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Review article

Cardiomyocyte autophagy and cancer chemotherapy

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ABSTRACT

Autophagy, an evolutionally conserved process of controlled cellular cannibalization, plays a vital role in cardiac physiology. Perturbations in cardiomyocyte autophagy contribute to the pathogenesis of a wide range of cardiac diseases, many of which culminate in heart failure. With recent advances in cancer chemotherapy and consequent improvements in cancer survival, drug-induced toxicity to the heart has assumed greater importance. As a number of prominent cellular pathways are critical to the survival of both tumor cells and heart cells, it comes as little surprise that therapies targeting those pathways have consequences in both tissues. Little is known presently about cardiomyocyte autophagy, a prominent cellular response to stress, in the setting of chemotherapy, but preliminary evidence suggests an important and context-dependent role. Dissecting the role of autophagy in "onco-cardiology" will likely yield insights into mechanisms underlying cardiomyopathy and may lead to novel means to protect the myocardium from chemotherapy-induced injury. This article is part of a Special Issue entitled "Protein Quality Control, the Ubiquitin Proteasome System, and Autophagy".

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Contents

1. Introduction

Recent advances in oncologic medicine, including early diagnosis and novel therapies, have significantly improved the long-term survival of patients with cancer [1]. This is especially true in pediatric oncology, as most children diagnosed with cancer today are expected to be long-term survivors [2]. However, as a consequence of these successes, cancer therapy-related complications are replacing tumor recurrence and secondary neoplasia as major clinical issues. Among those complications, cardiotoxicity has emerged as a prominent cause of chemotherapy-related co-morbidity and mortality [3], presenting as a spectrum of clinical manifestations that includes left ventricular dysfunction,

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arrhythmia, ischemia, and pericarditis. In addition, in many cancer patients concomitant cardiovascular comorbidities exist which synergize with the stress of chemotherapy. Also, emergence of new anti-cancer drugs and the prominence of combination therapies together heighten the concern for potential untoward cardiac toxicities.

Our understanding of mechanisms underlying chemotherapy-induced cardiotoxicity is limited. Further complicating the picture is the fact that these mechanisms vary widely. Drugs such as anthracyclines and HER-2 receptor inhibitors provoke direct cardiomyocyte injury, while others such as anti-metabolics cause indirect cardiac effects by inducing hypertension or thrombotic events. Among the direct toxicities, accumulation of reactive oxygen species (ROS), mitochondrial damage, endoplasmic reticulum (ER) stress, disruption of pro-survival signaling pathways, and metabolic alterations have been implicated [4,5]. Recent reviews have discussed molecular mechanisms associated with cancer chemotherapy [6]. Here, we focus specifically on autophagy, a less appreciated aspect of chemotherapy-induced cardiotoxicity. Although the possible role of cardiomyocyte autophagy in cancer therapy-induced cardiotoxicity is uncertain and numerous contradictory observations have been reported in literature, there are strong hints suggesting a significant contribution.

1.1. Autophagy and its molecular regulation

Autophagy, an evolutionarily conserved cellular cannibalization process, has gained increasing recognition in recent years for its vital role in cardiac physiology and pathology [7]. Autophagy is a generic name for different routes of delivery of cytosolic materials to the lysosome for degradation [8]. Three major forms of autophagy have been described: macroautophagy, microautophagy, and chaperone-mediated autophagy [8]. Macroautophagy, the most extensively studied type and hereafter termed autophagy, involves sequestration of cellular contents into double-membrane autophagosomes followed by cargo delivery to lysosomes for bulk degradation. At present, nothing is known about possible involvement of microautophagy or chaperone-mediated autophagy in chemotherapy-induced cardiomyopathy.

Autophagy is critical to cellular survival under baseline, resting conditions, serving to maintain cellular homeostasis, recycle cellular constituents such as mitochondria and ER, and eliminate misfolded, dysfunctional proteins. In response to cellular stress, such as starvation, autophagic activation is up-regulated, recycling macromolecules to replenish essential substrates for energy production [8,9]. In animal models, defective autophagy leads to perinatal death due to severe nutritional deficiency prior to proper feeding [9]. In later stages of life, defective autophagy accelerates aging, promoting end-organ damage and reduced lifespan [10–12].

