Manipulation of autophagy in cancer cells: an innovative strategy to fight drug resistance

Autophagy is a catabolic process activated by stress conditions and nutrient deprivation, to which it reacts by promoting the degradation of damaged organelles and misfolded/aggregated proteins, as well as generating new energetic pools. Paradoxically, in cancer cells, which signal the dangerous microenvironment occurring during clinical therapies, autophagy could promote their proliferation and sustain drug resistance. Special attention is given to autophagy manipulation in order to counteract drug resistance of cancer cells. This article describes the basic properties of autophagy and focuses on the strategies of manipulating it.

**Autophagy: a new entry in the cancer community**

- **Relevance of apoptosis & autophagy in cancer**
  The analysis of the molecular bases of carcinogenesis revealed that drug resistance of cancer cells is often due to the impaired activation of programmed cell death (PCD), and, in particular, of apoptotic pathway(s) [1]. Thus, it has been assumed that eradication of cancer could be obtained through the successful reactivation of apoptosis [2,3]. However, this dogma is not absolute, given that it has been recently discovered that cancer cell survival could be promoted by autophagy [4–6], a process in charge of maintaining normal cellular homeostasis and ensuring the regular turnover of cellular components [7,8].

- **General properties of autophagy**
  In fact, autophagy is a genuine prosurvival process that protects cells under stress conditions (e.g., nutrient deprivation, starvation and damaged organelles) by ‘eating’ part of their own components and degrading macromolecules [9,10,11]. The main function of autophagy is the prevention of necrosis and inflammation, which can lead to genetic instability, and the production of new fuel for the synthesis of molecules essential for facing a critical status [12]. However, according to a recent 'Janus’ process definition [13], the beneficial role of autophagy could ‘turn on’ the prodeath role, when stress conditions persist and autophagy may act as type II (or autophagic) PCD [14,15], which occurs in the absence of the caspase activation, typical of the more common type I PCD, and is characterized by the formation of autophagosomes and lysosomal degradation of intracellular content.

- **Dual role of autophagy in cancer**
  The dual role of autophagy implies on the one hand the promotion of tumor growth, and on the other tumor-suppressor activity. The signaling for autophagy in cancer cells originates from the so-called ‘tumor microenvironment’, which is characterized by hypoxia, extracellular pH changes, nutrient deprivation and oxidative stress sustained by reactive oxygen species (ROS) production. Autophagy normally senses these signals by degrading damaged organelles, DNA and proteins, and providing new energy, which can be instrumental to sustain cell proliferation [16–18]. This ensures the removal of unwanted/exhausted factors, the selective degradation of toxic cellular components, thus facing drug-induced chromosomal instability and sustaining cancer cell proliferation [16,19–22]. When unfavorable conditions (e.g., hypoxia, ROS and nutrient deprivation) persist, autophagy turns to the tumor-suppressor role, by blocking the cell cycle and killing cells [23]. In a context of defective autophagy, the accumulation of damaged mitochondria and ROS results in massive oxidative stress, favoring malignant transformation, having genotoxic and mutagenic effects and triggering an inflammatory processes (reviewed in [24]). The complex impact of autophagy on cancer cell metabolism is schematized in Figure 1.

- **Cell-in-cell structures: the proof of cannibalism**
  A peculiar feature of autophagy can provide a further connection with cancer. Autophagy can be considered a sort of ‘self-cannibalism’, being able to engulf and digest its own cell components, which is the ‘canonical’ role of autophagy. However, the discovery of the so-called ‘cell-in-cell
structures’, based on the detection of vacuole-like structures with viable cells enwrapped into the plasma membrane of nonphagocytic cells [25], has stimulated the new concept of xenocannibalism (or entosis) exerted by autophagy. In this context, ‘xenophagy’ [26] promotes the digestion of living cells [27,28], including the components of the immune system [29]. Recently, it was also found that xenocannibalism could promote polyplody, as an internalized cell may interfere with cytokinesis of engulfing cell host, possibly promoting tumorigenesis [30]. A further link between entosis and cancer is represented by the evidence that metastatic cells could engulf and digest surrounding normal cells; however, the nature of the signals governing this event is still unknown and little is known regarding the dynamics of the entire engulfing process, probably activated by starvation conditions that force the cell to find alternative routes to restore energetic metabolism [29].

