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Autophagy and cancer therapy cardiotoxicity: From molecular mechanisms to therapeutic opportunities☆

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ABSTRACT

Cardiotoxicity is a major drawback of anticancer therapies, often hindering optimal management of cancer. Among the most cardiotoxic agents are anthracyclines (AC) that, despite being cardiotoxic, are highly effective in the treatment of a wide variety of cancers, spanning from hematological malignancies to solid tumors. Modern imaging techniques can identify patients at risk of developing cardiotoxicity, but treatment options are still limited and ineffective, partly because the molecular mechanisms underlying AC cardiac side effects are still incompletely understood. Although AC cardiotoxicity was initially ascribed to the trigger of cell-damaging oxidative stress, antioxidants fail to prevent anthracycline-induced cardiotoxicity (AIC), suggesting the involvement of additional mechanisms. Among these, the cellular recycling process, named autophagy, recently emerged to play a key role in AIC, but whether autophagy activation is beneficial or detrimental in this context is still controversial. This review will summarize recent evidence on the role of autophagy in AIC in the attempt to reconcile the controversial findings in the field. Finally, we will describe major regulator of cardiac autophagy that may represent good candidates for therapeutic intervention in AIC.

1. Introduction

In recent years, the use of more effective anticancer therapies greatly improved the prognosis and survival of cancer patients, but at the expense of the health of their cardiovascular system. Cardiotoxicity represents the major drawback of both radiotherapy and chemotherapy, often requiring early discontinuation of the anticancer regimen. In addition, in most cases, cardiotoxicity manifests long after completion of the treatment, commonly within one year, impairing the quality of life of cancer survivors, and ultimately leading to heart failure. Once cardiotoxicity is diagnosed, patients receive standard heart failure pharmacotherapy which, however, appears to be quite ineffective, likely because of the peculiar pathogenesis of this subtype of heart disease [1].

The recent advent of a cardiology subspecialty, named Cardio-Oncology, has prompted cardiologists, oncologists, and basic scientists to combine their efforts with the aim of better understanding the molecular mechanisms underlying this pathology. Most of the mechanistic proofs accumulated so far refer to the cardiac side effects of anthracyclines (AC), such as doxorubicin (DOX), epirubicin and daunorubicin, which represent a cornerstone in the treatment of a wide variety of tumors, and that are among the most cardiotoxic anticancer drugs. Originally, AC cardiotoxicity has been ascribed to the ability of these molecules to accumulate within mitochondria, disrupt iron metabolism and generate excessive reactive oxygen species (ROS) [2]. However, the finding that anti-oxidants fail to prevent anthracycline-induced cardiotoxicity (AIC) led to the hypothesis that additional mechanisms may be involved [3]. Among these, the major cellular recycling process, namely autophagy, has received great attention in the last years, partly because the importance of such detoxifying mechanism in other types of cardiac disease started to emerge and is nowadays well established [4]. However, as in the case of other conditions, such as ischemia, the role of autophagy in AIC appears to be controversial, and there is still no clear consensus on whether activating

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or inhibiting this process may be beneficial for AIC patients.

The aim of this review is to summarize the recent knowledge on the role of autophagy in the cardiotoxicity of anticancer agents, with a major focus on anthracycline-induced cardiotoxicity (AIC). We will discuss the controversial findings in the field, in the attempt of highlighting the reasons that may account for conflicting positions. Finally, we will describe in detail key regulators of autophagy, with a main emphasis on those that may be therapeutically exploited to prevent AIC.

2. Autophagy at a glance

Autophagy is a highly conserved biological process with a relevant role in both physiological and pathological conditions. The main function of autophagy is to maintain cellular homeostasis after stress stimuli, such as nutrient starvation, energy depletion and impairment of the redox-state, promoting the removal of damaged/long-lived organelles, misfolded/aggregated proteins and intracellular pathogens [5]. Importantly, autophagy can also play an essential role in the mobilization of energy stores, such as lipids and glycogen [6]. Three different forms of autophagy are described, namely micro-autophagy, chaperone-mediated autophagy, and macro-autophagy; the latter, according to literature, is commonly used to indicate the process of autophagy [7].

The autophagic process is composed of five steps: initiation, nucleation, elongation, maturation and degradation [8] (Fig. 1). Autophagy begins with the formation of double membrane vesicles, named autophagosomes, that engulf and carry the target molecules to lysosomes, where they are degraded by lysosomal acid hydrolases. The products of the digestion, such as amino acids, fatty acids and glucose, are exported by lysosomal permeases and transporters out to the cytoplasm, where they can be re-used for building new macromolecules and for cellular metabolic reactions [9].

Autophagy-related genes (Atgs) are known as the main proteins involved in the induction of autophagy. Currently, 32 different Atgs have been identified in yeast, and many of them are conserved in plants, flies and mammals [10]. Atg1, also known as activated unc-51-like autophagy activating kinase 1 (Ulk-1), is the first protein recruited during autophagy initiation. The phosphorylation of Beclin-1 by Ulk-1 [11], and the consequent activation of vesicle-mediated vacuolar protein sorting 34 (VPS34) complexes, are crucial for the initiation of the process [12]. VPS34, which belongs to class III phosphoinositide 3-kinase (PI3K), drives the production of phosphatidylinositol 3-phosphate (PI3P), and promotes the recruitment of further Atgs necessary for the nucleation and elongation of the autophagosome [13]. The activity of microtubule-associated protein 1 light chain 3 (LC3) is essential for the subsequent step of maturation of the autophagosome. Its conversion to LC3-I by a proteolytic cleavage at C-terminus, and the subsequent conjugation with phosphatidylethanolamine (PE), produces an insoluble form, called LC3-II, that is able to insert into the double membrane of the forming vesicle [14]. The activation of LC3, which is mediated by the Atg complex formed by Atg12–Atg5-Atg16 and Atg8–PE, enables LC3-II to recruit different proteins carrying an LC3-II-interacting domain (LIR) on the autophagosome [15]. Sequestosome-1 (SQSTM1, also known as ubiquitin-binding protein p62) is the main

