Sirtuin 3 (SIRT3) Prevents Doxorubicin Induced Dilated Cardiomyopathy: Investigating Mitochondrial Protein Acetylation, Cardiac Lipids and Metabolic Dysfunction

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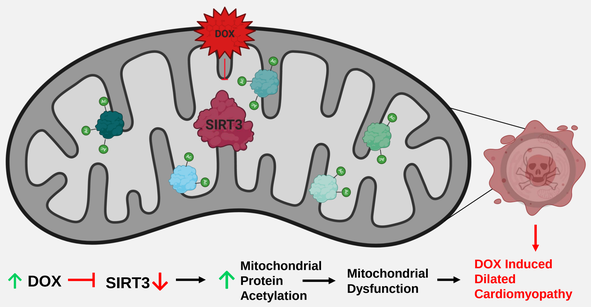
Introduction

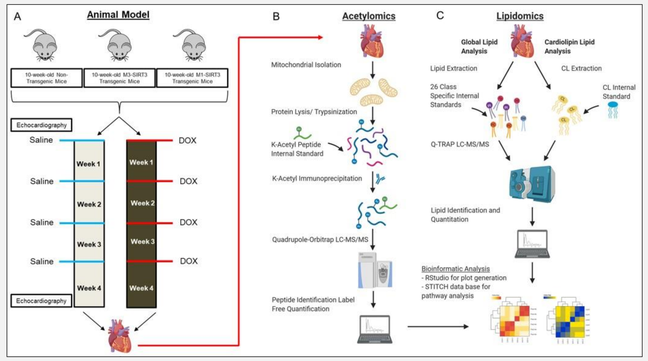
- Doxorubicin (DOX) is a chemotherapeutic with dose-dependent cardiotoxic effects that limits its use in pediatric cancer patients.
- Sirtuins (SIRT) are a class of lysine deacetylases. SIRT3 is the main mitochondrial lysine deacetylase which regulates mitochondrial proteins.
- Previously we showed that DOX decreases expression of SIRT3 in H9c2 rat cardiomyocytes and in the mouse heart.
- Down regulation of SIRT3 with DOX treatment results in an increase in mitochondrial protein acetylation.
- SIRT3 overexpression in H9c2 rat cardiomyocytes increases cardiolipin mass and prevents DOX induced mitochondrial dysfunction.



Hypothesis

- Increased SIRT3 expression *in vivo* could attenuate DOX-induced cardiac dysfunction via alterations of protein acetylation to enzymes involved in lipid remodeling and metabolic processes.

Experimental Animal Model and Workflow



- 10-week-old mice with cardiac restricted expression of full length (M1-SIRT3) which is localized to the mitochondria or truncated (M3-SIRT3) which lacks the mitochondrial localization signal by muscle creatine kinase (MCK) and myosin-heavy chain (MHC) promoters respectively were treated with DOX.

Statistics

Statistics are Student's T-Test or Two-way ANOVA with Tukey Post-Hoc Analysis. Data represented as mean ± SD unless otherwise indicated. P-values indicated multiple comparisons shown on graph. * p<0.05 in comparison to control group.

Results

1. Mitochondrial M1-SIRT3 Expression Prevents DOX-induced Dilated Cardiomyopathy

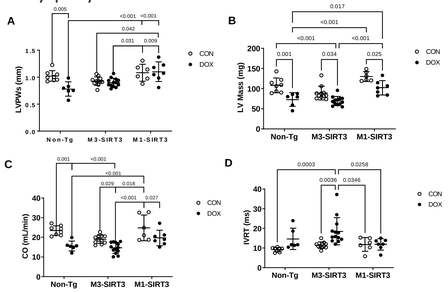


Figure 1. Echocardiography of M3-SIRT3, M1-SIRT3 and non-transgenic controls treated with 8.0mg/kg of DOX or saline once a week for four weeks. **(A)** Left ventricular posterior wall thickness during diastole **(B)** Left ventricular mass. **(C)** Cardiac output. **(D)** Isovolumetric relaxation time. Male mice n=6-13.

