Targeting the methyltransferase SETD7 prevents myocardial ischemic injury: a translational study

Samuele Ambrosini[1], Fabrizio Montecucco[2], Detmar Kolijn[3,4], Alexander Akhmedov[1], Daniela Pedicino[5,6], Shafeeq A. Mohammed[1,] Peter Brown[7]; Fabio Rossi[8], Attila Kiss[9], Antonio Paolo Beltrami[10], Filippo Crea[5,6] ,Thomas F. Luscher[1,11], Nazha Hamdani[3,12],Sarah Costantino[1], Francesco Paneni[1]

[1]Center for Molecular Cardiology, University of Zürich, Switzerland; [2]First Clinic of Internal Medicine, Department of Internal Medicine and Centre of Excellence for Biomedical Research (CEBR), University of Genoa, Genoa, Italy; [3]Institute of Physiology, Ruhr University, Bochum, Germany; Molecular and Experimental Cardiology, Ruhr University, Bochum, Germany, [4]Department of Cardiology, St-Josef Hospital, Ruhr University, Bochum, Germany, [5]Dipartimento di Scienze Cardiovascolari e Toraciche, Università Cattolica del Sacro Cuore, Rome, Italy, [6]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, [7]Structural Genomics Consortium, University of Toronto, Toronto, ON, Canada M5G 1L7, [8]Biomedical Research Centre, Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada, [9]Ludwig-Boltzmann-Institute for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Austria, [10]Department of Medicine (DAME), University of Udine, Udine, Italy, and Department of Medical Surgical and Health Sciences, University of Trieste, Italy, [11]Royal Brompton and Harefield Hospitals and Imperial College, London, United Kingdom, [12]Department of Cardiology, St-Josef Hospital, Ruhr University, Bochum, Germany.

Introduction

Despite significant advances in coronary revascularization, acute myocardial infarction remains the leading cause of heart failure and death worldwide. Upon cellular stress, the Hippo pathway is activated leading to cytosolic retention and degradation of the pro-survival transcription factor YAP. Post-translational modifications, namely methylation, have been shown to regulate YAP activity. The protein SET domain-containing lysine methyltransferase 7 (SETD7) - which induces a specific mono-methylation of both histone and non-histone proteins – is emerging as a pivotal modulator of protein functionality and gene expression.

Hypothesis

The present study investigates the role of SETD7 in modulating the Hippo pathway during myocardial ischemia.

Results

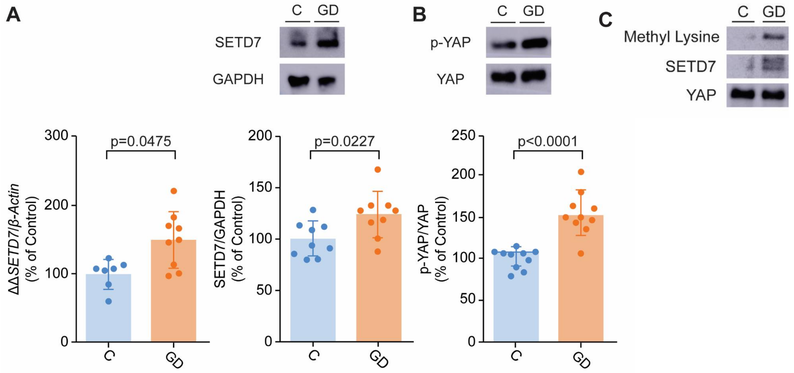
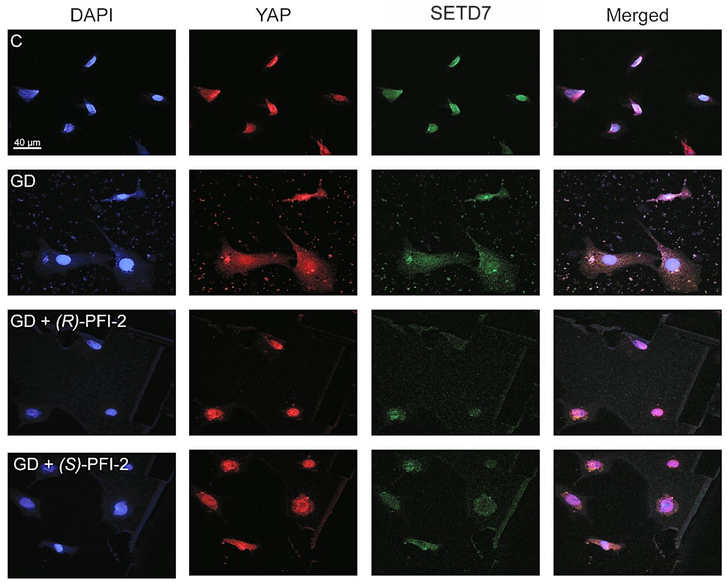


Figure 1: Glucose deprivation increases SETD7 expression and activates the Hippo pathway. A) SETD7 expression was assessed by real-time PCR and Western blot in NRVMs exposed to normal glucose concentrations or glucose deprivation for 15 h. **B**) Western blot showed YAP phosphorylation at Ser127 in C and GD-treated NRVMs. **C**) Glucose deprivation induces SETD7 and YAP binding. Data are expressed as mean ± standard deviation. A p-value <0.05 vs control by t-test was considered as statistically significant. C: control, GD: glucose deprivation.



