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Review article

Non-coding RNAs as orchestrators of autophagic processes

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ABSTRACT

Autophagy is an important quality control mechanism present in all cells to maintain their cellular homeostasis. An imbalance in the autophagic process had been reported in numerous diseases including cardiovascular disease and is associated with serious consequences. Thus, knowledge of key regulators of cardiac autophagy is helpful to regain a balanced autophagic activity and to maintain healthy myocardial function. In this review we summarize all microRNAs which had been reported to regulate cardiac autophagy to date. In addition, we discuss long noncoding RNAs and circular RNAs as potential modulators of autophagy. Altering non-coding RNAs *in-vivo* by novel therapeutics offers a promising approach to treat autophagy-related diseases.

Contents

1. Introduction

Cells rely on two degradation pathways for quality control to dispose and renew their cellular junk. One is the ubiquitin-proteasome system which specifically degrades the ubiquitin labeled proteins. Apart from the proteasomes, autophagy maintains the bulk degradation and renewal of non-functional proteins and organelles. The term autophagy was coined by Christian de Duve in 1963 and the existence of autophagy in the heart was first reported in 1976 with detection of autophagic vacuoles in fetal heart kept in organ culture [1,2]. Despite the discovery of cardiac autophagy in 1976, an understanding about the molecular regulators appeared only in the last decade. The well-known regulators of autophagy were termed as autophagy-related genes (*Atg*) and there are around 32 *Atg* genes known to regulate the process of autophagy in mammals [3]. Among them, *Atg5*, which together with *Atg12* & *Atg16L* constitutes the ubiquitin conjugation complex is involved in expansion of autophagosomes, has been studied in detail for its role in the heart [3]. Cardiac specific deletion of *Atg5* leads to an early aging phenotype and to death starting at six months of age [4]. *Atg5* knockout mice

Abbreviations: Atg, autophagy related gene; AAV, adeno-associated virus; $CryAB^{R120G}$, αB-crystallin R120G mutation; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; 3-MA, 3 methyladenine; lncRNA, long non-coding RNA; HUVECs, human umbilical vein endothelial cells; HAECs, Human aortic endothelial cells; Ox-LDL, oxidized low density lipoprotein.

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shows massive cardiac hypertrophy, fibrosis and abnormal mitochondria at older ages compared to wildtype controls [4]. Knockout mice at a younger age had normal function at baseline, but were more susceptible to cardiac stress like pressure-overload and isoproterenol-infusion [5]. Additionally, deletion of *Atg5* in adult mice leads to cardiac remodeling and impaired cardiac function [5]. These data show the necessity of maintained baseline autophagy in order to combat cardiac stress. In contrary, overexpression of *Atg5* in mice lead to activated autophagy in various organs including the heart and to an extended lifespan [6]. Despite of these findings, conclusive data regarding beneficial effects of *Atg5* in cardiac disease models are still missing. It will be interesting to see whether overexpression of *Atg5* mediated by AAVs (adeno-associated viruses) could have beneficial effects in pressure-overload or myocardial infarction models opposite to knockout phenotypes and thus these approaches could be of important therapeutic interest. Interestingly, such studies would also clarify some of the controversial roles of autophagy in cardiac complications.

Overexpression of another *Atg* gene *Atg7* showed beneficial effects in a desmin-related cardiomyopathy model. *Atg7* overexpression led to increased autophagy, delayed death and better cardiac function in CryAB^{R120G} (αB-crystallin R120G mutation). In addition exercise, another inducer of autophagy, showed further benefits [7]. Similar to *Atg5*, overexpression studies evaluating the effect of *Atg7* overexpression in a pressure-overload or infarction model are lacking but could shed more light on the exact role of autophagy during these conditions.

Another well-known regulator of autophagic process is the mTORC1 complex, which functions as nutrient sensor and inhibits autophagy in nutrient rich conditions by phosphorylating the ULK kinase complex [8]. Deletion of *Raptor* (regulatory associated protein of *mTOR* complex 1) in adulthood led to increased autophagy but with detrimental consequences [9]. *Raptor* deletion showed failure in adaptation to pressure overload as well as under physiological conditions of exercise and sedentary [9]. It resulted in activation of the fetal gene program and a maladaptive switch to glucose metabolism with decreased fatty acid oxidation [9]. In contrary, inhibition of mTORC1 by rapamycin inhibits ongoing as well as established cardiac hypertrophy and improves cardiac function [10,11]. These opposite findings could be explained as the *mTOR* pathway is a master coordinator and deletion of this complex could affect several pathways, while on the other hand rapamycin could also have off-target effects.

In addition, numerous other genes like, des-acyl ghrelin, *FoxO3a*, *KLF4* and many others had been reported to regulate cardiac autophagy and show an effect on cardiac physiology [12,13,14]. However, most of these *Atg* genes and other pathways are ubiquitously present in all cell types and thus specific modulation of cardiac autophagy by any molecule which targets them may have serious consequences on other organs and could limit their therapeutic use. Therefore, there is a serious need to identify tissue specific modulators of autophagy to harness autophagy as therapy for cardiac diseases as well as others.