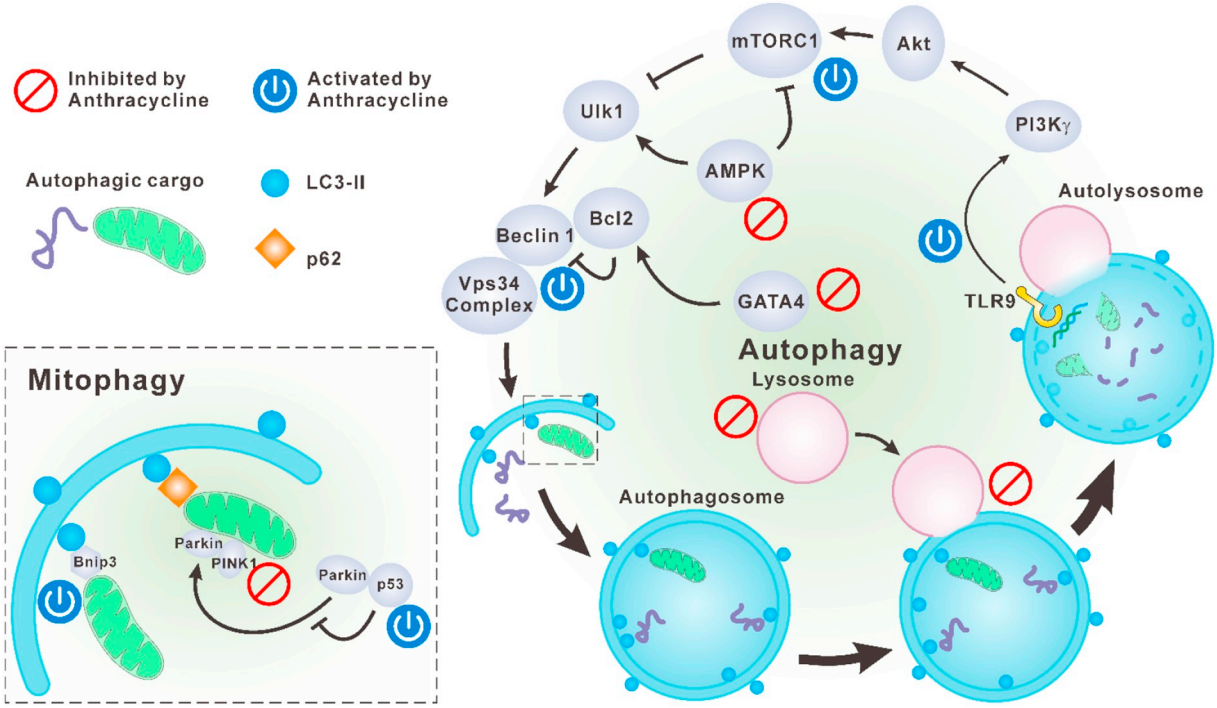

Fig. 1. Anthracyclines affect different steps of autophagy and mitophagy.
Anthracyclines (AC) block autophagy initiation by controlling mTORC1 and AMPK. AC activate mTORC1 that, in turn, inhibits autophagy by phosphorylating and inhibiting the autophagy initiator Ulk1. At the same time, AC inhibit the activity of the autophagy inducer AMPK, which is known to induce autophagy *via* inhibition of mTORC1 or activation of Ulk1, ultimately resulting in energetic and oxidative stress. On the other hand, AC can upregulate Beclin 1, another regulator of autophagy initiation, eventually leading to the accumulation of the autophagic markers, LC3-II and p62. Beclin 1 activation likely stems from inhibition of the transcriptional factor GATA4, which in normal conditions promote the interaction of the anti-apoptotic and anti-autophagic protein Bcl2 with Beclin 1, ultimately driving Beclin1 inhibition. The accumulation of LC3-II and p62 also results from AC-mediated impairment of autophagosome-lysosome fusion and of lysosome function. Undegraded cargos contained in autolysosome, such as mitochondrial DNA, can in turn activate lysosomal TLR9 and the downstream PI3Kγ/Akt/mTOR/Ulk1 signaling, converging on feedback inhibition of autophagy. (**inset** - **Mitophagy**) AC-injured mitochondria represent the major autophagic substrate in AIC. PINK1 recruits Parkin to the mitochondrial outer membrane of damaged organelles, facilitating the recognition by p62 and LC3-II and the ensuing engulfment by autophagosomes. AC activate p53, which can directly interact with Parkin and inhibit its translocation to damaged mitochondria, thus blocking mitophagy. On the other hand, AC activate Bnip3 and may promote Bnip3-mediated mitochondria recognition by LC3-II and autophagosome formation.

protein recruited *in situ* by its LIR and ubiquitin-binding domain, and functions by sequestering all the ubiquitinated proteins destined to degradation within the autophagosome [16]. Similar to p62, other proteins carrying a ubiquitin-binding domain, including "neighbor of BRCA1 gene 1" (NBR1), the TNF receptor- associated factor 2 (TRAF2) and the Smad ubiquitin regulatory factor 1 (SMURF1) participate to this mechanism [17].

As mentioned above, the initiation of the autophagic process is under the control of Ulk-1 complex-induced phosphorylation of Beclin-1 [11]. The kinase activity of Ulk-1 is finely regulated by a series of activating and inhibiting phosphorylation events, mediated by AMP activated protein kinase (AMPK) and mammalian target of Rapamycin (mTOR), respectively [18]. AMPK is the main metabolic sensor of the cell. Its activation is triggered by phosphorylation of Thr172 by Liver Kinase B1 (LKB1) and can be mediated by two different mechanisms, known as nucleotide-dependent and nucleotide-independent regulation. The first mechanism is sensitive to changes in AMP:ATP or ADP:ATP ratio, induced by different cell states, such as starvation, exercise, ischemia and mitochondrial damage. AMP and ADP molecules bind different domains of AMPK, facilitating the phosphorylation by LKB1 [19]. The nucleotide-independent regulation is better understood and is mediated by calcium/calmodulin-dependent kinase kinase 2 (CAMKKβ). Different hormone stimuli promote this mechanism, leading to activation of CAMKKβ and AMPK phosphorylation [19].

In presence of high levels of energy substrates, aminoacids or growth factors, AMPK activity is antagonized by mTOR. When activated, the mTOR pathway leads to formation of two distinct complexes, called mTORC1 and mTORC2, although only mTORC1 is linked to ULK-1 inhibition and autophagy block [20].

Since autophagy represents the main recycling mechanism of the cell, it is not surprising that autophagy deregulation underpins different pathological conditions. However, the role of this process in certain diseases, such as for example cardiovascular diseases, has long remained controversial, probably as a consequence of the complexity of the different regulatory mechanisms and actors involved. In the following sections, we will describe the role of autophagy in AIC, highlighting the major controversies in the field and describing putative molecular targets for autophagy modulation for therapeutic purposes.

3. Role of autophagy in anthracycline-induced cardiotoxicity (AIC)

The role of cardiac autophagy in AIC has long been a matter of debate. Some studies report that AC chemotherapy induces autophagy which, in turn, exacerbates cardiotoxicity, whereas other work reveals that autophagy is protective and prevents AIC (Table 1). Here, we summarize the conflicting views about autophagy regulation by AC therapy and discuss the possible reasons of these contradictory findings.

3.1. View 1: autophagy causes AIC

Early studies report that AC therapy increases autophagy *in vivo* and *in vitro* and mediates maladaptive cardiac remodeling. Lu et al. [21] first reported that AC stimulate the autophagy marker Beclin 1, and autophagic vacuole formation. This was confirmed by Kobayashi et al. [22], showing that AC increase Beclin 1 and LC3-II, another key marker of autophagosome formation, and by later studies demonstrating that AC enhance the expression of different autophagy-related markers, like LC3-II, ATG5, ATG7, p62 [23–27]. In line with these observations, overexpression of Beclin 1 exacerbates AC-induced apoptosis, whereas inhibition of Beclin 1 prevents autophagy activation and reduces cardiomyocyte apoptosis [22]. Overall, these studies point to the activation of autophagy as a primary cause of cardiomyocyte programmed cell death in response to AC and suggest inhibition of autophagy as a possible means to prevent AIC.

