Abstract: Duchenne muscular dystrophy leads to compromised genomic stability in stem cells and depletion of cardiac progenitors in failing heart

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Topic(s):
Stem Cells, Cell Cycle, Cell Senescence, Cell Death

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S66

Funding Acknowledgements:
Supported by: GACR CR P302/12/G157, MEYS CR: LQ1605 and CEITEC 2020 (LQ1601), AFM 20225, FRM; SPF20130526710, Duchenstem no. 28379TE, INSERM

Duchenne muscular dystrophy (DMD) is a genetic condition characterized by the lack of functional dystrophin. The progressive muscular pathology has devastating effect on the quality and lifespan of male patients. Majority of the DMD patients develops heart muscle fibrosis and cardiomyopathy, eventually leading to heart failure and premature death. Although several molecular mechanisms leading to the DMD cardiomyocyte death were described during the recent decades, the link between dystrophin deficiency and delayed onset of cardiomyopathy is still unclear. Recent evidence suggests involvement of progenitor population failure: thus we focused on studying DMD stem cells.

First we used mdx mouse model lacking dystrophin, to analyze c-­kit+ cardiac progenitor cells (CPCs) depletion and its mechanism. We observed dramatic increase in CPCs population in young adult (2-­3 months) mdx mice hearts, followed by steep decrease in mature mdx adult (5-­6 months) animals, which is in contrast to WT mouse hearts, where the CPCs population size in both young and mature hearts is stable. The analysis of double strand breaks demonstrated that CPCs depletion in mdx animal hearts is associated with elevated nuclear DNA damage.

In order to dissect the mechanism of CPCs depletion in humans, we used DMD patient specific induced pluripotent stem cell model and human embryonic stem cells with dystrophin mutation introduced by CRISPR/Cas technology (DMD hPSC for both models). We observed that absence of dystrophin in DMD hPSC leads to dysregulation of nitric oxide synthase (NOS) activity, resulting in significantly elevated reactive oxygen species (ROS). Inhibition of NOS activity results in lowering ROS level in DMD hPSC. Elevated ROS level in DMD hPSC was further associated with increased DNA damage. Both the inhibition of NOS, as well as ROS scavenging, results in DNA damage reduction. Further, we observed that DMD phenotype is associated with increased mutation frequency of the hPSC, which suggests causal link leading from dystrophin deficiency, via NOS dysregulation, to the elevated ROS and subsequent DNA damage. Finally, we showed compromised genomic stability of DMD stem cells by analysis of mutant frequency.

Based on these results, we hypothesize that dystrophin deficiency leads to elevated proliferation of CPCs, presumably by cardiomyocyte damage/death or inflammatory response. We suggest that elevated proliferation together with NOS induced-­ROS mediated-genomic instability leads to CPCs depletion, and subsequently to limited regenerative capacity of the heart muscle. In contrast to plain cardiomyocyte damage, this chain of events could explain the delayed onset of cardiac symptoms.