2. Non-coding RNAs as autophagy regulators

Non-coding RNA comprises nearly 98% of the human transcriptome and with few exceptions previously had been labeled as junk DNA until recently. Based on the size criteria of 200 nucleotides, noncoding RNAs are divided into long and short non-coding RNAs [15]. Among the short non-coding RNAs, microRNAs are the most extensively studied and reported to play an important role in cardiovascular pathophysiology. Excellent review articles summarizing role of microRNAs in cardiac disease and development are already present, and will not be discussed here [16–18]. Contrary to protein coding genes, many of these microRNAs and other non-coding RNAs show tissue specific distribution pattern and thus could be an ideal target molecule for tissue or cell type specific therapy [19,20]. Identification of cardiac specific (or enriched) microRNAs which could regulate autophagy can be potentially used to alter cardiac autophagic activity without disturbing the autophagic flux of other organs.

3. AutophagomiRs regulating pathological cardiac hypertrophy

Similar to protein coding genes, microRNAs regulating autophagy (autophagomiRs) were also described to have contradictory effects on autophagy and hypertrophy. Some reports had profoundly put forward data arguing anti-autophagic microRNAs miR-212/132, miR-199a and miR-221 to be pro-hypertrophic [21–23]. The overexpression of the miR-212/132 cluster resulted in massive hypertrophy, impaired autophagy and death due to heart failure. Deletion of the miR-212/132 cluster resulted in activated autophagy and also rescued from the development of hypertrophy in response to pressure-overload [21]. MiR-212/132 directly targets the pro-autophagic and anti-hypertrophic transcription factor *FoxO3a* and thus inhibits cardiac autophagy [21]. Similarly overexpression of another microRNA miR-199a, results in cardiac hypertrophy and cardiac dysfunction because of lower autophagic activity [22]. Alike miR-212/132, miR-199a also targets a pro-autophagic and anti-hypertrophic factor *GSK3-β* and also activates the *mTOR* pathway [22]. Interestingly rapamycin mediated activation of autophagy attenuates ongoing hypertrophy and decreased *mTOR* activity in transgenic hearts [22]. Both the targets of anti-autophagic microRNAs, *FoxO3a* and *Gsk3-β* are also inhibitors of calcineurin/NFAT (Nuclear factor of activated T cells) pathway in the heart and thus it would be interesting to check the effect of calcineurin/NFAT activation on cardiac autophagy. Another in depth study had been performed with miR-221, where miR-221 cardiac overexpression resulted in heart failure, cardiac hypertrophy and lower autophagic activity [23]. MiR-221 targets the cell cycle inhibitor *p27* which led to activation of *Cdk2* and thus activation of the *mTOR* pathway [23]. Reactivation of autophagy by inhibition of the *mTOR* pathway *via* rapamycin or Raptor silencing reverses the effect of miR-221 on cardiac hypertrophy and provided evidence for indispensable role of autophagy inhibition for cardiac hypertrophy [23].

In contrary several studies had linked activated autophagy to the development of cardiac hypertrophy [24]. MiR-30 showed as cardiac autophagic suppressor was found to be downregulated in rodent hearts subjected to either Angiotensin II or pressure overload [25]. Angiotensin II infusion was found to activate autophagy which was blocked with either the autophagy inhibitor 3-MA (3-methyladenine) or miR-30 mimic demonstrating the anti-autophagic effect of miR-30 [25]. MiR-30 targets the well-known autophagy regulator *Beclin-1* and *Beclin-1* deletion led to lower autophagy and suppression of remodeling genes while overexpression had opposite effects [25]. As miR-30, miR-34a was also reported to be decreased in angiotensin II treated rat hearts with activation of autophagy [26]. MiR-34a was found to inhibit cardiomyocyte autophagy and hypertrophy by targeting an *Atg* gene *Atg9* [26]. In fact, miR-34a had been demonstrated to be induced during cardiac aging. Thus its anti-autophagic function and the regulation of *Atg9* expression would be pertinent to its aging effect [27]. Strikingly, no microRNAs regulating cardiac autophagy and thus aging have been studied so far, although lower autophagic activity has been associated with aging. The phenotype of cardiac aging was reversed by deletion of endothelin A receptor through activation of autophagy [28]. This in principle demonstrates the possibility to identify an "autophagomiR" which could reverse the cardiac aging process and thus avoid aging associated diseases.

A similar finding was also reported in a model of hypertrophic cardiomyopathy, where the autophagic inhibitor miR-451 was found to be downregulated in the heart, and thus led to activation of autophagy [29]. A summary of autophagomiRs regulating cardiomyocyte hypertrophy is presented in Fig. 1 and Table 1.

4. AutophagomiRs regulating cardiomyocyte death

Autophagy has been described as adaptive as well as maladaptive relating to cardiomyocyte death and survival. The *Atg* gene *Atg7* increases