Autophagy proceeds through different steps

- **Morphological changes**

Sequential steps regulate the complex process leading to the formation of the active autophagic entity, that is, the autolysosome. First, a double-membrane structure derived from the endoplasmic reticulum, Golgi, endosome, mitochondrial and plasma membrane [16], known as the phagophore, is formed. Under the control of an ubiquitin-like system, the ATG5–ATG12 heterodimer, in the presence of an ATG16 homodimer, constitutes a protein complex that associates to the phagophore assembly site [31], which then seals to enclose cytosolic cargos, such as long-lived/misfolded proteins and damaged organelles, thus, forming the autophagosome [78]. The subsequent vesicle elongation and autophagosomal membrane completion are regulated by two ubiquitin-like systems allowing the conjugation of ATG12 to ATG5 [32], and of LC3-I to the lipid phosphatidylethanolamine to produce the isof orm II (LC3-II) that is the most specific marker for autophagosome formation, being incorporated into the autophagosomal membrane during its elongation. Finally, the autophagosome, which contains structures of various size and shape, fuses with the lysosome to form the autolysosome, where the lysosomal content is degraded to release lipids, sugars, proteins and nucleotides in the cytoplasm, potentially reutilized or used as an energy source [31–33].

- **Biochemical reactions**

The initial stage of autophagy is characterized by the formation of the ULK complex with FIP200 and ATG13 [34]. A multimeric PI3K complex, controlled positively by UVRAG and negatively by Rubicon [35], is then formed, including Beclin-1, Vps15/34 and Ambra1 [36]; the latter factor being the positive regulator of Beclin-1 [36]. A number of kinases, including MAPKs, ERK 1/2, DAPK, PI3K/AKT, p53/AMPK, p38 and JNK, are implicated in the tight regulation of autophagy by acting on the negative regulator of autophagy mTOR, a conserved Ser/Thr kinase that has to be switched-off to permit autophagy occurrence [4,5,32,37–39]. On the whole, autophagy appears to be a finely regulated process [40].

Autophagy deregulation in cancer cells

- **Altered expression of crucial autophagy factors**

The main autophagy actors involved in discriminating the impact of autophagy in cancer cells are Beclin-1 and Atg5; their enforced loss proved to cause increased DNA damage, gene amplification and aneuploidy, in parallel with enhanced tumorigenicity [41]. Also UVRAG-experimentally depleted cells showed impaired centrosome stability, chromosome segregation and spindle formation [42]. Accordingly to in vitro data, many cancers were found to be characterized by deregulated...
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Autophagic genes or proteins (reviewed in [43,44]). Namely, in 40–75% of human breast, ovarian, and prostate cancers, the Beclin-1 gene is mono-allelically deleted [45,46]. In gastric and colorectal cancers, UVRA and other ATG genes (Atg2B, Atg5, Atg9B and Atg12), showed frameshift mutations with microsatellite instability [47,48]. The autophagic marker LC3-II is highly expressed in more than 50% of human gastric cancer, and in general is deregulated in various cancer types [49–51]. Autophagy may also result in the exhaustion of lysosomal enzymes and overload of undigested cytoplasmic constituents, which become evident as the so-called ‘stone-like’ structures (SLS), that are dense, dark brown, spheroidal structures, 1.2–12 μm in diameter, typically enclosed within cytoplasmic vacuoles [52]. A high number as well as a large size of SLS have been associated with very aggressive tumors and poor prognosis in a variety of epithelial malignancies, including breast, endometrial, colorectal, urothelial and pulmonary carcinoma [52]. From the above considerations, to assess autophagy in human patient samples, new strategies based on immunocytochemistry procedures able to monitor LC3-II or SLS have been developed [50–52].