3.2. View 2: autophagy prevents AIC

In contrast, other studies report that autophagy is inhibited by AC. Kawaguchi et al. [28] found that acute high-dose AC administration induces the accumulation of both LC3 and p62 in mouse hearts. Further analysis of the autophagic flux revealed that AC impair autophagosome-lysosome fusion. Furthermore, AC inhibit the phosphorylation of AMPK, an upstream positive regulator of autophagy initiation, suggesting that AC dampen autophagy by both inhibiting autophagy initiation and impairing the autophagic flux. Starvation prior to AC treatment was found to restore AMPK signaling and autophagy, and ultimately to protect the heart against AC-induced contractile dysfunction. In agreement with this study, we and others have shown that LC3 and p62 accumulate and that autophagy is inhibited after AC treatment [29–33]. In addition, we found that AC inhibit autophagy through the activation of PI3Kγ/Akt signaling and that PI3Kγ inhibition reactivates autophagy and protects the heart against AIC [33]. Another recent study [34] confirms that AC block the autophagic flux and this likely stems from inhibition of the activity of vacuolar-type H+ -ATPase on lysosomes. This in turn leads to impaired lysosome acidification and blocks autophagosome-lysosome fusion, resulting in Beclin1, LC3-II and p62 accumulation. Thus, AC appears to affect later steps autophagy, such as cargo degradation, in addition to inhibiting autophagy initiation. In line with this notion, inhibition of autophagy initiation in Beclin $1^{+/-}$ mice successfully prevents chronic AC toxicity, while boosting autophagy initiation without correcting lysosome acidification defects, exacerbates cardiotoxicity.

Overall, these studies reveal that autophagy is impaired by AC and that correction of the autophagic flux attenuates the heart injury induced by AC.

3.3. Possible explanations to the conflicting findings on the role of autophagy in AIC

Whether AC increase or decrease autophagy and whether autophagy is protective or detrimental in AIC is still debated. Different factors may account for the discrepant findings described above.

First, most studies lack an accurate analysis of the autophagic flux. As described above, autophagy initiates with the formation of autophagosomes that engulf damaged or dysfunctional cellular components, progresses with the fusion of autophagosomes with lysosomes, and ends with the degradation of the cargos within autolysosomes. LC3-II is the main molecule that directly binds cargos and intermembrane of autophagosome or autolysosome and, as such, it is considered the gold standard marker of autophagy level. However, increased LC3-II levels may reflect either enhanced autophagosome formation or a block of the fusion between autophagosomes and lysosomes [35]. Therefore, the analysis of LC3-II levels in the presence of inhibitors of autophagosome-lysosome fusion, such as chloroquine and bafilomycin A1, is critical to discern between these two possible scenarios. Other tools, such as tandem fluorescence RFP-GFP-LC3 reporter, can be additionally used to measure the autophagic flux [35]. Instead, most studies claiming that AC increase autophagy do not include autophagic flux analyses and, in these cases, the simple observation of the expression levels of the autophagic markers LC3-II and p62 could lead to incorrect conclusions [21,23–27]. A comprehensive analysis of the work described above supports a model where AC-dependent increase of LC3-II in cardiomyocytes or hearts stems from an impairment of the autophagic flux.

Second, the effect of AC on autophagy may depend on the dose and the duration of the treatment. Several studies use *acute high-dose AC* regimens, which do not accurately mimic the clinical situation where patients are exposed to repeated doses of chemotherapy over time and develop cardiotoxicity even long after the completion of the treatment. On the contrary, in the attempt to be closer to the clinical situation, other studies have set up protocols of *chronic low-dose AC*, characterized by low mortality and leading to the development of a cardiomyopathy

Table 1
In vivo studies of autophagy in models of acute and chronic AC treatment.

Animal model	Treatment protocol	Endpoint	Autophagy marker change	Analysis of autophagic flux	Effects of autophagy targeting	Role of autophagy	References
Acute treatment							
Rat	6 × 2.5 mg/kg i.p. over 2 weeks	1 day	Beclin 1 increase	No	3-MA inhibits autophagy and protects against AIC	Detrimental (AC induce autophagy)	2009. Lu et al. [21]
Mouse	20 mg/kg	4 days	LC3-II increase	No	Nrdp1 overexpression enhance autophagy and exacerbates AIC	Detrimental (AC induce autophagy)	2011. Zhang et al. [23]
Mouse	2 × 10 mg/kg i.p. at 3 day intervals	2 days	LC3-II/I and p62 increase, AMPK phosphorylation decrease	Yes	Prior starvation increases AMPK signaling as well as autophagy and protects against AIC	Protective (AC blocks the autophagic flux)	2012. Kawaguchi et al. [28]
Mouse	2 × 10 mg/kg i.p. at 3 day intervals	10 days	LC3-II/I decrease, p62 increase	Yes	Rapamycin increases autophagy and protects against AIC	Protective (AC block the autophagic flux)	2013. Sishi et al. [29]
Mouse	2 × 10 mg/kg i.p. at 3 day intervals	4 days	LC3-II and p62 increase, AMPK phosphorylation decrease	Yes	Macrophage migration inhibitory factor (MIF) deficiency augments DOX-induced autolysosome formation. Rapamycin prevents AIC	Protective (AC impair autolysosome formation)	2013. Xu et al. [30]
Rat	10 mg/kg i.p.	1 day	LC3-II, p62 and Beclin 1 increase	No	Caloric restriction combined with resveratrol enhances autophagy and protects against AIC	Protective (AC impair the autophagic flux)	2014. Dutta et al. [31]
Mouse	3 × 6 mg/kg i.v. every 3 days	1–14 days	PINK1 and Parkin decrease since 2–8 days, increase at 14 days after treatment	No	Heme oxygenase-1 overexpression preserves mitophagy and protects against AIC.	Protective (AC inhibit mitophagy)	2016. Hull et al. [40]
Rat	12.5 mg/kg i.p.	3 days	Increase of autophagy vacuoles	No	NA	Detrimental (AC induce autophagy)	2018. Luo et al. [24]
Mouse	4 × 6 mg/kg i.p. at 3 day intervals	5 days	LC3-II, ATG5, p62 and Beclin 1 increase	No	Ginsenoside Rg1 inhibits autophagy and protects against AIC	Detrimental (AC induce autophagy)	2018. Xu et al. [25]
Mouse	20 mg/kg i.p.	5 days	LC3-II, ATG5 and ATG7 increase	No	Carvedilol and carnosic acid, alone or in combination, decrease autophagy and protects against AIC	Detrimental (AC induce autophagy)	2019. Zhang et al. [26]
Chronic treatment							
Mouse	8 × 3.5 mg/kg i.p. weekly	40 days	LC3-II/I, Beclin 1, Lamp 1 and p62 increase	No	TLR4 inhibition suppresses autophagy and exacerbates AIC	Protective (AC impair autophagy)	2012. Ma et al. [58]
Mouse	5 × 2.5 mg/kg i.p. over 2 weeks	8 weeks	Mitochondrial PINK1, Parkin and p62 decrease	No	p53 deficiency or Parkin overexpression preserves mitophagy and protects against AIC	Protective (AC inhibit mitophagy)	2013. Hoshino et al. [41]
Mouse	4 × 8 mg/kg i.p. weekly	1 weeks	LC3-II increase	No	Ghrelin or 3-MA reduces autophagy and protects against AIC	Detrimental (AC induce autophagy)	2014. Wang et al. [27]
Mouse	4 × 5 mg/kg i.v. weekly	4 weeks	LC3-II, p62 and Beclin 1 increase	Yes	Beclin 1 blockade inhibits autophagy initiation and protects against AIC	Protective (AC block the autophagic flux)	2016. Li et al. [34]
Mouse	3 × 4 mg/kg i.p. weekly	4 weeks	LC3-II increase	Yes	PI3Kγ inhibition enhances the autophagic flux and protects against AIC	Protective (AC block the autophagic flux)	2018. Li et al. [33]
Mouse	4 × 5 mg/kg i.v. weekly	1 weeks	LC3-II increase	Yes	HDAC6 inhibition promotes the autophagic flux and protects against AIC	Protective (AC block the autophagic flux)	2018. Song et al. [32]

