SIRT1 protects the heart against doxorubicin-induced cardiotoxicity by mediating the DNA damage response via deacetylation of histone H2AX

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Funding Acknowledgement: Type of funding sources: None.

Background: Doxorubicin induces DNA damage not only in tumor cells but also in the cardiomyocyte, and accumulation of damaged DNA has been implicated in doxorubicin-induced cardiotoxicity. We previously found that cardiomyocyte-specific deletion of SIRT1, a NAD+-dependent histone/protein deacetylase, worsens doxorubicin-induced cardiotoxicity in mice. However, its molecular mechanism remains unclear. Phosphorylation of histone H2AX at Ser139 catalyzed by ATM (mutated in ataxia-telangiectasia) at the sites of DNA damage is a critical mediator for DNA repair.

Purpose: Here, we tested the hypothesis that deacetylation of H2AX by SIRT1 mediates DNA damage response to counteract doxorubicin-induced cardiotoxicity.

Methods and results: Wild-type (WT) mice and tamoxifen-inducible cardiomyocyte-specific SIRT1 knockout (SIRT1-cKO) mice at 3 month of age received doxorubicin (4 IP injections of 5 mg/kg/week) or a vehicle. Immunoblotting of myocardial lysates from mice 1 week after final doxorubicin showed that doxorubicin increased phospho-Ser139-H2AX level by 1.6-fold in WT, but such a response was blunted in SIRT1-cKO. Ser1981-phosphorylations of ATM induced by doxorubicin were similar in WT and SIRT1-cKO. DNA fragmentation evaluated by TUNEL staining revealed that the increase in TUNEL-positive nuclei by doxorubicin was more in SIRT1-cKO (0.13% to 0.38%) than those in WT (0.07% to 0.19%), suggesting higher DNA damage in SIRT1-cKO.

In H9c2 cardiomyocytes, knockdown of SIRT1 also abolished the doxorubicin-induced Ser139-phosphorylation of H2AX without changing phospho-ATM levels. Increases in DNA damage evaluated by comet assay and cleavage of caspase-3 by doxorubicin were also enhanced in SIRT1-knockdown cells. Immunostaining for acetyl-Lys5-H2AX in the heart sections revealed that acetyl-Lys5-H2AX levels were increased in SIRT1-cKO by 58% compared with those in WT. In H9c2 cells, acetyl-Lys5-H2AX level was also increased by SIRT1 knockdown and reduced by expression of wild-type SIRT1. To test the role of the increased acetyl-Lys5-H2AX level under SIRT1 inhibition, we generated a mutant in which Lys5 was substituted to glutamine (K5Q H2AX) as a mimic of acetylated Lys5. In COS7 cells expressing WT or K5Q H2AX, Ser139-phosphorylation induced by doxorubicin was suppressed in K5Q mutant. In addition, doxorubicin-induced cleavage of caspase-3 was enhanced in H9c2 cells expressing K5Q H2AX as well as S139A H2AX, that cannot be phosphorylated at Ser139, compared with cells expressing WT H2AX.

Conclusions: These findings suggest that the increased Lys5 acetylation of H2AX via SIRT1 inhibition interferes Ser139 phosphorylation, leading to accumulation of damaged DNA and promotion of the apoptotic response. Such regulation of the DNA damage response contributes to protection by SIRT1 against doxorubicin-induced cardiotoxicity.