FGFR Inhibition protect synapses from microglial reactivity in mild TBI.

Introduction:
Microglial reactivity is a major event in the acute phase of traumatic brain injury (TBI) shaping the severity of the neuronal damage suffered in the long term and in the end the recovery of the patients. So far, in the acute treatment of TBI no medication is used to target the microglial reactivity specifically. Recent publication on the other hand indicate a trauma specific upregulation in tyrosine kinase receptors including FGFRs in the acute phase, indicating a potential entry point to alter microglial reactivity via FGFR Inhibition. This FGFR Inhibition could result in the repurposing of already existing cancer medication to enhance the recovery after TBI and reduce the resulting damage.

Methods: To mimic a possible first aid response a pan-FGFR inhibitor was administered to mice 30min after the TBI was applied to completely inhibit FGFR signalling. For subacute time points the FGFR inhibitor administration was repeated each day. Phosphor-RTK Arrays were employed to study the phosphorylation of several RTKS including FGFR2, FGFR3 and FGFR4 immidiately 3h after the injury. In addition, pFGFR1 and pFGFR3 immunostaining was used for validation. The effect of FGFR inhibition on microglia was characterized by immunostaining for the markers CD11c, CD68 and CST7 at 1dpi and 7dpi. General effects of FGFR inhibition were monitored by a proteome analysis at 3dpi and 7dpi. As outcome parameters of the TBI injury immunostainings for NeuN, VGLUT1, SHANK2+3, VGAT and Gephryn at 7dpi were employed.

Results: Inhibition of FGFR signalling during TBI successfully restricted phosphorylation of FGFR1, FGFR3, FGFR4 and ErbB4. Phosphorylation of FGFR1 and FGFR3 during TBI was confirmed to occur mainly in microglia. This downregulation co-exists with a restrict in microglial reactivity in terms of density, their expression of microglial CD11c, CST7, which are markers related to Disease-Associated-Microglia (DAM), and their phagocytic activity via lysosome protein CD68 expression at 1dpi and 7dpi compared to TBI without FGFR inhibition. In fact, our proteome analysis at 3dpi revealed a strong repressive nature of the FGFR inhibitor where proteins related to the immune system and the cytokine response were severely downregulated (Axl, CD40L, CXCR3, CCL4, CCR4, ILR6, MMP3 and OPG). Throughout the experiments FGFR Inhibitor treatment exhibited a neuroprotective effect. This neuroprotective effect includes a preserved neuronal density at 7dpi and the protection of synaptic density especially in form of excitatory presynaptic VGLUT1 and inhibitory postsynaptic Gephryn.

Discussions: In this study, we have shown the relevance of FGFR signalling in controlling the microglial reactivity after TBI. In fact, inhibiting FGFR signalling results in a reduction of the inflammatory reaction, shown by a reduction in microglial activation and proteomic changes. This reduction is followed by a better outcome in the form of an increased preservation of neurons and their synaptic networks, indicating the potential of FGFR Inhibitors as a possible repurposed medication for TBI patients.