Abbreviation: i.p. indicates intraperitoneal injection; i.v. indicates intravenous injection; NA indicates non-analyzed.

similar to that observed clinically (Table 1). It is conceivable that the effect of AC on autophagy is dose-dependent and that the cardiac outcome may vary depending on the extent of autophagy activation. In agreement with this view, independent studies suggest that, in certain conditions, including those of cardiac stress, a physiological level of autophagy is protective, while over-activation of autophagy may be detrimental [36,37].

In models of *chronic low-dose AC* treatment (Table 1), the autophagic flux was found dampened. Inhibition of autophagy initiation, *e.g. via* Beclin 1 blockade [34], or restoration of a physiological autophagic flux, *e.g. via* inhibition of PI3Kγ [33] and HDAC6 [32], protects the heart against AIC. On the other hand, in *acute high-dose AC* studies (Table 1), a detrimental role of autophagy is reported, as inhibition of autophagy correlates with protection against AC cardiotoxicity [21,25–27], while promotion of autophagy augments heart injury induced by AC [23]. Nevertheless, in these latter studies, an accurate analysis of the autophagic flux is missing, and no clear conclusion can be drawn on the protective *vs* detrimental role of autophagy in *acute high-dose AC* models. Comparative studies of the effects of AC on autophagy in *chronic low-dose AC vs acute high-dose AC* models are awaited to clarify the scenario.

Studies carried out in models of cardiac stress different from AIC have already confirmed that autophagy may be either protective or detrimental depending on the level of activation. For instance, cardiac autophagy plays distinct roles during the two phases of ischemia and reperfusion. In the ischemic phase, autophagy is induced and is accompanied by the activation of AMPK and a decrease of mTOR signaling. Inhibition of AMPK and autophagy exacerbates ischemia-induced cell death, thus suggesting that autophagy is protective during ischemic stress. In contrast, in the reperfusion phase, energy supply and mTOR activity are restored, while Beclin 1 is upregulated and promotes autophagy hyperactivation and heart injury. Beclin $1^{+/-}$ mice show reduced autophagy and are protected against the reperfusion insult, implying that hyperactivation of autophagy during reperfusion is a primary cause of heart injury [36]. This study also reveals that AMPK and Beclin 1 independently control autophagy and differentially contribute to cell survival/death in cardiomyocytes [36]. Interestingly, studies in *C. elegans* show that, under starvation, inhibition of autophagy aggravates food deprivation-induced death, suggesting that physiological activation of autophagy is essential to overcome the nutritional deficiency. Nevertheless, G-protein β subunit gbp-2 mutants, in which muscarinic acetylcholine receptor signaling fails to be downregulated, are more sensitive to starvation-induced death, with overactivated and unrestrained autophagy, whereas inhibition of autophagy improves the survival of gpb-2 mutant *C. elegans* in response to starvation [37]. This study suggests that physiological activation of autophagy is pro-survival, while overactivation of autophagy is pro-death.

Taken together, the discrepant findings on the protective *vs* detrimental role of autophagy in AIC may due to the lack of an accurate analysis of the autophagic flux in different studies, but also depend on the dose and the duration of treatment. Despite no consensus has been reached so far on the role of autophagy in AC cardiotoxicity, most studies support the view that AC impair the autophagic flux and, as a consequence, the efficient recycling of damaged and potentially toxic cellular components. Among these are mitochondria, where AC preferentially accumulate, as a consequence of their ability to bind cardiolipin, a key component of the inner mitochondrial membrane [38]. In the next paragraph, we will summarize our current knowledge of the effects of AC on mitochondrial autophagy, also defined as mitophagy.

4. Role of mitochondrial autophagy in AIC

In AIC, AC-injured mitochondria represent a major source of cell-damaging ROS, ultimately leading to cardiomyocyte death. Interestingly, cardiomyocytes are particularly susceptible to mitochondrial damage as they contain a high number of these organelles and lower antioxidant resources than other tissues [2]. Therefore, mitochondrial autophagy, namely mitophagy, plays a pivotal role in the maintenance of functional mitochondria and, as a consequence, of a healthy heart after AC treatment.

Several studies have investigated the mechanisms by which damaged mitochondria are recognized and engulfed by autophagosomes (reviewed in ref. [39]). Specific proteins at the outer mitochondrial membrane serve as labels that allow the recognition of dysfunctional mitochondria by LC3 and p62 to initiate mitophagy. Bnip3 (BCL2/adenovirus E1B 19 kDa interacting protein 3), Nix/Bnip3L (NIP3-like protein X), FUNDC1 (FUN14 domain containing 1) and Cardiolipin function as direct receptors for LC3 on the outer mitochondrial membrane, while PINK1 (PTEN-inducible putative kinase 1) and Parkin (Parkinson juvenile disease protein 2) mediate the ubiquitination of mitochondrial proteins and their recognition by p62 and LC3 [39]. Of note, PINK1/Parkin and Bnip3 have been shown to independently regulate mitochondrial autophagy during AC treatment.

