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TOR-autophagy signaling in adult zebrafish models of cardiomyopathy

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The target of rapamycin (TOR) kinase is part of an evolutionarily conserved signaling pathway that coordinates cell growth, survival, and autophagy. Previously, pharmacological studies using rapamycin have suggested a cardioprotective effect of TOR signaling inhibition on cardiomyopathy. We found that rapamycin exerts a conserved cardioprotective effect in two adult zebrafish models of cardiomyopathy of different etiology, and provided the first genetic evidence to support a long-term cardioprotective effect of TOR signaling inhibition. Moreover, we detected dynamic TOR-autophagy activities along different stages of cardiomyopathy. This needs to be considered when developing TOR-autophagy-based therapeutics for cardiomyopathy.

Keywords: target of rapamycin, cardiomyopathy, anemia, doxorubicin, cardioprotective, autophagy, zebrafish

Abbreviations: DOX, doxorubicin; TOR, target of rapamycin

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In response to numerous stresses, the heart initially compensates to meet the needs of the body by adjusting its structure. However, sustained stress leads to pathological cardiac remodeling, which becomes decompensational and causes cardiomyopathy. Heart failure is an end stage of cardiomyopathy affecting millions of American each year with an estimated five-year survival of 50%. To develop novel therapeutics, it is crucial to elucidate the signaling pathways during the remodeling process.

Zebrafish is an emerging vertebrate model that offers new research opportunities. To leverage its genetic potential, we recently generated two adult zebrafish cardiomyopathy models induced by either chronic anemia or doxorubicin (DOX). The chronic anemia was induced by a Band 3 mutation that affects the survival of erythrocytes, which sequentially imposes high-output stress to the heart and eventually induces cardiomyopathy. In contrast, DOX is an anthracyclin compound that has been clinically used to treat cancer patients; however, overdose leads to acute cardiac toxicity and/or cardiomyopathy at later stages. We found that injection of a single bolus of 20 μg/g body mass DOX in adult *casper* fish induces progressive and pathological cardiomyopathy. The pathogenesis differs from that in anemia-induced cardiomyopathy, likely due to different etiology.

Previously in rodent models, pharmacological studies using rapamycin, a specific inhibitor of target of rapamycin (TOR) signaling, prompted a cardioprotective effect of TOR signaling inhibition. However, this effect has not been validated by genetic studies. In contrast, loss-of-function studies of TOR suggested a deleterious effect of TOR depletion on cardiomyopathy. The general goal of our study was to assess the therapeutic potential of TOR signaling in cardiomyopathy using the novel zebrafish model. We first validated the anti-hypertrophy effects of short-term TOR signaling inhibition via rapamycin treatment in both adult fish models of cardiomyopathy. We then assessed the long-term effect of TOR signaling inhibition by utilizing a *tor* mutant that was isolated from an insertional mutagenesis screen. Similar to that in rodents, homozygous zebrafish *tor* mutants were embryonic lethal. However, heterozygous zebrafish *tor* mutants attenuated cardiomyopathy phenotypes and increased survival in both adult fish models of cardiomyopathy. Therefore, our study provides the first genetic evidence to support

the cardioprotective function of TOR signaling inhibition. Importantly, our data suggested that the dose of TOR signaling inhibition is crucial for its cardioprotective effect, which might explain the controversial conclusions in rodents.

To decipher the cardioprotective effects of TOR signaling inhibition on cardiomyopathy, we assessed both the phosphorylated S6K and the autophagy levels, two major downstream targets of the TOR signaling pathway. In addition to the dynamic activities of phosphorylated S6K level in both cardiomyopathy models, we found that the autophagy level is oppositely regulated at different stages of pathogenesis in the DOX model. At 3 d post-DOX injection, we detected significantly activated autophagy. This is likely a maladaptive response to DOX-induced acute cardiotoxicity, as supported by the observation that rapamycin treatment further elevates autophagy activity and leads to higher mortality. In contrast, a suppressed autophagy activity was detected at 12 weeks post DOX injection, when fish exhibit decompensational cardiomyopathy phenotypes. Rapamycin treatment at this later stage improves both cardiac function and survival, suggesting a cardioprotective function of autophagy activation. Together, our data suggested that TOR-autophagy signaling needs to be modulated differently at different stages of pathogenesis to achieve the best therapeutic benefits against cardiomyopathy.

As a highly conserved process to degrade and recycle proteins and organelles, autophagy can act as a protective cellular mechanism to remove harmful protein aggregates and damaged organelles. Dysregulation of this crucial cellular process, either defective or excessive, might lead to cardiac dysfunction and heart failure. While depressed autophagy results in accumulation of damaged cytoplasmic components and promotion of cell death such as apoptosis, overactivation of autophagy destroys a large fraction of the cytoplasmic organelles, leading the affected cardiomyocyte to autophagic cell death. Therefore, TOR-autophagy signaling must be delicately modulated to gain therapeutic benefits. Our studies of the DOX model in zebrafish suggested that the autophagy level at the early acute stages of pathogenesis needs to be reduced, but the compromised autophagy level at late stages of cardiomyopathy needs to be tuned up to a certain threshold to protect the heart. In summary, our results validated the therapeutic potential of TOR signaling inhibition, as well as underscored the need to optimize both the dose and the stage-specific treatments when developing a TOR-based therapy for cardiomyopathy.