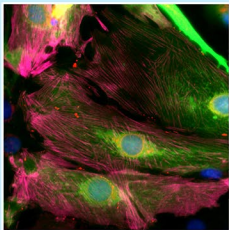




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Cardiac Fibroblasts Undergoing Reprogramming
(green: alphaMHC-GFP, pink: cardiac troponin T,
blue: nuclei, red: connexin 43)

Autophagic Factor Beclin1 Inhibits Direct Cardiac Reprogramming

Fibroblasts can be directly reprogrammed into induced cardiomyocytes (iCM) by expression of cardiac transcription factors Mef2c, Gata4, and Tbx4 (collectively called MGT). Since fibroblasts are readily available, this approach holds great potential for cardiac regeneration. However, the molecular basis underlying this conversion is still largely unknown. Overexpression of MGT promotes the degradation of p62, which is also degraded in autophagy and is an indicator of autophagic flux. This suggests that autophagy plays an important role in iCM reprogramming. Here, we tested whether autophagy is induced during iCM reprogramming by transducing fibroblasts with either MGT or lacZ as a negative control. The resultant levels of p62, as well as the autophagosome marker LC3-II were quantified using Western blot and immunocytochemistry (ICC) imaging. Overexpression of MGT lead to LC3-II accumulation as well as delayed p62 degradation, indicating that autophagy is activated in iCM reprogramming. To determine if transcription of autophagy-related genes changed significantly over the course of iCM reprogramming, mice cardiac fibroblasts (CFs) were transfected with MGT. RNA was extracted at five time points and qPCR was used to determine that transcription levels of autophagy related genes. Surprisingly, autophagy genes were not changed as a result of iCM reprogramming. Therefore, post-translational modifications may be responsible for the activation of autophagy during reprogramming. To determine the role of autophagy in iCM reprogramming, we depleted the key autophagy factor Beclin1 and its upstream regulators, Ulk1 and Ambra1, from mice CFs. In sum, we found that silencing these factors increased iCM reprogramming efficiency. Direct cardiac reprogramming will ultimately lead to therapies that can address the loss of contracting cardiomyocytes and restore heart function.