The histone deacetylase SIRT1 mediates the proper DNA repair response by targeting histone H2AX to protect against doxorubicin-induced cardiotoxicity

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Background: Failure of DNA repair and accumulation of damaged DNA have been implicated in the pathogenesis of doxorubicin-induced cardiotoxicity. SIRT1, an NAD+-dependent histone deacetylase, is known to positively regulate DNA repair. One of the earliest events in DNA damage response (DDR) is phosphorylation of histone H2AX on Ser139 catalyzed by kinases including ATM (mutated in ataxia-telangiectasia). However, it remains unknown whether SIRT1 protects the heart from doxorubicin-induced cardiotoxicity by regulating histone H2AX.

Purpose: In this study, we investigated whether SIRT1 plays a role against doxorubicin-induced cardiotoxicity by regulating histone H2AX and therefore DDR.

Methods and results: We used tamoxifen-inducible cardiomyocyte-specific SIRT1 knockout (SIRT1-cKO) mice. Knockout was induced at 2 month of age, and mice without Cre recombinase served as wild type (WT). Mice were treated with vehicle (Veh) or doxorubicin (4 IP injections of 5 mg/kg/week) starting at 3 month of age. Echocardiography showed that fractional shortening (FS) before doxorubicin was similar in WT (34%) and SIRT1-cKO (34%). However, FS at 1 week after final doxorubicin was lower in SIRT1-cKO than WT (26% vs. 30%, $P<0.05$). Myocardial ANP mRNA level was 2.4-fold higher in SIRT1-cKO than WT after doxorubicin. Apoptotic cells analyzed by TUNEL-positive nuclei were similar in Veh-treated SIRT1-cKO and WT (0.125 vs. 0.073%) but were more increased by doxorubicin in SIRT1-cKO than WT (0.384 vs 0.194%, $P<0.05$). Immunoblotting showed that doxorubicin significantly increased myocardial levels of phospho-Ser139-histone H2AX (p-H2AX) and phospho-Ser1981-ATM (p-ATM) in WT. However, the doxorubicin-induced increase in p-H2AX level was significantly attenuated in SIRT1-cKO despite similar p-ATM levels after doxorubicin between WT and SIRT1-cKO.

In H9c2 cardiomyocytes, doxorubicin treatment (10 μM) increased both p-ATM and p-H2AX levels, but siRNA-mediated knockdown (KD) of SIRT1 attenuated doxorubicin-induced phosphorylation of H2AX without changing p-ATM level. Cell death after doxorubicin was enhanced in SIRT1-KD cells compared to control cells (13.2% vs 8.6%, $P<0.05$). Immunostaining showed that SIRT1 colocalized with histone H2AX in the nucleus. Treatment with a SIRT1 inhibitor Ex527 increased level of acetylated H2AX at 5th lysine residue, suggesting that SIRT1 regulates histone H2AX via deacetylation.

Conclusion: These data suggest that SIRT1 plays a protective role against doxorubicin-induced cardiotoxicity via regulation of H2AX phosphorylation to mediates proper DDR.