In addition, to support the suppressor role played by autophagy in early tumor stages, it was recently observed that mice with systemic mosaic deletion of Atg5 or liver-specific deletion of Atg7 have a high incidence of benign liver adenoma [53]. Finally, the protein p62/SQSTM1 accumulates in immortalized baby mouse kidney autophagy-deficient cells after metabolic stress, leading to an increase of damage at mitochondria and DNA, as well as of oxidative stress through the activation of NF-kB [54–56].

Of note, the intrinsic meaning of the altered expression of autophagic factors is far from to be elucidated. As reviewed by Carew et al. [57], in some cancers the loss of a specific protein coincides with a poor prognosis for one type of cancer and a favorable prognosis for another, indicating that, to date, there is no univocal prognostic value.

The analysis of the correlation between autophagy and cancer focused not only on the genes and proteins directly involved in autophagy pathway execution, but also on factors that can regulate autophagy.

- **Bcl-2 family proteins**

An example is represented by the proteins involved in the apoptotic machinery and also shown to act as regulators of autophagy, such as the members of the Bcl-2 family, which are active players in cell death, as well as important inhibitors/inducers of autophagy. In particular, the overexpression of Bcl-2/Bcl-XL (or the loss of BH3-only proteins) may contribute to oncogenesis by inhibiting autophagy, favoring genomic instability and tumor progression [58].

- **Hypoxia**

Among the different signaling pathways starting from the tumor microenvironment to modulate autophagy through Bcl-2 proteins, hypoxia, a common feature of solid tumors caused by low oxygen levels, plays a crucial role. The activation of HIFs, which regulate metabolic pathways and stimulate the transcription of drug resistance associated molecules (e.g., Bcl-2/Bcl-XL and survivin), can indirectly regulate autophagy machinery [59,60]. These conditions allow cancer cells to readapt to the changing environment and to rearrange metabolic pathways in order to provide a proliferative advantage to transforming cells and promote metastatization.

The Bax-binding protein-1 Bif-1 (also known as endophilin B1 or SH3GLB1) is involved in the modulation of membrane shape during various processes and forms a complex with autophagic proteins during autophagosome biogenesis [61]. Furthermore, some NF-kB family members have a role as autophagy modulators in the context of tumorigenesis, by regulating the transcription of some pro- and/or anti-autophagic genes [62].

On the whole, all these observations are helpful to define the regulation of autophagy in cancer metabolism and underpin the intricate nature of the process, which requires further investigations aiming at identifying an experimental strategy able to control the impact of autophagy on cancer.

**Autophagy manipulation: pros & cons**

Different approaches have been used to manipulate (either to inhibit or stimulate) autophagy [63]; some of them are illustrated in Figure 1.

- **Stimulation of autophagy**

The role of autophagy in mediating survival of chemotherapy-resistant cells provides the rationale for using autophagy inhibitors alone or in combination with drugs. Many attempts at targeting a number of factors having either a direct or indirect role in the autophagic pathway have been carried out [64–67]. The strategies for targeting mTOR, the proteasome, angiogenesis and p53 are summarized below.
mTOR inhibitors

The negative regulator of autophagy is mTOR, the inhibition of which is essential to drive autophagy. This property has been exploited to search for pharmacological inhibitors of mTOR, possibly hyperactivating autophagy in cancer cells and, thus, converting this process into an efficient killer [68]. The first-generation mTOR inhibitors (rapamycin and analogs, termed rapalogs; Figure 2) act by establishing a complex with FKBP12, which interferes with mTOR activity [69]. Rapalogs are currently under evaluation in Phase III clinical trials directed against various cancers [201], as single agents or in combination. Among them, everolimus and temsirolimus (Figure 3) have been approved for the treatment of pancreatic neuro-endocrine tumors and mantle cell lymphoma, respectively, and both for metastatic renal cell carcinoma [70–72].

