TLR4 Deficiency Alters Platelet-Derived Exosome Release and Increases Mortality in a Model of Liver Regeneration

Nijmeh Alsaadi[1], Hamza Yazdani[1], Matthew D. Neal[1], Roberto Ivan Mota Alvidrez[1,2,3]
[1]Department of Surgery-University of Pittsburgh, [2]McGowan Institute for Regenerative Medicine-University of Pittsburgh, [3]Vascular Medicine Institute

Introduction: Platelets have a strong role in liver regeneration; however, the exact mechanisms are still unknown. A novel mechanism proposes platelet-derived exosomes (PDE), small particles with high biological activity, to be activators of inflammatory and regenerative molecules via activation of the HMGB1/TLR4 pathway. We hypothesize that mice deficient of TLR4 will have decreased PDE release after 24hr polytrauma, resulting in decreased liver regeneration compared to WT mice.
Methods: Following 24hr polytrauma to activate platelet HMGB1, PDEs were isolated from adult male WT and TLR4-KO mice using a kit-based preparation. Nanosight was used to assess exosome concentration and size, and PDE characterization was performed by ImageStream system. Adoptive transfer of $2x10^6$ PDEs was carried out subsequent to 70% hepatectomy in WT and TLR4-KO mice. Hepatocyte proliferation was evaluated using Ki67 MUSE. Data is represented as mean±SEM, and statistical analysis was performed using one- and two-way ANOVA with Tukey's correction.
Results: All exosomes were effectively CD41+CD63+, confirming their platelet origin. Nanosight analysis showed that following polytrauma, TLR4-KO mice have decreased PDE concentration with size remaining unaffected. Following hepatectomy and PDE injection, WT mice showed increased hepatocyte proliferation 48 hours after, while TLR4-KO did not show any proliferation and exhibited a >80% mortality rate.
Conclusions: These data suggest that PDE release is dependent on HMGB1 signaling, and TLR4 deficiency severely increases mortality and halts hepatocyte proliferation following liver resection. Future studies will be aimed at understanding the high mortality in this model and to comprehend the exact mechanism behind the results.
Financial Support: Department of Surgery Funding for RIMA, 1R35GM119526-01 from NIGMS to MDN