2. DOX Induces Changes to Mitochondrial Ultrastructure and SIRT3 Expression Alters Mitochondria Cristae

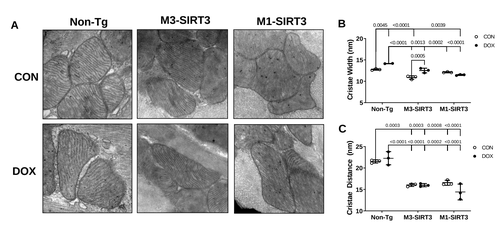


Figure 2. Transmission Electron Microscopy of DOX treated Cardiac Tissue. (A) Representative images. (B) Cristae Width (nm). (C) Distance between cristae (nm). n=3 Male mice.

3. DOX Causes Alterations to the Acetylation of Mitochondrial Enzymes

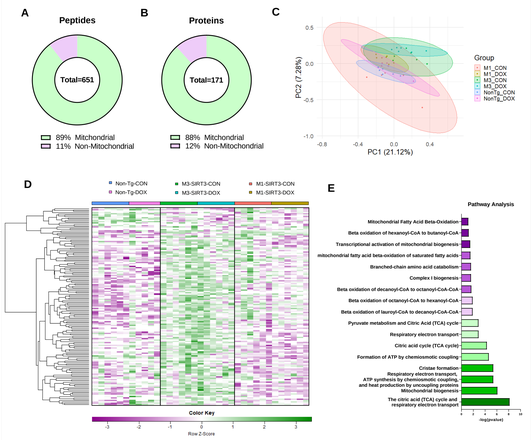


Figure 3. Mass Spectrometry of cardiac mitochondrial acetylated peptides from DOX treated Non-Tg, M3-SIRT3, M1-SIRT3 mice. **(A)** Number of mitochondrial and non-mitochondrial acetylated peptides. **(B)** Number of mitochondrial and non-mitochondrial proteins **(C)** PCA plot of normalized dataset. **(D)** Heatmap of differentially acetylated mitochondrial proteins in Non-Tg, M3-SIRT3 and M1-SIRT3 saline and DOX treated mice. Data represented as Log2 value of normalized peak intensity where values have been z-scored. Green indicates hyperacetylated peptide. Purple represents hypoacetylated peptides. Statistics are 2-Way-ANOVA. **(E)** KEGG Pathway Analysis. P<0.05, n=5-6 male mice

4. Characterization of Cardiac Lipids from DOX Treated Non-Tg and SIRT3 Transgenic Mice

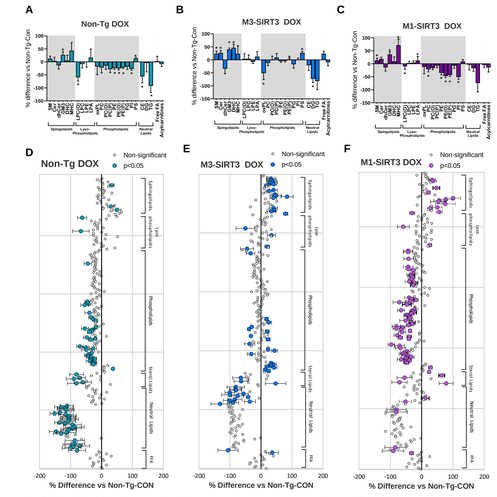


Figure 4. Global lipidomic analysis by mass spectrometry from DOX Treated Non-Tg and SIRT3 Transgenic Mice. Lipids in cardiac tissue of **(A)** Non-Tg-DOX **(B)** M3-SIRT3-DOX **(C)** M1-SIRT3-DOX mice expressed as a percent difference compared to Non-Tg-CON. * p<0.05. **(D-F)** Forest plot of individual lipids in cardiac tissue of **(D)** Non-Tg-DOX **(E)** M3-SIRT3-DOX **(F)** M1-SIRT3-DOX mice expressed as a percent difference compared to Non-Tg-CON. p<0.05. n=6 for all groups (4 males and 2 females). Values are mean ± SEM. Statistics are Student unpaired T-test.