Results

Figure 2: Pharmacological inhibition of SETD7 restores YAP nuclear localization under glucose deprivation conditions. Representative images of NRVMs exposed to control and glucose deprivation conditions for 15 h, in the presence or in the absence of the SETD7 pharmacological inhibitor *(R)*-PFI2 or its inactive enantiomer *(S)*-PFI-2 (10 μM). Cardiomyocytes were stained for YAP (red), SETD7 (green) and DAPI (blue). All images were acquired by confocal microscopy. C: control, GD: glucose deprivation.

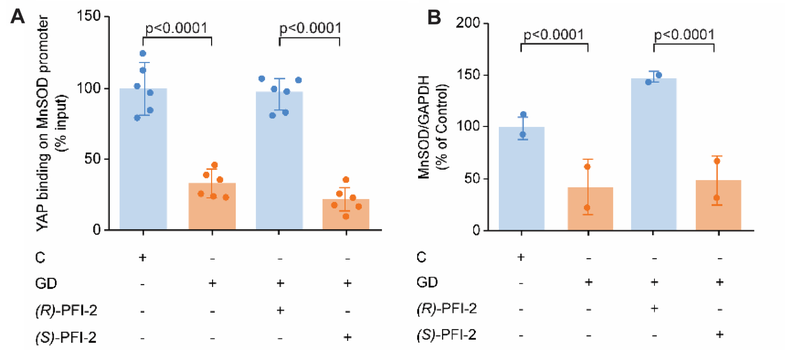


Figure 3: Pharmacological inhibition of SETD7 increases YAP binding to the promoter of anti oxidant genes. A) YAP binding to the promoter of anti-apoptotic genes was assessed by chromatin immunoprecipitation in NRVMs exposed to control and glucose deprivation conditions for 15 h, in the presence of the SETD7 pharmacological inhibitor *(R)*-PFI-2 or its inactive enantiomer *(S)*-PFI-2 (10 μM). **B)** Western blot quantification of anti oxidant genes expression following treatment.

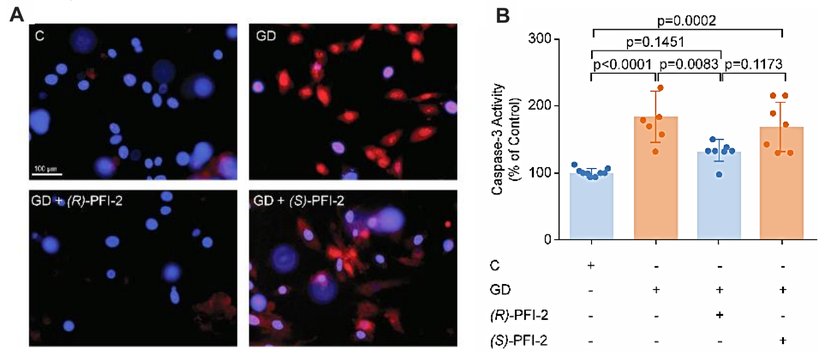


Figure 4: Pharmacological inhibition of SETD7 activity reduces oxidative stress and Caspase-3 activation. A) Mitosox assay in NRVMs exposed to control and glucose deprivation conditions for 15 h, in the presence of the SETD7 pharmacological inhibitor *(R)*-PFI-2 or its inactive enantiomer *(S)*-PFI-2 (10 μM). **B)** Caspase-3 activity in the four experimental groups. Data are expressed as mean ± standard deviation and expressed as percentage of control. A p-value <0.05 by ANOVA and post-hoc analysis with Bonferroni correction was considered as statistically significant. C: control, GD: glucose deprivation.