PINK1/Parkin-mediated mitochondrial autophagy is the most well-described mechanism. In depolarized mitochondria, PINK1 fails to insert into the inner mitochondrial membrane, accumulates at the outer mitochondrial membrane and is activated. Activated PINK1, in turn, recruits Parkin, which ubiquitinates multiple proteins of the mitochondrial outer membrane. Ubiquitinated proteins can be then recognized by p62, consequently leading to the initiation of mitochondrial autophagy [39]. In AC-treated hearts, PINK1 and Parkin are downregulated in early phases (2–8 days post treatment) but significantly upregulated at later stages (14 days post treatment), suggesting that mitophagy is suppressed during AC treatment, but it is restored in the long run and likely contributes to the elimination of accumulated damaged mitochondria [40]. Nevertheless, how AC inhibit mitophagy is still not clear. An early study indicates that AC activate the apoptotic protein p53, which directly binds and sequester Parkin in the cytosol, thereby preventing its translocation to mitochondria and inhibiting mitophagy initiation [41]. In keeping with this mechanism, p53-deficient mice and Parkin-overexpressing cardiomyocytes are resistant to AC-induced inhibition of mitophagy and cell death [41].

Outer mitochondrial membrane proteins Bnip3 and Bnip3L/Nix can also bind to LC3, when activated, and guide the engulfment of damaged mitochondria by the autophagosome. Different from PINK1/Parkin, the translocation of Bnip3 and Nix to mitochondria does not require mitochondrial permeability transition pore opening and depolarization. Acute high-dose AC trigger the translocation of Bnip3 to mitochondria which in turn promotes the permeability transition pore opening and an increase in ROS production in mouse hearts. Conversely, Bnip3 inhibition *in vitro* in cardiomyocytes, with either shRNA against Bnip3 or expression of a mutant Bnip3 that fails to target mitochondria, rescues AC-induced mitochondrial respiratory chain defects. Bnip3 knock out mice ($Bnip3^{-/-}$) are also protected against AC-induced alteration of mitochondria morphology and cardiac dysfunction, implying a maladaptive role of Bnip3 during AC cardiotoxicity [42]. Notably, Bnip3 activation can mediate both necrosis and mitophagy [39], but which one between these two mechanisms underlies Bnip3-mediated AC cardiotoxicity remains unclear. Evidence supports a primary role of Bnip3-dependent necrosis, as necrotic phenotypes, including loss of nuclear HMGB1, and release of LDH and cTnT, were detected in AC-treated cardiomyocytes [42]. In this view, AC appear to induce Bnip3 activation, and promote necrosis but not mitophagy.

Overall, the two markers of mitophagy, PINK1 and Parkin, are downregulated, whereas Bnip3, a dual marker of mitophagy and necrosis, is upregulated by AC. Current evidence supports a model where AC inhibit PINK1/Parkin-mediated mitophagy and promote Bnip3-dependent necrosis. Instead, whether Bnip3-mediated mitophagy is involved in AC cardiotoxicity still needs to be clarified. Unfortunately, as described above for general autophagy, infusion of chronic low-dose AC [40,41] and acute high-dose AC [42] was differentially used in these

studies, thus preventing to reach a conclusion on whether mitochondrial autophagy is detrimental or protective in AIC.

5. Role of chaperone-mediated autophagy in AIC

Another major target of AC besides mitochondria is represented by lysosomes. As mentioned above, AC directly inhibit the vacuolar-type H^+-ATPase on lysosomes, resulting in impaired lysosome acidification and a block of autophagosome-lysosome fusion, thus disrupting the process of macro-autophagy [34]. Lysosomes are also critically involved in another type of autophagy, namely chaperone-mediated autophagy (CMA), but whether AC interrupt this process has not been explored in detail. During CMA, selective soluble cytosolic proteins can be directly targeted to lysosomes for degradation, without additional vesicle formation and cargo engulfment. CMA starts with the recognition by the cytosolic chaperone hsc70 (heat shock cognate protein of 70 kDa) of cargo protein substrates, which specially contain the pentapeptide motif sequence KFERQ, leading to the formation of the chaperone-substrate complex. This complex consequently binds to LAMP-2A on the lysosomal membrane which guides the unfolding of protein substrates by hsc70 and other co-chaperones on the lysosomal membrane. Once bound and activated by chaperone-substrate complex, LAMP-2A monomers are assembled to form multimers, which serve as the translocation complex allowing unfolded protein substrates to pass through the lysosomal membrane. The heat shock protein 90 in lysosomal lumen, namely lysosomal-associated hsp90 (lys-hsp90), is essential for the assembly and stabilization of the LAMP-2A translocation complex, whereas lysosome-associated hsc70 (lys-hsc70), a variant of cytosolic hsc70 located in the lysosomal lumen, further facilitates the translocation of protein substrates for degradation [43]. Interestingly, cytosolic hsc70 also mediates the disassembly of the LAMP-2A complex, further promoting LAMP-2A degradation by cathepsin A in lysosomal membrane microdomains, and thus rendering CMA a highly dynamic process [44].

Due to the limited number of studies, the role of CMA in AIC is still poorly understood. A first report showed that hsc70, a CMA key regulatory chaperone, is upregulated in chronic AC-treated rabbit hearts, implying that AC may stimulate a long-term CMA activation [45]. However, another work demonstrated that AC treatment in H9c2 cardiomyofibroblasts results in a decrease of hsp90 and LAMP-2A, supporting the opposite view, *i.e.* that CMA is suppressed by AC [46]. This suppression of CMA stems from an AC-mediated inhibition of transcription factor EB (TFEB), a key regulator of lysosome biogenesis, which controls the expression of hydrolases, lysosomal membrane proteins and autophagy genes [46,47]. Under physiological conditions, TFEB is phosphorylated by mTORC1 and Akt, independently, and is retained in the cytoplasm. In response to stress, such as nutrient depletion, TFEB is dephosphorylated, and translocates to the nucleus, thus regulating the expression of its target genes, and activates autophagy [47–49]. Intriguingly, AC treatment induces TFEB depletion and the consequent inhibition of proteins involved in macro-autophagy (Ulk1, LC3 and p62) and CMA (hsp90 and LAMP-2A), ultimately impairing lysosome function and autophagy flux [46]. Thus, AC appear to inhibit both macro-autophagy and CMA by suppressing TFEB. The mechanism of TFEB inhibition by AC likely involves mTORC1 activation, since inhibition of mTORC1 with Torin-1 restores TFEB and autophagy level, and ultimately protects H9c2 cells against AC-induced apoptosis and cell death [46].

Further studies are expected to provide more mechanistic insights on how AC affect CMA (*e.g.* LAMP-2 complex assembly and disassembly).