Molecular mechanisms of autophagy are highly conserved from yeast to human. Autophagy is initiated by formation of a phagophore, an isolated membrane that originates from the ER or other cellular membranes, such as mitochondria and plasma membrane [9,13]. The process initiates with the formation of a multiprotein complex containing Beclin 1, Atg14L, Vps34 and Vps15 (p150). Next, phagophore elongation is initiated by two ubiquitin-like conjugation cascades: a) the Atg5–Atg12 conjugation system, and b) the microtubule-associated light chain 3 (MAP-LC3/Atg8/LC3) conjugation system. As the phagophore elongates, it progressively engulfs a portion of the cytoplasm, including proteins and organelles. Ultimately, the phagophore membrane fuses on itself, forming the double-membrane autophagosome. Next, fusion of the autophagosome with a lysosome leads to the formation of an autolysosome and degradation of intravesicular materials together with the inner membrane (Fig. 1).

Several signaling pathways regulate the induction of autophagy [14]. The mammalian target of rapamycin complex 1 (mTORC1) is a major regulator of starvation-induced autophagy. mTORC1 suppresses autophagy induction mainly by phosphorylating ULK1/2, thereby inhibiting the cascade of autophagy induction. mTORC1 activity, in turn, is tightly controlled by availability of nutrients (e.g. amino acids), growth factors, and energy status as sensed by AMP-activated protein kinase (AMPK). AMPK also regulates autophagy by inhibiting mTORC1 and by phosphorylating Beclin 1 [15]. Further, phosphorylation of Bcl-2 and Bcl-X_L disrupts their interaction with Beclin 1, leading to release of Beclin 1 for autophagy induction [16,17].

Though less well characterized, autophagosome turnover, the second half of autophagy, is fundamental to the process. Disruption of autophagosome turnover has been observed in various human diseases, including Danon cardiomyopathy [18,19]. Recent studies have shown that late events in autophagosome processing are regulated. Transcription factor EB (TFEB) induces expression of genes coding for both autophagic and lysosomal proteins. As a consequence, TFEB governs autophagic initiation, autophagosome-lysosome fusion, and lysosomal degradation of internal cargo [20,21]. Other proteins, such as UVRAG [22], Rubicon [23], Rab7 [24], and Lamp2 [18], have also been suggested to play important roles in autophagosome-lysosome fusion.

1.2. Monitoring autophagy

There are multiple ways to measure autophagy, including assays useful for both in vitro and in vivo studies [25]. Atg8/LC3 detection either by Western blot or by cellular imaging, and protein turnover assays, are commonly used.

Atg8/LC3 detection assays serve as a measure of autophagosomes abundance. Conversely, conversion of cytosolic LC3-I to phosphatidylethanolamine-conjugated LC3-II is used as a marker for autophagosome formation. However, it is critical to bear in mind that autophagy is a highly dynamic process; abundance of autophagosomes can reflect either induction of autophagosome formation or a defect in downstream autophagosome degradation (or both occurring together). Obviously, the implications of these two different events are vastly dissimilar. Therefore, it is of critical importance to assess autophagic flux, as opposed to relying on a single “snapshot in time” of autophagosomes levels within the cell.

Turnover of long-lived proteins is the oldest and “classical” means of assessing autophagic activity. More recently, however, assays of long-lived protein degradation have been supplanted largely by monitoring degradation of protein p62. However, given this protein's multiple biological functions [26,27], its transcriptional regulation by stress [28], and its decreased solubility in some contexts [25], p62 levels cannot be used reliably in every scenario as a reflection of autophagic flux.

Another common method to probe autophagic flux involves autophagosome quantification in the presence versus absence of pharmaceutical manipulations to block autophagic degradation. E64D, pepstatinA, chloroquine and bafilomycin A are commonly used for this purpose.

A third method to quantify autophagic flux involves usage of tandem fluorescence probes of LC3, such as mRFP/mCherry-GFP-LC3 [29]. As GFP fluorescence is selectively quenched in the acidic environment of the lysosome, autolysosomes emit a red signal and autophagosomes shine yellow (red + green).

1.3. Autophagy in cardiovascular disease

The heart is a dynamic organ marked by a high metabolic rate, requiring continuous production of ATP to maintain a healthy contractile state. Under conditions of nutrient insufficiency, autophagy is required for energy production [30]. Viewed from another angle, the cardiomyocyte's abundance of mitochondria and high levels of oxidative phosphorylation render it susceptible to accumulation of ROS and injury from oxidative damage. Accumulation of ROS can lead to protein and membrane oxidation, organelle dysfunction, and ultimately cell injury and death. Working in concert with anti-oxidant enzymes, autophagy