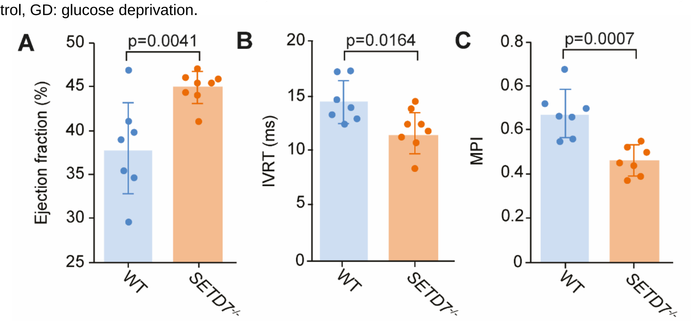


Figure 5: Genetic deletion of SETD7 protects against cardiac dysfunction after myocardial infarction. A) Ejection fraction in WT and *SETD7*$^{-/-}$ mice following I/R injury. **B**) Isovolumic relaxation time. **C**) Myocardial performance index. Data are expressed as mean ± standard deviation. A p-value <0.05 vs control by t-test was considered as statistically significant. IVRT: isovolumic relaxation time, MPI: myocardial performance index.

Results

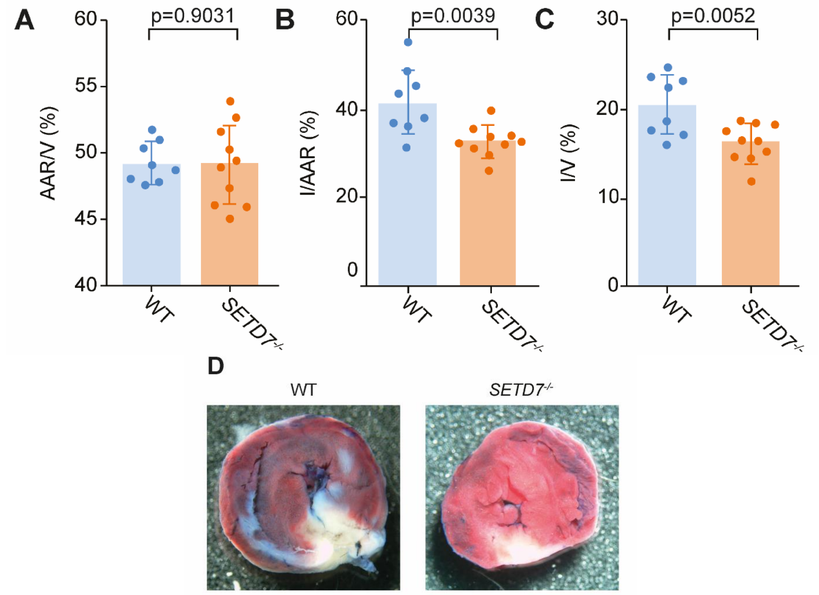


Figure 6: Genetic deletion of SETD7 protects against ischemia-reperfusion injury. A) Quantification of area at risk (AAR) per ventricle surface (V). **B**). Quantification of infarct size (I) per AAR. **C**) Quantification of infarct size (I) per ventricle surface (V). **D**) Representative images of TTC-stained middle heart sections of wild type and SETD7 knockout mice following myocardial infarction. Blue: continuously perfused tissue, red: stained ischemic viable tissue, white: unstained necrotic tissue. Data are expressed as mean ± standard deviation. A p-value <0.05 vs control by t-test was considered as statistically significant.

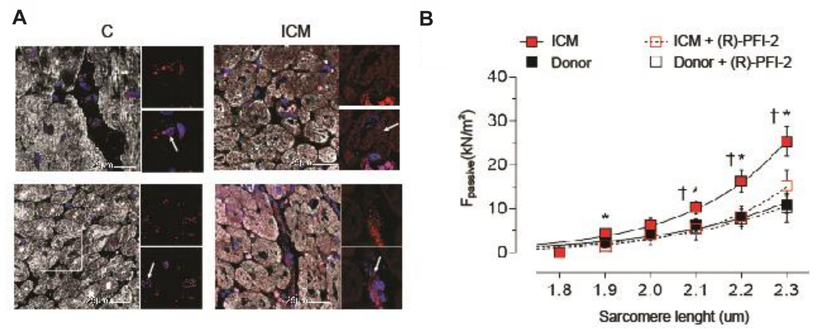


Figure 7: Activation of SETD7/YAP signaling in patients with ischemic cardiomyopathy. A) Confocal microscopy of YAP-positive nuclei showing YAP (red) cytosolic retention in ICM patients (n=10) vs. controls (n=9). Arrows on higher magnification fields indicate YAP nuclear retention in control hearts. **B)** Normalized passive stiffness measured at various sarcomere lengths from 1.8 to 2.3 μm in skinned cardiomyocytes isolated from patients with ICM and control donors, in the presence or in the absence of the SETD7 inhibitor *(R)*-PFI-2 (n= 3 patients).

Discussion

Our findings suggest that - in conditions of myocardial ischemia - SETD7 triggers cardiomyocyte apoptosis via increased YAP methylation and subsequent reduction of YAP-dependent anti-oxidant genes. Pharmacological modulation of the Hippo pathway by SETD7 may represent a novel therapeutic approach to prevent myocardial damage in patients with ischemic heart disease.

Disclosures: none