The second-generation mTOR inhibitors (ATP-competitive) include WYE-125132 and PI-103 (Figure 4) [73–75], the latter being a dual inhibitor of PI3K/mTOR useful to promote the apoptotic response in drug-resistant glioma cells [76]. Another dual PI3K/mTOR inhibitor, NVP-BEZ235 (Figure 4), is able to kill cancer cells by a genuine autophagy mechanism [77,78] but also by restoring apoptosis [79]. These controversial observations support the notion that apoptosis and autophagy are tightly (and dangerously) interconnected.

Critical interpretation of the results

The promising results obtained so far have to be carefully interpreted, given that it appears from the literature that autophagy can be efficiently induced only in a few rare tumors (listed above), and characterized by defective apoptosis machinery due to a general dysregulation of Bcl-2 family protein members or by the inactivation of the von Hippel–Lindau tumor suppressor gene, as it occurs in renal cell carcinoma cells [80–82]. The evidence that genetic determinants predispose cancer cells to be sensitive/resistant to rapalogs, suggests that a careful search for biomarkers to predict which cancer patients are susceptible to them could be desirable in order to develop ad hoc therapies.

Moreover, the clinical results obtained using rapalogs proved to be less potent than envisaged on the basis of encouraging preclinical research. In fact, the overall response to single-agent therapy not only has been modest in most tumors, but is also confined to few cancer types. Accordingly, their use in combination with other drugs, including PI3K inhibitors, has been developed [83,84].
Indirect inhibitors of mTOR have been studied. As described above, a network of kinases modifies the status and function of many factors involved in autophagy, including mTOR. For example, in cancer cells the elevated expression of AURKA is associated with and regulates the autophagic protein p62/SQSTM1; AURKA inhibition (by siRNA or chemicals) decreases the phosphorylation of mTOR and promotes the increase in the expression level of LC3-II in the autophagosomes, thus, enhancing the autophagic potential. However, this effect is not necessarily useful to kill cancer cells while, on the contrary, could promote drug resistance in breast cancer cells [85].

In conclusion, the net effect of mTOR inhibition on cancer cells could be linked either to the restoration of the apoptotic death, or to switch off the prosurvival activity of autophagy/switch on the death proficiency [65,86]. To date, the search for potent mTOR inhibitors remains a major issue, given that the long-term effects of mTOR inhibitors are not fully satisfactory, thus rendering their use disappointing in the clinics. Of note, the pharmacological use of rapalogs proved to potentiate the clearance of protein aggregates in experimental models of neurodegenerative diseases, allowing neuroprotection [87–89] and improving the phenotype of several animal models, including Gerstmann–Sträussler–Scheinker disease [90]. Accordingly, the induction of autophagy decreases the accumulation of polyglutamine-expanded protein aggregates and protects against misfolded protein neurotoxicity [91].

Proteasome inhibitors
Both autophagy and the ubiquitin–proteasome system (UPS) are responsible for protein degradation. Indeed, UPS first enzymatically modifies the proteins to be discarded and then promotes their transport and rapid degradation, so that the recognition signals of the cargo by the autophagosome could be mediated by ubiquitylation [92]. The best example of the joint activity of autophagy and UPS is represented by mitochondrial protein quality control, which is a fundamental process to avoid mitochondrial dysfunctions and consequently to maintain cellular homeostasis.

Mitophagy: a crucial event
In this context, mitochondrial autophagy (better known as mitophagy) degrades injured and dysfunctional mitochondria, possibly marked through the polyubiquitination of outer mitochondrial membrane proteins. For example, PINK1 recruits Parkin, an ubiquitin E3 ligase, to damaged mitochondria [93,94], where it mediates polyubiquitination and delivery to the autophagosome, also involving the adaptor protein p62/SQSTM1 and HDAC6 [95–97]. By consequence, UPS inhibition could affect protein targeting and autophagy efficiency.
through multiple pathways, including accumulation of misfolded proteins and inhibition of mTOR signaling. This leads to the excessive induction of autophagy, possibly resulting in autophagic cell death, suggesting that autophagy may contribute to the antitumor effect of proteasome inhibitors [98], such as the chemothrapeutic agent bortezomib (Figure 5), which inhibits reversibly the UPS degradation pathway and induces autophagic death in cancer cells [99]. This compound represents the best example of an innovative, US FDA-legitimated strategy to contrast cancer by a proteasome inhibitor [100]. However, recent observations point on the evidence that the proteasome inhibitor MG132 could induce a ‘non-canonical’ form of autophagy (Figure 5), that is, independent of Beclin-1, in ovarian [101] and thyroid cancer cells [102], thus, adding a further level of complexity to the scenario.