5. DOX Reduces Cardiac Triglycerides and Changes Cardiolipin Composition in Non-Tg and SIRT3 Transgenic Mice

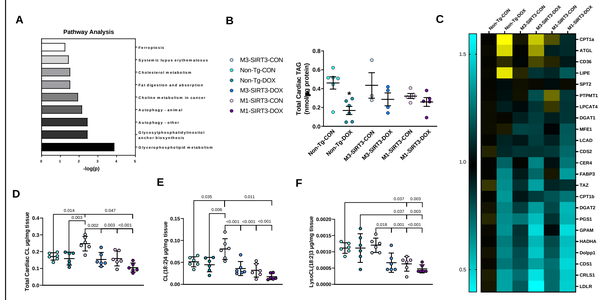


Figure 5. Lipidomic Validation and Cardiolipin Mass Spectrometry. **(A)** Lipid Pathway Enrichment Analysis. **(B)** Colorimetric Triglyceride Assay. Values are mean ± SEM. n=3-6 male **(C)** Expression of genes involved in fatty acid metabolism and cardiolipin biosynthesis by qPCR. Yellow indicates increased expression; blue indicates decreased expression. Boxes are mean values. Mass Spectrometry of **(D)** Total Cardiac CL. **(E)** Tetra-linoleic CL. **(F).** Lyso-CL. n=6 for all groups (4 males and 2 females). Statistics are 2-way ANOVA.

6. Correlation of Altered Lipid Levels with Echocardiography Parameters of Cardiac Dysfunction

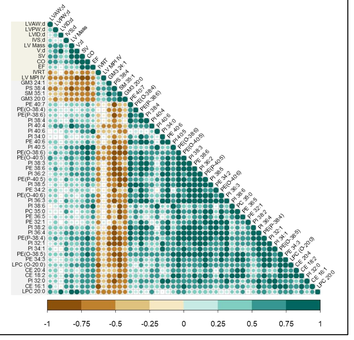


Figure 6. Spearman Correlation Analysis of Lipids and Echocardiography Parameters in Non-Tg Murine Hearts (A) Top 20 most significantly altered lipids correlated with echocardiography parameters in Non-Tg-DOX compared to Non-Tg-CON. Positive correlations are displayed in blue and negative correlations are in brown. Color intensity and size of circles are proportional to the correlation coefficients.

7. DOX Increases Cardiac Glucose Uptake in Non-Tg and M3-SIRT3 Mice

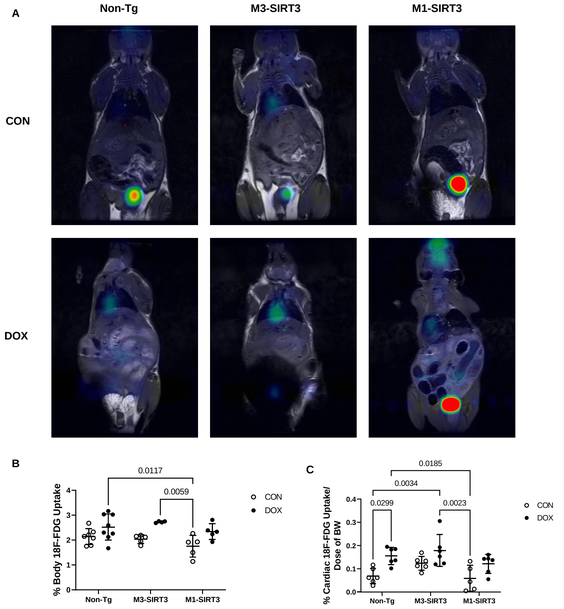


Figure 7. Glucose Uptake by 18F-FDG PET-MRI Imaging. **(A)** Representative PET-MRI Image overlays in Non-Tg, M3-SIRT3 and M1-SIRT3 animals treated with saline or DOX. **(B)** Radiation calibration measurements of total body 18F-FDG Uptake. **(C)** Radiation calibration measurements of cardiac 18F-FDG uptake. Values are mean ± SD. N=5-6 male mice.

Conclusion

- DOX reduced SIRT3 expression which altered acetylation of proteins involved in cardiac energy production and fatty acid metabolism.
- Increased SIRT3 expression in the heart rescues DOX-induced cardiac dysfunction.
- DOX-induced alterations in cardiac lipids are correlated with dilated cardiomyopathy phenotype.
- DOX increased glucose uptake in the heart characteristic of metabolic dysfunction.
- SIRT3 could be a potential therapeutic target for the treatment of chemotherapy induced cardiac dysfunction in pediatric patients.

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