MiR-155 ablation protects spinal cord (SC) from damage in a mouse model of ischemic SC injury

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PURPOSE: Thoraco-abdominal aortic aneurysm (TAAA) is a surgical procedure performed when the risk of aneurism rupture, and patient death as a consequence, is really high. Unfortunately 5-10% the TAAA repairs results in patient paralysis. MicroRNA 155 (miR-155), a pro-inflammatory microRNA is associated e.g. with neuro-inflammatory pathologies such as Alzheimer's disease[1] and multiple sclerosis[2]. In this study a mouse model of thoraco-abdominal aortic aneurysm (TAAA) repair was used to study a miR-155 ablation as a protection factor from spinal cord (SC) ischemic injury. MRI imaging was used for in vivo monitoring and as a prognostic factor of the paralysis event in wild type (WT) and miR-155 knockout (miR-155$^{-/-}$) mice.

METHODS: WT and miR-155 knockout (miR-155$^{-/-}$) mice underwent a descending aortic cross-clamping (ACC) procedure for a 7.5 min at the 34.5°C body temperature (as describe previously)[3]. T2-weighted images of spinal cord covering at least T6-L2 spinal cord level were obtained at 24-30 and 48-72h post ACC (RARE seq., TR/TE=2500/36 ms, Rare factor=8, slice thickness 0.5 mm, 78*66 µm resolution, Naver=4). T2 map was performed at 48h post ACC at the T12 SC level (with MSME sequence and following parameters: TR/TE=2200/10.87 ms, 16 echo images, and 1 mm slice thickness). Imaging was performed using 9.4T MRI system (Bruker BioSpin, Germany) and 4-channell phase array mouse brain coil as a receiver and 72 mm volume coil as a transceiver. Edema volume was calculated from manually-traced ROIs on the T2-weighted images (hyperintense signal) and 3D image was reconstructed using TeraRecon workstation. T2 relaxation values were calculated from the same ROI of the gray matter for each mouse. After the last MRI imaging session, the mouse spinal cords were prepared for an H&E staining. Data are represented as an Average±Standard Deviation.

RESULTS: The ablation of miR-155 reduced the incident of paralysis by about 46% in mice subjected to ACC procedure (Figure 1A). All paralyzed mice presented significant vacuolated gray matter on histological sections (Figure 1D). There was smaller region of damage in the gray matter and more skipping area in miR-155$^{-/-}$ in comparison to WT visible on histopathological slices.

MRI analysis (Figure 1B) showed edema throughout the central gray matter of the SC in paralyzed mice (volume=7.31±4.31 mm^3, N=8). There was no, or very small, volume of edema observed in non-paralyzed mice (0.23±0.36 mm^3, N=9). Eight animals have been subjected to MRI acquisition to obtain T2 map at the T12 SC level. Six mice from this group were paralyzed. Relaxation time was higher in paralyzed in comparison to not-paralyzed mice (T2=86±7.2 ms vs 50.5±19.1 ms, respectively).

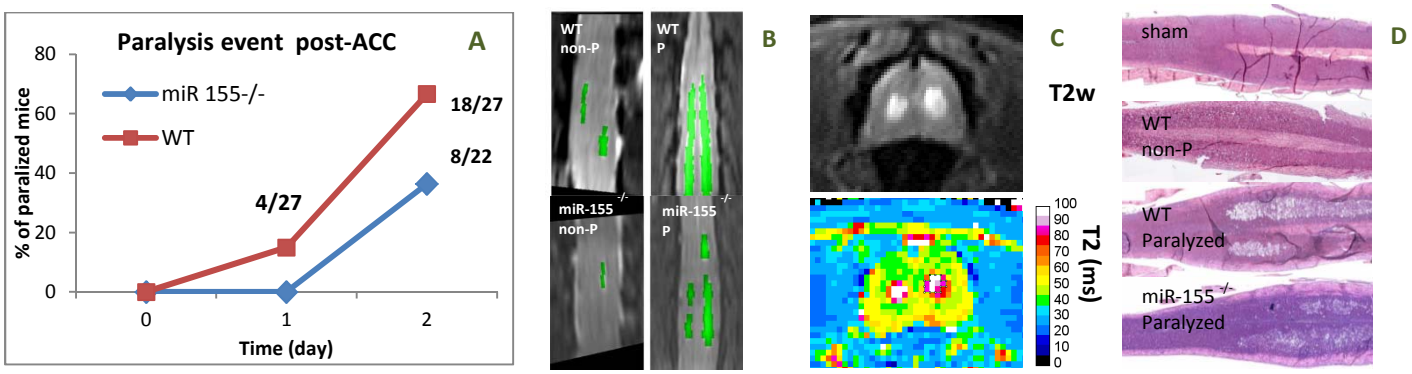

Figure 1. (A) Comparison of paralysis events in WT and miR-155$^{-/-}$ mice after aortic cross-clamping procedure (ACC); (B) 3D reconstruction of T2-weighted images with edema ROIs (green mask) of WT (upper) and miR-155$^{-/-}$ mice (below); P-paralyzed, non-P not paralyzed; (C) T2-weighted image (upper) and color T2 map (below) at the T12 SC level, 48 h post ACC. (C) H&E stained spinal cord of sham and animals subjected to ACC procedure.

DISCUSION/CONCLUSION: The significant reduction in paralysis incident after ACC in miR-155$^{-/-}$ and reduction in lesion size, observed on histological slices, may result from reduction of SC barrier leakage (reduction in edema), better control of homeostasis following ischemia/reperfusion, reduction in inflammatory response or a combination of all of these factors. It is important to note that TAAA is a planned procedure thus it is possible to administer preventive interventions to the patient to reduce a spinal cord injury. We believe that strategies that would allow blocking miR-155 up-regulation following TAAA may offer a new therapeutic opportunity for preventing paralysis in patients.

We are planning to continue the pre-clinical study with focus on (a) biochemistry of the miR-155 ablation and its protective effect on SC injury, (b) adding MR imaging at earlier time points and including MR imaging methods which can be more sensitive at the early changes post-surgery (DWI, DCE), as well as (c) using MRI methods for a monitoring the therapeutic effect of the intervention after TAAA outcome in SC injury in mice.

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