**Anti-angiogenic compounds**

The formation of new vessels from existing vessels, that is, angiogenesis, is a dangerous situation common to several disorders [103] representing a cancer hallmark that has to be targeted in order to reduce blood supply, and slow (or impede) cancer development [104]. In this respect, some experimental approaches for angio-prevention allowed the inhibition of proliferation and induction of autophagy of endothelial cells [105]. This is the case of the administration of resveratrol (trans-3,4,5-trihydroxystilbene; Figure 6), which is able to modulate cell death processes such as apoptosis and autophagy [106]. Given that in some instances resveratrol is neither completely bioavailable nor free from side effects [107], an active screening of new derivatives has been carried out. Recently, the analysis of the properties of trans-3,4-dimethoxystilbene in endothelial cells (Figure 6), revealed its ability to inhibit angiogenesis as well as induce autophagosome formation, possibly mediated by ROS overproduction [108]. Analogously, the kringle domain 5 of human plasminogen promotes both apoptosis and autophagy in endothelial cells [109].

**Undesired effects**

These promising observations have to be carefully elaborated, since anti-angiogenesis compounds are not selective mediators of autophagy but also drive apoptosis, thus rendering it difficult to delineate their molecular mechanism of action and the signaling routes. Moreover, accumulating evidence supports the correlation between cancer cell survival and the hypoxic condition caused by anti-angiogenesis therapy, possibly leading to the activation of protective autophagy [110,111].

**Modulation of p53 functions**

A link between p53 status and autophagy has been established [112–117]. The cytoplasmic form of p53 increases the synthesis of mTOR, thus, acting as a negative regulator of autophagy [118–120]; by consequence, it could be imagined to activate autophagy by blocking p53 itself [121]. Most tumors are characterized by p53 mutant accumulation, due to its ability to escape degradation by the proteasome; this event can be bypassed by inducing p53 mutant deacetylation, followed by degradation via autophagy, depletion of p53 and autophagy activation leading to cell death [122]. Strategies to modulate histone deacetylases have been developed, in the attempt of turning autophagy against cancer cells [123–125]. Emerging evidence points to the link between basic functions, such as DNA damage response, protein acetylation and autophagy, thus, supporting once more the concept that each metabolic pathway is not operating in a single compartment [126,127]. It has been demonstrated that p53 expression can be managed by glucose restriction in animal models, possibly suggesting an innovative strategy to be applied in cancer therapy [128]. In addition to the direct involvement of p53 in modulating autophagy, it also appears that proteins downstream of p53 could play a crucial role in governing the functions of autophagy in a particular context, as reported for a etoposide-induced 24 kB transcript that is involved in the response to the chemotherapeutic drug etoposide [129].
Inhibition of autophagy

Chloroquine

It is widely accepted that inhibition of autophagy in cancer cells could be beneficial in overcoming drug resistance [130]. Among the many attempts to block the unwanted role of autophagy, the use of antimalarial chloroquine (CQ) and its analog hydroxychloroquine (HCQ) (Figure 7), inexpensive oral drugs that cross the blood–brain barrier, have demonstrated promise in in vitro experiments and preclinical studies in mice [131–134]. To bypass the high toxicity of CQ, the derivative HCQ has been often employed; however, since its long half-life, requiring weeks to achieve peak concentration, and given that it has to be administered at micromolar concentrations, HCQ has a low potency as single agent [86]. Intriguingly, the effect of CQ on cancer cell lines proved to depend on the intrinsic level of autophagy, with CQ being more effective in cancers characterized by high basal autophagy, for example, Ras-driven pancreatic tumors, compared with samples with a low basal level of autophagy [135]. In fact, when used in combination with anticancer agents, HCQ improves the efficacy of the standard therapy by inducing autophagy [86]; this evidence justifies the approval by FDA and its current use in Phase I, II and III studies [57,86].

