of steps, including initiation, elongation and completion. During the initial step, a double membrane structure (phagophore) is generated, possibly by the extrusion of ER membranes; during the elongation step, the phagophore expands and wraps cytosolic organelles/molecules; after the closure of the phagophore, the generated autophagosome fuses with a lysosome (autophagolysosome) to breakdown and degrade the autophagosome inner membrane and cargo, allowing the recycling of catabolic products (completion).

Several genes, named Atg (autophagy-related), have been identified to be involved in the different steps of the assembly line that permits the formation of the autophagosome, the recognition of cargos and their delivery to the lysosome. The process is activated by a tightly regulated and intricate cascade of events involving the mammalian target of rapamycin (mTOR), the ULK1 (Atg1) kinase complex and the Class III phosphatidylinositol 3-kinase (PI3K) complex, (including Beclin 1, ATG14, UVRAG and Ambra1) which facilitate the formation of autophagosome and its targeting for lysosomal degradation. Metabolic activity and cell survival are consequently sustained by the recycling of degradation products(45).

Thus, autophagy functions mainly as a cytoprotective mechanism, favouring stress adaptation that avoids cell death. However, several reports also suggest that

autophagy contributes to cell death execution when apoptotic signalling is compromised(46). Although a physiological role of autophagy in cell death remains a matter for debate, there is evidence that autophagy may favour cell death by the selective removal of survival factors or by prolonged removal of cellular constituents, this resulting in the efficient demise of the cell(47-49).

Besides the pro-survival and pro-death roles of autophagy and apoptosis, what it is now well established is the existence of a complex regulatory interplay between the two processes. There is evidence to indicate that these processes share common regulatory factors such as, for example, BCL2 andBcl-X_L, which are able to bind Beclin 1 and inhibit autophagy(50). The crosstalk between autophagy and apoptosis is further highlighted by data showing mutual regulation of these pathways through modification of each other'sactivity. It has been demonstrated that crucial autophagy-related proteins are substrates of the apoptotic executioner proteases and the inhibition of their degradation favours the autophagy pro-survival function and counteracts cell death(51-53). On the other hand, the autophagic degradation of a subunit of the active CASP8 enzyme during TRAIL-induced autophagy, keeps the apoptotic response at bay(54). These data support the existence of cross-regulatory mechanisms between both cell fate-determining processes, so that only one process can prevail. Thus, it is becoming evident that the switch between pro-survival and pro-death pathways resides in the selective caspase-mediated degradation of key autophagic proteins to block autophagy and promote apoptosis(53).

Autophagy and cancer

The involvement of the autophagic process in several human diseases has been well documented(55), since it is known to be protective against some neurodegenerative diseases, such as Parkinson and Huntington(56), and infectious diseases(57), such as MTB(58), HCV(59) and HIV(60) infections. However, its role in tumorigenesis and cancer progression is intricate and more complex. Although many evidences indicated autophagy activation representing an anti-tumorigenic activity, it is now clear that the issue is more complicated than previously thought.

The role of autophagy as an important barrier for cellular transformation is supported by data demonstrating mice deficient for UVRAG, Bif-1, or heterozygous for Beclin1 are tumour-prone(61-63). The role played by Beclin 1 as tumour suppressor is also highlighted by the identification of its mediators, most of which are implicated in tumorigenesis, such as Bcl-2. The anti-apoptotic member of the bcl-2 family is believed to constitutively bind Beclin 1, inhibiting autophagy induction, and released by its direct JNK-mediated phosphorylation or through DAPK-mediate phosphorylation of Beclin 1(64). Furthermore, overexpression of the positive regulator of Beclin1 UVRAG activates autophagy and suppresses tumour cell growth, whereas its down-regulation results in decreases autophagy levels and triggers uncontrolled cell proliferation(65). Moreover, autophagy provides a protective function to limit necrotic cell death of transformed cells and consequent inflammation favouring tumour progression(66). Particularly important in this respect is the interplay taking place between Bcl-2 and the Beclin 1 complex(67, 68). It is also

important to note that ATG5 gene is altered in gastrointestinal cancers at the mutational or expressional level leading to the notion that somatic mutation and loss of expression of this gene might play a role in gastrointestinal cancer pathogenesis(69).

Whereas the prevailing view is that autophagy is basically a tumour suppressor process and induced by environmental stress, including nutrient deprivation and chemotherapeutic agents, it is now also emerging that some established cancers require autophagy to survive, thus describing a pro-tumour autophagy activity. Pancreatic ductal adenocarcinoma (PDAC) cells, indeed, are characterized by high constitutive basal autophagy both in vivo and in vitro and inhibition of autophagy is associated to ROS generation, DNA damage and altered cell metabolism, resulting in extensive tumour growth inhibition(70). Moreover, Ras-dependent cell transformation and tumorigenesis has been also associated to autophagy induction, promoting cellular(71).

Therefore, cellular stress occurring in established tumours can induce autophagy, which in turn constitutes a mechanism through which cancer cells protect themselves against stress and thus prolong their survival(39). The current model is therefore that autophagy suppresses tumour growth at early stages, but promotes growth later. While it is hard to track the autophagy process in human tumour tissue, emerging evidence suggests that autophagy allows advanced tumour cells to survive within the tumour microenvironment, likely due to the phenomenon of oncogene-associated "autophagy addiction"(72, 73).