6. Druggable pathways regulating autophagy in AIC

6.1. Class I PI3K

Class I PI3Ks, including PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ isoforms, negatively regulate autophagy through the activation of Akt and mTOR. In the heart, PI3Kα and PI3Kβ are the major isoforms, whereas PI3Kγ is lowly expressed in healthy hearts but results activated upon cardiac stress, such as pressure overload [50]. We recently demonstrated that PI3Kγ signaling is similarly triggered by AC [33]. In AIC, PI3Kγ/Akt signaling activation results from accumulation of AC-damaged mitochondria which cannot be properly disposed by autophagy. The mitochondrial DNA (mtDNA) contained in autolysosomes is detected by the lysosomal CpG DNA sensor Toll-like receptor 9 (TLR9), recruits PI3Kγ and inhibits the autophagic flux in a feedback loop, thus exacerbating AC-induced cardiotoxicity [33]. Genetic or pharmacological inhibition of PI3Kγ reactivates the autophagic flux, facilitates the elimination of damaged mitochondria and ultimately prevents AIC. In contrast, inhibition of the autophagic flux by hydroxychloroquine or bafilomycin A1 completely abolishes the protective effect of PI3Kγ inhibition, further supporting a key role for PI3Kγ in the regulation of autophagy in response to AC. From a therapeutic perspective, PI3Kγ blockade thus appears a promising strategy to protect the heart against AIC, provided that means are available to achieve isoform selective inhibition. Pan-PI3K inhibition may exacerbate AC-induced cardiomyocytes apoptosis [51], since PI3Kα and PI3Kβ are indispensable for cardiomyocyte metabolism and survival. Luckily, PI3Kγ selective inhibition is feasible, since compounds like IPI145 or IPI549 are currently under clinical evaluation for cancer treatment and our study in preclinical models already showed the potential of this molecule “to kill two birds with one stone” in cancer patients, *i.e.* protecting against AIC and, at the same time, delaying tumor growth.

6.2. mTORC1

The role of mTORC1 in autophagy regulation is well described, but how mTORC1-mediated autophagy functions during AIC is still not fully understood. Zhu et al. reports that phosphorylation of mTORC1, and of its downstream signal eIF4G, is downregulated by acute high-dose AC (20 mg/kg DOX). In line with this, mice with cardiomyocyte-specific constitutively active mTORC1 (MHC-mTORca) are protected against acute AC-induced cardiac dysfunction [52]. Nevertheless, in this study the role of mTORC1 in AIC is not linked to the regulation of cardiac autophagy. In contrast, other studies demonstrate that inhibition of mTORC1 by rapamycin increases autophagy and protects the heart after AC treatment [29,30], suggesting that restoring autophagy by inhibition of mTORC1 may be beneficial against AIC. We and others further confirmed that mTORC1 and its downstream signaling, such as phospho-S6K and phospho-Ulk1 (Ser757), are upregulated by chronic AC treatment [33,34]. We reveal that mTORC1 is upregulated by AC-induced TLR9/PI3Kγ/Akt activation and inhibits autophagy *via* a feedback loop, thus exacerbating cardiotoxicity [33]. Intriguingly, despite mTORC1 activity is essential for lysosomal biogenesis and function [53], Li et al. demonstrated that mTORC1 inhibition restores autophagy initiation, but it does not release the block of the autophagic flux induced by AC [34], suggesting that AC inhibit autophagy initiation by activating mTORC1, but block the autophagic flux by inhibiting lysosome acidification independently of mTORC1.

Overall, these studies suggest that mTORC1 could be a potential target to prevent AIC. Since mTOR inhibitors, such as rapamycin and everolimus, are widely studied for cancer therapy, the combinatorial use of mTOR inhibitors and AC may provide novel strategies to treat cancer and at the same time protect the heart. Future investigation is awaited to prove this assumption.

6.3. AMPK

AMPK is a central sensor and mediator of nutrient and energy signaling. Despite AC-induced energetic, genotoxic and oxidative stress are supposed to stimulate AMPK signaling, numerous studies found that AMPK is instead inhibited by AC [28,30,54–56]. In further support of this view, MEFs derived from AMPKα1 depleted mice ($AMPK\alpha1^{-/-}$) are more susceptible to AIC [55], whereas AMPK pre-activation by multiple strategies, including prior starvation, 2-deoxyglucose (2-DG) treatment, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) treatment and adenovirus-mediated AMPK constitutive activation (AMPK-CA), attenuate cardiomyocyte and heart injury induced by AC [28,55,57]. In this regard, the energetic stress associated to AIC seems to result from AC-mediated inhibition of AMPK, rather than serving as a stimulus for AMPK activation. In this context, AMPK inhibition further leads to the blockade of autophagy and results in cardiotoxicity [28,57]. Moreover, AC-mediated AMPK inhibition can also activate mTORC1 signaling [54], which in turn may further inhibit autophagy and exacerbate AC-induced energetic stress. Conversely, AMPK activation by prior starvation or 2-DG treatment appears to protect against AC cardiotoxicity by restoring autophagy [28,57].

Overall, these investigations suggest that AMPK signaling is inhibited by AC and may consequently inhibit autophagy-based nutrient supply. Reactivation of autophagy by pharmacological activation of AMPK may serve as a potential strategy to limit AIC.

6.4. Beclin 1/Vps34 signaling

In spite of the debate on whether AC induce or block cardiac autophagy, most studies consistently found that Beclin1, a key mediator of autophagy initiation, is upregulated by AC [21,22,25,34,58]. Due to the fact that Beclin 1 knock out is embryonic lethal, Beclin 1 heterozygous-deficient mice ($Beclin\ 1^{+/-}$) were used to investigate the role of Beclin 1 in autophagy *in vivo*. Li et al. [34] found that Beclin 1 blockade reduces the initiation of autophagy, thus limiting the accumulation of damaged cellular components that cannot be properly digested by AC-injured lysosomes. As a result, $Beclin\ 1^{+/-}$ hearts are protected against the structural and functional damage induced by AC. A similar protection is observed *in vitro* in cardiomyocytes where Beclin 1 is silenced with short hairpin RNA adenovirus (AdBCN) [22]. In contrast, mice with cardiomyocyte-specific Beclin 1 overexpression show upregulation of autophagy markers in the heart and exacerbated AC-induced systolic dysfunction. Targeting VPS34 (Class III PI3K), a lipid kinase that binds directly to Beclin 1 and regulates the initiation of autophagy, display a similar cardiac protection to Beclin inhibition. 3-MA, a selective inhibitor of VPS34, significantly improves cardiac function and reduces mitochondrial injury in response to AC, by inhibiting autophagy initiation [21,22,27].

Taken together, targeting Beclin 1/Vps34 signaling could limit autophagy initiation during AC treatment and may provide a useful tool to reduce the side effect of AC chemotherapy.

6.5. GATA4

The transcription factor GATA4 is essential for cardiac development and survival of the embryo. It is highly expressed in adult cardiomyocytes, where it works together with other cardiac transcription factors, such as Nkx2-5 and Tbx5, to control cardiac gene expression and regulate cardiomyocyte survival and growth [59,60]. GATA4 expression has been shown to be suppressed by AC and promote cardiac inflammation [61]. Another study demonstrated that GATA4 is also involved in the regulation of autophagy during AIC. GATA4 silencing in cardiomyocytes triggers autophagy *per se* and aggravates AIC, whereas overexpression of GATA4 rescues AC-mediated dysregulation of autophagy and reduces cardiomyocyte death [22]. GATA4 appears to participate to autophagy regulation by directly upregulating Bcl2 [22], a protein that was originally found to be anti-apoptotic, but that also inhibits autophagy by binding and blocking Beclin 1 [62]. In this view, preserving GATA4 expression may prevent AIC by inhibiting both apoptosis and autophagy. GATA4 is thus a promising molecular target to prevent AC-induced cardiomyopathy. In agreement, silencing of miR-208a, a microRNA that direct targets GATA4, salvages GATA4 and Bcl2 level and attenuates AC-induced heart dysfunction [63].