Another CQ analog, mefloquine (Figure 7), revealed interesting properties with respect to autophagy inhibition [136]. Nevertheless, the pleiotropic action of CQ/HCQ on many diseases, as well as nonhomogeneous results [137], impose a cautious optimism towards their use.

An innovative strategy based on miRNAs

miRNAs are noncoding, short (approximately 22 nucleotide) RNAi molecules generated in the nucleus and able to modulate basic processes [138]; this property has stimulated the search for strategies useful to inhibit their processing, often based on siRNA/shRNA. Among the multiple intriguing roles attributed to miRNAs [139], their involvement in autophagy has been recently described [140–145], supported by the observation that miR-18a is overexpressed under autophagy induction by drug or radiation [146]. The miR212/132 family, which is overexpressed in cardiac diseases, exerts anti-autophagic activity, thus limiting the basic role of autophagy to solve the problems coming from cardiac failure and contributing to the disease [147]. After drug treatment of cancer cells, the miRNA level is usually downregulated, as it happens for miR-199a-5p in colon carcinoma cells treated with cisplatin, where protective autophagy occurs and cells become drug resistant [148].

The mechanism through which miRNAs can affect autophagy is largely unknown, although possibly correlated to the reduced expression of Beclin-1, ATG2B, ATG4C, ATG4D, ATG5, ATG7, DICER1 and LC3-II [149–154]. Conversely, recent evidence indicates that autophagy is concerned in the modulation of the activity of proteins in charge for miRNA processing, stabilization and loading, for example, DICER, AGO-1 and -2, thus, supporting the existence of an interplay between the autophagy and miRNA universes [155,156].

Open questions

On the whole, this new aspect establishing a correlation between miRNAs and autophagy opens new perspectives of research, exploiting
the easy silencing procedure by the complementary antagomir, as well as the inhibition through different forms of oligos designed for a specific miRNA [202]. Of course, this approach is not completely free of off-target effects, given that miRNA manipulation does not solely affect autophagy but also other unintended basic processes. Moreover, the uptake, delivery (either systemically or local, often through viral vectors) and intracellular trafficking of RNAi are still far from being completely efficient and require more accurate and innovative strategies to couple high sequence specificity to stability in order to develop standard procedures based on stable RNAi [157,158].

Conclusion
A major goal in autophagy research is to decipher the molecular pathways of the process, and possibly identify the mode of modulating them, in order to obtain beneficial effects in many diseases. Several groups are investigating the role of autophagy in the complex network regulating cell proliferation and death and its opposite roles (prosurvival/prodeath), in the attempt to define its enrollment in several basic functions. From the accumulating literature, it is now clear that autophagy properties depend on intra- and extra-cellular signals, including those governing cancer development and drug resistance; however, how prodeath and prosurvival decisions are made is largely unknown. In a simplified view, autophagy is efficient to face acute stress by producing new energy, but is unable to sustain prolonged stress conditions, thus turning to a prodeath factor [94,159,160]. The search for new strategies able to overcome drug resistance has delineated the complex relationship between cancer and autophagy, which impacts on cancer metabolism in different ways, mostly depending on cancer type and stage of progression [46,161].

Many attempts have been made to modulate autophagy, some of them successful in killing cancer cells, while others are unable to bypass drug resistance. Modulation of autophagy could have deleterious effects [124,162,163]; for example, considering the immunological features of cancer cells, it is obvious that a strategy affecting the cancer microenvironment could also decrease the immune response against cancer cells, which is instrumental to beat the disease [164].

