MicroRNA-34a regulates doxorubicin induced toxicity in rat cardiac progenitor cells

R. Russo, E. Piegari, A. De Angelis, D. Cappetta, G. Esposito, K. Urbanek, F. Rossi, L. Berrino

Dept. of Experimental Medicine, Section of Pharmacology 'L. Donatelli', Second University of Naples, Via S.Maria di Costantinopoli,16 80138 Naples, Italy

Rationale: MicroRNAs (miRNAs) are small (~22 nucleotides), noncoding RNAs that regulate gene expression primarily by inhibiting the translation of a specific mRNA target into a functional protein and/or promoting mRNA degradation (Valencia-Sanchez, 2006). MiRNAs have emerged as crucial regulators of cardiovascular function and pathogenesis (Small, 2011). At present, there is a growing interest in recognizing the potential of miRNAs both as biomarkers and therapeutics for cardiovascular diseases (Desai, 2014). MicroRNA-34 family members (miR-34a, -34b, and -34c) are up-regulated in the heart in response to stress (i.e. MI) and contribute to the age-dependent decline in cardiac function (Bernardo, 2012; Boon, 2013). In particular miR-34a is the predominantly expressed member in the heart and regulates a plethora of target proteins, which are involved in cell cycle, apoptosis, senescence, differentiation, and cellular development (Chen, 2012).
Objectives: The aim of the present study was to investigate the role of miR-34a in doxorubicin (DOXO)-induced cardiotoxicity. Our previous studies demonstrated that DOXO exposure severely affects the population of resident cardiac progenitor cells isolated from rat and human heart (De Angelis, 2010; Piegari, 2013). Therefore we evaluated if: 1. miR-34a could contribute to DOXO toxicity of Rat Cardiac Progenitor Cells (rCPCs) and 2. miR-34a modulation could protects DOXO exposed rCPCs.
Methods: rCPCs were treated for 24 and 48 h with DOXO (0.5 μM) and expression profiling of miR-34 family was evaluated by real time PCR. The levels of miR-34a target mRNAs were measured by real time PCR in DOXO treated rCPCs. Inhibition of miR-34a was induced transfecting the cells with antimiR-34a and vitality, proliferation, apoptosis and senescence of rCPCs were evaluated after DOXO administration.
Results: MiR-34 family was upregulated in DOXO treated rCPCs. The expression of protective genes Sirt-1, Bcl-2, PNUTS, important miR-34a target mRNAs, was significantly reduced after DOXO administration. To investigate whether inhibition of miR-34a can provide therapeutic benefit in DOXO exposed rCPCs, the cells were transfected with antimiR-34a. The expression of antimiR-34a increased vitality and proliferation of DOXO-treated rCPCs at 24 h. Moreover, antimiR-34a significantly reduced rCPCs apoptosis subsequently to DOXO administration at 24 and 48 h. Finally, the fraction of β-Gal positive cells was significantly decreased in cells co-treated with antimiR-34a and DOXO at 24 h, suggesting that miR34a inhibition could protect rCPCs from DOXO-induced senescence. In conclusion our data demonstrate that miR-34a is involved in DOXO induced toxicity of rCPCs and its modulation seems to protect the anthracycline exposed cells. Further experiments are ongoing to assess the pathways regulated by miR34a inhibition in DOXO treated rCPCs.

Bernardo B.C. et al. PNAS 2012;109(43):17615-20.
Boon R.A. et al. Nature. 2013;495(7439):107-10.
Chen F., Hu S.J. J Biochem Mol Toxicol. 2012;26(2):79-86.
De Angelis A. et al. Circulation. 2010 Jan 19;121(2):276-92.
Desai V.G. et al. Toxicol Appl Pharmacol. 2014;281(2):221-9.
Piegari E. et al. Basic Res Cardiol. 2013;108(2):334.
Small E.M., Olson E.N. Nature. 2011;469(7330):336-42.
Valencia-Sanchez M.A. et al. Genes Dev 2006;20:515-524.