7. The role of autophagy in other cancer therapy-induced cardiotoxicity

In contrast to AIC, less information is available on the role of autophagy in the cardiotoxicity associated to other types of chemotherapy. Among the most cardiotoxic drugs is the humanized monoclonal antibody trastuzumab, which remains the standard of care in combination with other drugs for patients diagnosed with HER2 positive breast cancer. The molecular mechanisms underlying trastuzumab cardiotoxicity are still incompletely understood but may include the production of ROS and, potentially, activation of apoptotic pathways (reviewed in ref. [64]). The role of autophagy in trastuzumab-induced cardiotoxicity has been poorly investigated. A recent study reveals a previously unappreciated mechanism by which trastuzumab suppresses autophagy in cardiomyocytes and results in increased ROS production [65]. Trastuzumab treatment in human primary cardiomyocytes disrupts the level of the major autophagic markers (increased LC3 II/I, Atg 5-12, Atg7, Atg14, Beclin 1, and decreased p62) in a time-dependent manner. Mechanistically, trastuzumab drives the phosphorylation of HER1 at Tyr-845 and HER2 at Tyr-1248, which consequently activate Erk/mTOR/Ulk1 pathway and inhibit autophagy initiation. The activation of mTOR/Ulk1 signaling depends on Erk but not Akt signaling, since Akt phosphorylation is unaffected by trastuzumab in human primary cardiomyocytes [65]. Interestingly, another anti-HER2 monoclonal antibody, pertuzumab, fails to regulate HER2/Erk/mTOR signaling and to inhibit autophagy, implying that inhibition of cardiac autophagy is a specific mechanism of trastuzumab-dependent cardiotoxicity. As a conclusively proof of the mechanistic link between trastuzumab, autophagy and ROS production, rapamycin treatment has been shown to restore autophagy level and to prevent trastuzumab-enhanced ROS production [65].

Notably, in the clinic trastuzumab is often used in conjunction with AC and co-administration of trastuzumab and AC markedly increases the incidence of heart failure [1]. While trastuzumab seems to inhibit autophagy initiation by activating HER2/mTOR signaling, AC may both inhibit autophagy induction and impair lysosome function. This complex regulation of autophagy by trastuzumab/AC cocktails thus poses a challenge to autophagy modulation as a strategy to prevent the associated cardiotoxicity.

Apart from the monoclonal antibodies trastuzumab and pertuzumab, small molecule tyrosine kinase inhibitors (TKIs), such as imatinib, sunitinib, pazopanib, sorafenib, dasatinib, lapatinib and nilotinib, are reported to induce cardiotoxicity [1]. The mechanisms of TKIs-induced cardiotoxicity primarily include “on-target” inhibition of kinases, “off-target” inhibition of non-kinases, induction of ER stress and mitochondrial dysfunction [66,67], but may also involve the dysregulation of cardiac autophagy in cardiomyocytes.

Imatinib, which targets Bcr-Abl tyrosine kinase and is employed for the treatment of chronic myeloid leukemia, was initially reported to induce left ventricular contractile dysfunction [68]. The mechanism of imatinib-induced cardiotoxicity appears to be independent of the targeting of the kinase, since genetic suppression of c-Abl activity by RNA interference does not induce cardiotoxicity. In contrast, imatinib structural analogs that do not have appreciable c-Abl inhibition (c-Abl inactive analogs) induce ER stress and cytotoxicity, further implying that imatinib induces cardiotoxicity independently of c-Abl inhibition. Intriguingly, imatinib and its inactive analogs were found to accumulate in cardiomyocyte lysosomes, resulting in the accumulation of LC3

and p62, and a block of autophagy [69]. Another study reported an increase of LAMP-2 staining in imatinib-treated rat hearts, further suggesting that imatinib may induce cardiomyocyte dysfunction through disruption of autophagy and induction of ER stress [70]. Nevertheless, these early studies did not perform comprehensive analysis of the autophagy flux, leaving the role of autophagy during imatinib treatment still uncertain. Moreover, a long-term study on a large population, involving the observation of 942 gastrointestinal stromal tumor patients for 24 months, declared that, different from what suggested initially, imatinib does not significantly induce cardiac left ventricular failure [71]. Instead, imatinib as an add-on therapy for pulmonary arterial hypertension improves exercise capacity, hemodynamics, right ventricular function and left ventricular early diastolic relaxation in patients [72,73]. These latter studies thus critically questioned the clinical relevance of imatinib cardiotoxicity and diminished the interest of basic scientists in understanding if and how imatinib affects cardiac function. On the other hand, efforts are still required to explore the potential link of autophagy with other types of TKIs, such as sunitinib and pazopanib, which are associated with congestive heart failure [74].

8. Cancer-induced cardiac cachexia and autophagy

While the cardiotoxicity induced by antineoplastic treatments has been largely studied, the direct influence of cancer on the heart has received less attention. Despite the fact that cancer cells rarely invade the heart, numerous studies indicate that the tumor can have systemic effects and promote cardiac cachexia. This pathological perturbation leads to metabolic changes and cardiac muscle atrophy, associated with arrhythmias, fibrosis and inflammation [75–78]. In 1968, Burch et al. firstly found that patients died from advanced malignancy show a remarkably small size of the heart [79]. Further studies in rodent models of cancer cachexia demonstrated that this process is driven by cellular atrophy rather than apoptosis [75,80].

Our current understanding of the mechanisms underlying cancer-induced cachexia benefits mostly from the study of skeletal muscle wasting [81], underrating for a long time its impact on the heart. The major cause of muscle mass loss during cancer is due to metabolic imbalance between synthesis and protein degradation [82]. Many factors, such as hormones, miRNA and pro-inflammatory cytokines, that are released or induced by the tumor, mediate the process of muscle wasting [76,83]. In this context, autophagy was initially considered not relevant and only recently received attention, although its role on skeletal muscle wasting is still controversial [84]. Catabolic/proteolytic pathways, such as calcium-dependent calpains and ubiquitin-proteasome system (UPS), were initially reported to be mainly involved in the process of both cardiac and skeletal muscle cachexia. Nevertheless, a growing number of studies show a significant activation of autophagy in the heart of different cachectic rodent models [75,80,85], coordinated with a marked UPS activity [86,87] and decreased protein synthesis [80]. Evidence of aberrant cardiac autophagy was found in a mouse model of colorectal cancer, in which interleukin-6 (IL-6) overproduction leads to an increase of the autophagy marker Beclin 1 and of AMPK activity, along with a decrease of Akt-independent mTOR phosphorylation [80]. Other inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-12 (IL-12), tumor necrosis factor α (TNF-α) and interferon γ (IFNγ) are also reported to promote chronic activation of autophagy in tumor-bearing cachectic rats [85,88–90]. These results suggest that humoral factors, released or induced by the cancer, indirectly trigger a deleterious activation of autophagy, which contributes to cardiac cachexia during cancer development.