- A drawback: cross-talk with apoptosis
Finally, it has to be kept in mind that a cross-talk relation between apoptosis and autophagy exists [4,5,161,165–167], making it difficult to selectively affect a single process without interfering with the other. Among the many factors implicated in both processes, Beclin-1 is cleaved and inactivated by caspase 3, thus, being subtracted to the autophagy pathway and entering in the apoptotic one, given that its C-terminal fragment promotes the release of apoptotic factors from mitochondria [168–170]. ATG5 function is modulated by the cleavage of apoptotic proteases in order to be directed to mitochondria, where it interacts with Bcl-XL and controls cytochrome c release [171]. Proteolysis of ATG6 by caspasizes contributes to regulate its functions in the autophagy pathway cascade [169]. Caspase 8 has been proved to interact with some autophagic factors, such as pH2 and ATG5, and form a complex with Fas-associated protein with death domain, as well as ATG5 on ATG16- and LC3-positive structures, suggesting a role of the autophagosomal membrane as a platform for the activation of caspase 8 and initiation of apoptosis [168]. The situation is even more intricate because of the existence of additional, nonclassical programmed death processes that can be relevant in managing the response of cancer cells to clinical therapies [172–175].

Future perspective
In the past 10 years, autophagy has received growing attention because of its involvement not only in physiological maintenance of homeostasis and energy supply, but also for its dual role in cancer. The change of feeling with respect to autophagy is reminiscent of the situation that occurred for apoptosis, when this process was not exclusively viewed as fundamental during the development but equally responsible for drug resistance.

In parallel, with the attempts to modulate apoptosis, the authors assisted an active search for possible targets within the autophagic pathway, leading to the identification of the apical factor mTOR as a target for turning autophagy to a prodeath process. The results of clinical trials, although encouraging, revealed that rapalogs as single agents are far from specific and potent. In this respect, we can imagine that in the near future the combinatorial strategy will be more and more attempted, possibly coupling mTOR inhibitors with other compounds, including proteasome inhibitors. However, this attempt requires a solid medicinal chemistry approach that can lead to the development of highly specific inhibitors, deleterious for cancer cells and not for their normal counterparts, given that a
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general inhibition of kinases could be unwanted for noncancerous cells. The response to inhibitors is actually mediated by the genetic background of the different cancers, some of them being more responsive than others. This limitation of the efficacy of the treatment points to the absolute need to develop the bases for the so-called personalized therapy, which is to date a myth more than a reality. The next 5–10 years will be instrumental to understand if and how this strategy will be available.

The recent discovery that miRNAs could be relevant in the regulation of autophagy opens new perspectives of research, thus stimulating the search for their expression. However, more in-depth experiments have to be carried on in order to discover the mechanisms through which these short RNAs could impact the decision between proliferation/death properties of autophagy. As for the molecular approaches to be applied in order to inhibit unwanted autophagy, the near future will be characterized by the setting of protocols for stable RNAi.

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Executive summary

How the autophagic machinery works
Autophagy is a catabolic mechanism activated by stress conditions and nutrient deprivation; it proceeds through the formation of the autophagosome, a double membrane structure that encloses cytosolic cargos, such as long-lived/misfolded proteins and damaged organelles. Autophagy ensures a regular intracellular turnover and provides energy derived from catabolic digestion.

Autophagy deregulation in cancer
When stress conditions persist, autophagy may promote cell death, thus, having opposite roles in cancer progression. Indeed, on the one hand the autophagy stimulates tumor growth, and on the other could have a tumor-suppressor activity. This feature is supported by experimental results reporting the alteration of several autophagic genes or proteins in many cancer types, suggesting a regulatory role of autophagy in cancer metabolism.

Strategies to manipulate autophagy
To find new weapons to fight cancer, many experimental approaches aiming at autophagy manipulation have been developed, based on either stimulation or inhibition of the autophagic machinery. This goal was achieved mainly by the use of chemicals as single agents or in combination with other antitumor drugs, as well as by miRNA-based strategy. However, the effectiveness of these procedures is highly tumor-context dependent, pointing the need to develop ad hoc therapies.

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• Describes the roles of Bcl-2 family members.


• Reports the effect of mTOR inhibition in cancer.


• Summarizes the strategies to manipulate autophagy.


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