Effect of DPP3 expression on oxidative stress in MEFs

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INTRODUCTION

Dipeptidyl peptidase III (DPP3), the sole member of the M49 family of metalloproteases, is a zinc-dependent aminopeptidase that specifically cleaves dipeptides at the N-terminus. DPPIII is mainly found as a cytosolic protein although some studies suggest a potential localization in membranes. DPP3 is associated with important physiological functions in mammals and it is widely distributed in several tissues such as brain, liver, kidney, pancreas, spleen and lungs. A variety of small peptides such as Met-enkephalin and angiotensin I and II are targets of the enzyme, however the full range of substrate peptides remains undefined. There is accumulating evidence for the involvement of DPP3 in oxidative stress, pain, cell cycle regulation, carcinogenesis and inflammation, however the exact role of DPP3 in physiology and disease-related processes remains elusive. In addition, recent data have indicated that DPPIII also affects the Keap1-Nrf2 signaling pathway by competing with Nrf2 for binding to Keap1 through a highly conserved ETGE motif found both in DPPIII and Nrf2. Binding of DPP3 to Keap1 may enhance the function of Nrf2 by blocking its ubiquitination, which usually leads to uncontrolled transcriptional activation of Nrf2. To address the involvement of DPPIII in oxidative stress, a dpp3-knockout mouse model was generated and a mouse embryonic fibroblast cell line (MEF) was established.

MATERIALS AND METHODS

Two MEF cell lines from were generated from wildtype and knockout mice. Cells were cultured, up to 4 passages, in DMEM medium 10% FBS and 1% primocin. The genotype of the generated cells was determined by the PCR method and further analysis of the amplified gene was performed by agarose gel electrophoresis. The DPP3 activity was determined by fluorometrically measuring (ex. 332nm; em. 420nm) the release of 2-naphthylamine from the artificial substrate Arg-Arg-naphthylamide at 37 ˚C . The concentration of H_2O_2 was determined using the Pierce™ Quantitative Peroxide Assay Kit (ThermoFischer Scientific) and absorbance at 595 nm was measured. The intracellular ROS level was detected by using 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) and fluorescence was determined at 485 nm excitation and 525 nm emission. Level of lipid peroxidation in cells was determined by measuring concentration of malondialdehyde (MDA) present in the sample measuring the absorbance at 532 nm.

RESULTS

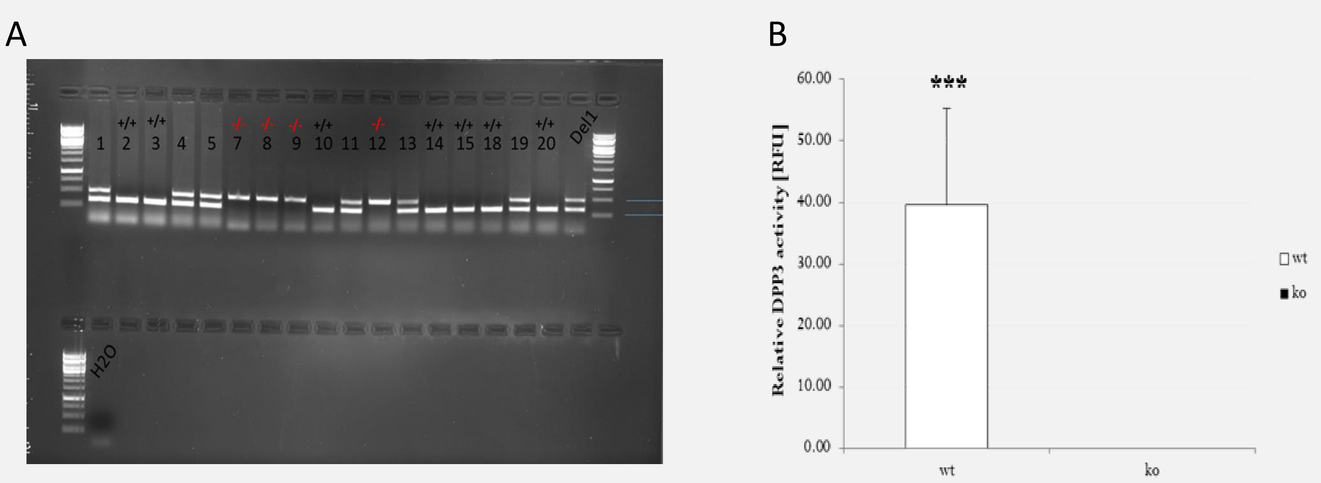

Figure 1: Characterization of the generated mouse embryonic fibroblasts: Representative gel of PCR analysis of the presence of a gene sequence for the *dpp3* (wildtype 200 bp; knockout 500 bp; heterozygos 200+500 bp)(**A**). Relative DPP3 activity (***p < 0.0001,versus wildtype mice based on unpaired two-sided Student's t-test)(**B**).