Linking ER stress to autophagy in cancer: a vicious circle ?

As described above, ER stress and autophagy are pathways activated by cells under stress conditions primarily to sustain survival and suppress cell death. Although their induction, execution and termination seems apparently independent, it is now clear that they are instead interlinked at multiple levels(74), since, among the others, UPR can stimulate the induction of autophagy(75), and the latter is involved in the ER remodelling when the UPR has been turned off(76, 77). This interconnection becomes particularly interesting in circumstances of tumour development and progression, as demonstrated by the intensive research in the field.

Indeed, although the autophagic process represents a barrier against cell transformation by removing damaged organelles such as mitochondria potentially harmful due to ROS production and consequently DNA damage, and furthermore demonstrated by its frequent inhibition during early oncogenesis, by carcinogenic aberrations in oncogenes and tumour suppressor genes, both processes are positively modulated during tumour progression, when transformed cell overgrowth generates a microenvironment devoid of oxygen and nutrients, to sustain tumour growth.

More importantly, many reports contributed to demonstrate a direct link between UPR and autophagy induction, relaying on the activity of UPR mediators and calcium signalling.

In fact, activated IRE1 causes the recruitment of the adaptor TRAF2 and the subsequent activation of ASK1 and its downstream target JNK, resulting in the

phosphorylation of Bcl-2 and the release of Beclin 1 with consequent induction of autophagy(78, 79). The activation of the PERK/eIF2α/ATF4 branch determines, conversely, the up-regulation of Trb3, that in turn induces autophagy inhibiting the Akt/mTOR axis(80), and up-regulating Atg12 expression(81). ER activation is also generally associated to calcium release from the ER compartment into the cytosol, leading to the activation of several calcium-dependent signalling pathways(82). Increased cytosolic calcium may therefore link the ER stress to autophagy through: i) activation of DAPK that phosphorylates Beclin 1, thus promoting the release of the inhibitory partners Bcl-2 or Bcl-XL(83, 84); ii) activation of PKCθ that induces autophagy, although the mechanism is still unknown(85); and iii) activation of AMPK through the LBK1/CaCMKKβ/TAK1 pathway, resulting in mTOR inhibition and autophagy stimulation(86).

Finally, it has been reported that the autophagic process counterbalances ER expansion during the UPR(76), suggesting that it plays an important role in cell survival after ER stress(78), closing the circle.

Hence, the emerging intricate scenario strongly support the hypothesis that autophagy and ER stress are synergistically interconnected and play a pivotal role during tumour progression, in order to ensure cancer cell survival (Figure 4).

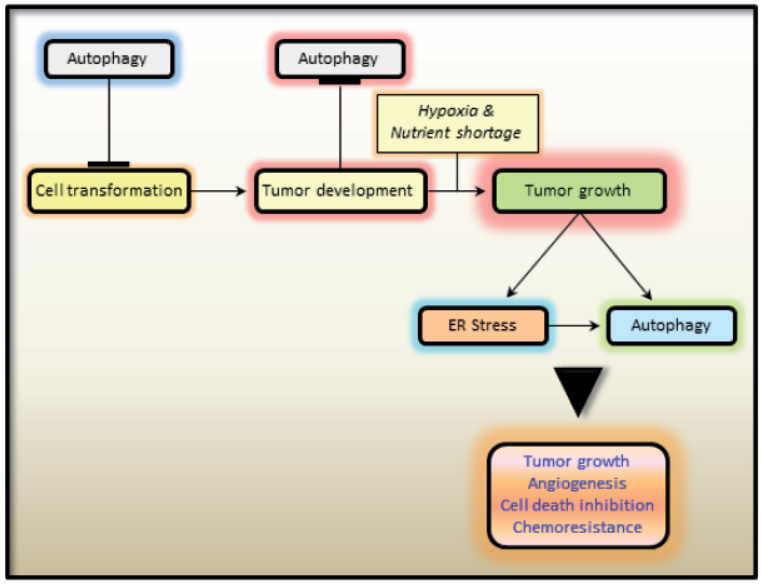

Figure 4. Role of ER stress and autophagy in cancer. Autophagy represents an important barrier for cellular transformation, inhibiting cancer initiation; however, many oncogenic signalling pathways are known to inhibit autophagy, while commonly mutated tumour suppressors are pro-autophagic; thus, tumour development may result in autophagy inhibition. Hypoxia and nutrient shortage, associated to tumour microenvironment, are responsible for both ER stress and autophagy induction to sustain tumour growth, typical in advanced stages of cancer. The close relationship between the two pathways further contribute to tumour growth strongly inhibiting cancer cell death induction and supporting chemoresistance.

Conclusions

ER stress and autophagy are both induced by tumour cells to keep them alive and reduce the effectiveness of chemotherapeutic agents, suggesting that their inhibition is potentially useful to inhibit tumour growth and improve cancer therapy. However, the concept is still highly debated and seems to be strongly related to the tumour type, since autophagy induction has been demonstrated to be required for a successful treatment of such tumours, while ER stress aggravation seems to be a promising strategy to induce tumour cell death in other malignancies(80, 87-89).

Therefore, the mutual and intricate cross-regulation of UPR and autophagy during cancer development and progression deserves a more in-depth and intensive study as it virtually opens new scenarios to better understand the molecular mechanisms of tumorigenesis and to design new specific and more efficient therapeutic approaches.

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