Currently, there are no specific treatments approved for cancer-induced cachexia. A recent study suggests insulin supplementation as a strategy to activate Akt signaling and reduce cardiac autophagy and cachexia during melanoma and colon carcinoma in mice [78]. A clear understanding of the events leading to maladaptive activation of autophagy by the tumor could help identifying new promising targets. However, given that boosting the autophagy flux could be beneficial to overcome the cardiotoxicity associated to anti-cancer treatments, a key issue would be to find the balance between activating autophagy to limit drug-induced cardiotoxicity on one hand, and inhibiting autophagy to avoid cardiac cachexia on the other hand.

9. Models to investigate cancer therapy cardiotoxicity

Our current understanding of the molecular mechanisms underlying cancer therapy cardiotoxicity stems from the availability of suitable pre-clinical models. Rodent animal models and cultured neonatal/adult cardiomyocytes are the most commonly used systems to study the cardiotoxicity of anti-cancer drugs. Recently, human-induced pluripotent stem cells (hiPSC) have emerged as an additional and useful platform to assess drug toxicity for personalized and precision cardiovascular medicine [91].

As mentioned above, the choice of the preclinical model to investigate the molecular mechanisms of cardiotoxicity is critical. Chronic models with continuous low dose AC injection and acute models with high dose AC treatment were indifferently used and this led to controversial findings, especially as regards the role of autophagy in AIC. Since high dose AC treatment has been associated to higher risk of cardiovascular events in the clinic, repeated low doses of AC now represent the current clinical practice in the attempt to reduce acute cardiotoxicity [92]. Also in animal models, chronic low-dose AC treatment appears to better mimic the clinical situation, being characterized by low acute mortality and by the development of late-onset cardiomyopathy. Furthermore, the use of tumor-bearing animals should be considered when investigating the mechanisms of cancer therapy cardiotoxicity for at least two reasons. First, recent studies reported that the cancer *per se* induces cardiac atrophy and cardiomyopathy [78], further complicating the cardiotoxicity of anti-cancer drugs. Second, strategies aiming at reducing the cancer therapy-associated cardiotoxicity may interfere with tumor growth or with the anti-cancer action of the drug and this should be carefully evaluated. Cardioprotective treatments should not only protect the heart, but at least preserve or even raise the antitumor efficacy of cancer therapy.

Nevertheless, animal models could be inaccurate for assessing human cardiac responses to anti-cancer drugs, due to the fact that electrophysiological properties of murine, canine and rabbit cardiomyocytes are different from those of human cells [93]. On these grounds, hiPSC-derived cardiomyocytes (hiPSC-CMs) recently emerged as a powerful tool to investigate individual cardiac response to anti-cancer therapy (reviewed in ref. [91]). hiPSC-CMs exposed to AC display the typical hallmarks of AC cardiotoxicity, including apoptotic and necrotic cell death, ROS production and mitochondrial dysfunction [94]. In hiPSC-CMs, genetic disruption of topoisomerase 2β (Top2β) recapitulates the cardioprotective effect detected in animal models [95] by reducing AC-induced double stranded DNA breaks and cell death [94]. Burridge et al. further demonstrated that hiPSC-CMs derived from breast cancer patients who developed AIC are more sensitive to AC toxicity than hiPSC-CMs from patients who did not experience AIC [96], thus suggesting hiPSC-CMs as a suitable platform to identify the molecular mechanisms of AIC and to predict the risk of cardiotoxicity in patients. A recent study further extends the application of hiPSC-CMs, hiPSC-derived endothelial cells (hiPSC-ECs) and hiPSC-derived cardiac fibroblasts (hiPSC-CFs) to screen cardiotoxicity of sets of TKIs. This high-throughput screening identified a group of TKIs targeting VEGFR2/PDGFR, including sorafenib, regorafenib and ponatinib, characterized by high cardiotoxic activity, and figured out that these TKIs induce cardiotoxicity by disrupting insulin and insulin-like growth factor (IGF) signaling [97]. Therefore, hiPSC could be further exploited in the future not only to predict cardiotoxicity but also to investigate the role of autophagy in cancer therapy cardiotoxicity and to explore the possibility of targeting this process pharmacologically for therapy.

10. Concluding remarks and perspectives

Studies in recent years have revealed autophagy as an important mechanism underlying AIC, though leaving an open controversy on whether AC increase or decrease autophagy and whether autophagy is protective or detrimental in this context. This uncertainty primarily stems from the lack of a systematic and accurate analysis of the autophagic flux in most of the studies as well as from the use of different models of AIC (acute high-dose *vs* chronic low-dose), potentially affecting the process of autophagy at different steps and extents. Despite the existence of controversial findings, the emerging and most accepted view in the field is that autophagy is essential to maintain a healthy heart and that this mechanism is impaired by AC. This is in line with the well-established beneficial role of autophagy in other types of cardiac diseases. Activation of autophagy has been proven to protect the heart from different types of stress, including oxidative stress-induced toxicity [98] and protein misfolding-induced cardiomyopathy [99]. In contrast, impairment of cardiac autophagy *per se* results in cardiomyopathy [100] and could further exacerbates other stress-induced heart failure [101]. In the context of cancer therapy cardiotoxicity, and especially of AIC, cardiomyocytes likely activate autophagy in the attempt to cope with the cardiac damage elicited by chemotherapy and to ensure survival. However, in the end autophagy fails to protect from toxicity due to 1) impaired lysosomal function [34] and 2) activation of an inhibitory feedback loop, involving PI3K and mTORC1 [33] (Fig. 1). In this view, correction of the autophagic flux could serve as a potential strategy to limit cancer therapy cardiotoxicity.

A growing number of autophagy regulators in the context of AIC have started to be uncovered, and include PI3Kγ, mTORC1, AMPK, Beclin 1, GATA4 as well as mitophagy-specific molecules like PINK1, Parkin and Bnip3 (Fig. 1). Nevertheless, how these signals are interconnected in the regulation of autophagy is still mysterious and additional work is awaited to provide proof-of-concept of pharmacological targeting of these enzymes as a therapeutic option for AIC. In this view, another relatively unexplored area is represented by the role of autophagy in other cardiac cell subtypes which are critically involved in AIC, including for example endothelial cells, fibroblasts and cardiac progenitor cells (CPCs). These latter, for instance, have been shown to experience premature senescence in response to AC, characterized by DNA damage, increased p53 expression, telomere attrition, and apoptosis [102], with a consequent reduction in their ability to promote cardiac repair. A recent study showed that AC also activates mTOR signaling eventually impairing autophagy in CPCs, but how this is linked to AIC development is yet to be determined [103]. Finally, since autophagy may promote the resistance of cancer cells to chemotherapy [104], targeting autophagy for cardioprotection may result in unwanted pro-tumorigenic effects. Thus, extensive investigation is required to conclusively clarify the feasibility of targeting cardiac autophagy to prevent AIC.

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Transparency document

The Transparency document associated with this article can be found, in online version.

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