Abstract: P318

Phosphoinositide 3-kinase gamma inhibition as a novel strategy to reactivate targeted autophagy and limit Doxorubicin-induced cardiotoxicity

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Background: Anthracyclines, like doxorubicin (DOX), are among the most potent antitumor drugs. However, their clinical use is hampered by severe cardiotoxicity. We previously demonstrated that inhibition of phosphoinositide 3-kinase (PI3K?) protects against anthracycline-induced cardiomyopathy (manuscript under revision), but the underlying molecular mechanisms are still unexplored.

Purpose: Here, we further test the hypothesis that anthracycline-damaged mitochondria activate Toll-like receptor 9 (TLR9)/PI3K? signaling, which in turn inhibits protective autophagy, thus exacerbating anthracycline cardiotoxicity.

Methods: Neonatal cardiomyocytes (NCMs) were isolated from mice expressing a kinase inactive PI3K? (PI3K? kinase-dead; KD) and wild-type (WT) controls, and treated with DOX (1 mM) or TLR9 agonist ODN1826 (1 µg/ml) ± PI3K? inhibitor AS605240 (500 nM) or TLR9 antagonist ODN2088 (1 µg/ml), for 1 hour before analyzing Akt/mTOR/Ulk-1 signaling. For in vivo studies, WT and KD mice were treated with 4 mg/kg DOX weekly for 3 weeks. Cardiac function was analyzed with echocardiography 6 weeks after the first injection. Electron microscopy (EM) study of morphology and signaling transduction were studied 3 days after the treatment. To investigate the role of protective autophagy in KD hearts, mice were treated with hydroxychloroquine (HCQ) or AAV9-shATG7, which silences the autophagy regulator ATG7 specifically in cardiomyocytes, together with DOX, as described above.

Results: In NCMs, DOX significantly increased the phosphorylation of PI3K downstream targets and autophagy inhibitors, Akt, mTOR and Ulk-1. These effects were completely prevented by the TLR9 antagonist ODN2088, the PI3K? selective inhibitor AS605240 and genetic PI3Kg inactivation (KD NCMs). Notably, the TLR9 agonist ODN1826, mimicking mitochondrial DNA (mitoDNA), similarly upregulated Akt/mTOR/Ulk-1 signaling in WT but not in KD NCMs. These results suggest that DOX activates PI3K? through mitoDNA/TLR9. In vivo, EM studies showed more abundance of autolysosomes containing injured mitochondria in KD than WT hearts, highlighting the efficient autophagy-dependent disposal of DOX-damaged mitochondria in KD mice. Enhanced activation of PI3Kg-dependent pathway correlated with a significant blunt of autophagy in DOX-treated hearts, whereas inhibition of PI3K? promoted autophagy and decreased DOX-induced contractile dysfunction. Finally, inhibition of autophagy by HCQ or AAV9-shATG7 erased the protection in KD mice.
Conclusion: Overall, this study demonstrates that PI3Kγ prevents autophagic disposal of DOX-damaged mitochondria, resulting in cardiomyopathy. We propose PI3Kγ inhibition as a novel strategy to reactivate targeted autophagy and limit cancer therapy-related heart disease.