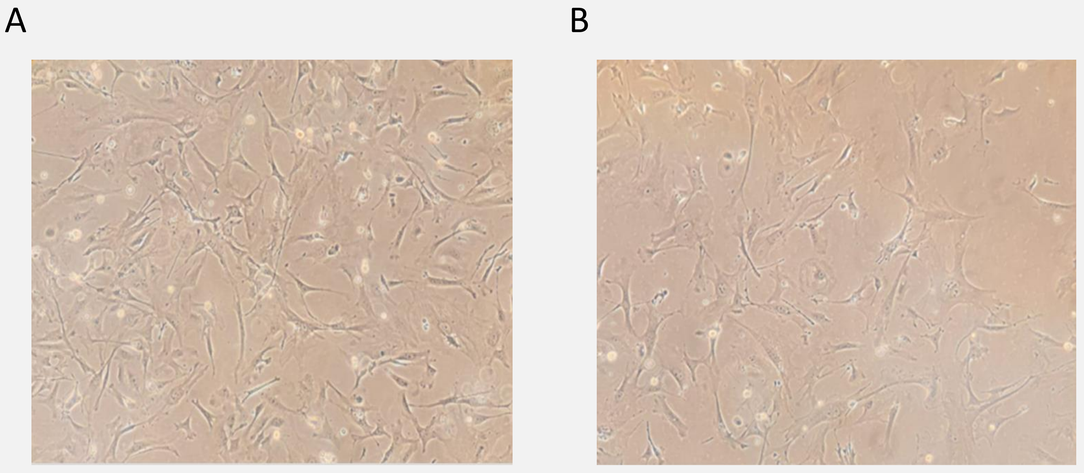

Figure 2: Microscopic presentation of mouse embryonic fibroblasts (MEFs) in cell culture at 20x magnification: Wildtype MEF cells with normal *dpp3* expression, $dpp3^{+/+}$ (**A**). Knockout MEF cells with lack of *dpp3* expression, $dpp3^{-/-}$ (**B**).

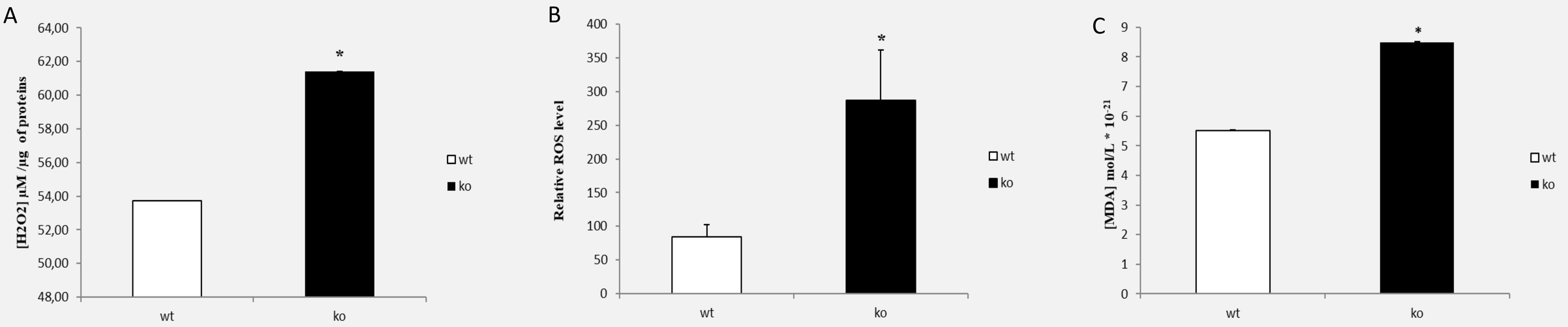

Figure 3: Oxidative stress markers in mouse embyonic fibroblasts: The absence of DPPIII increases the hydrogen peroxide concentration but also the relative level of reactive oxygen species (ROS) in the MEF when compared to the wild type where the levels of hydrogen peroxide and ROS are significantly lower (**A, B**). This is supported by the increased concentration of malondialdehyde (MDA), which is a marker of lipid peroxidation (**C**) (*p < 0.05,versus wildtype mice based on unpaired two-sided Student's t-test).

CONCLUSION

Oxidative stress parameters such as ROS generation and lipid peroxidation are significantly higher in cells lacking *dpp3* expression, and thus *dpp3*-knockout mouse embryonic fibroblasts experience more oxidative stress then cells with normal expression of *dpp3*. These results suggest an important role for DPPIII in maintaining homeostasis, which will be further investigated to unravel the molecular pathways that connect DPP3 with the production of ROS.

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