PDGF Increases Hepatocyte-HMGB1 Synergistically with Stimulation of Heparan Sulfate in-Vitro and Alters Hepatocyte Exosome Release in TLR4 Deficient Mice

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Introduction: Exosome release has unique effects depending on the tissue of origin. PDGF is a strong stimulant of multiple tissue processes like exosome release. Increase in HMGB1 via TLR4 receptor activation has been proposed to induce extracellular vesicle release. Our hypothesis is that Heparan Sulfate (a known TLR4 ligand) will be able to evidence PDGF dependence on TLR4 to promote exosome release in hepatocytes.
Methods: Isolated primary WT and TLR4-KO hepatocytes were stimulated for 24hrs with PDGF, supernatant was evaluated with a Nanosight for exosome release quantification. Primary hepatocytes were also stimulated 24hrs with the following conditions:Heparan Sulfate,PDGF and Heparan Sulfate+PDGF. Hepatocytes were lysed for protein quantification by Western Blot(n=3). Data is represented as mean±SEM. Statistical analysis was performed using one-way ANOVA with Tukey's correction.
Results: Hepatocyte exosome concentration is increased in vitro in the presence of PDGF after 24hrs in WT hepatocytes, however in TLR4-KO mice, exosome particle concentration did not differ similar to levels observed in unstimulated WT hepatocyte exosomes. As for exosome size, 24hr-PDGF stimulation increased WT hepatocyte exosome size but not in TLR4-KO hepatocytes. Western blot analysis showed that the highest increase in HMGB1 protein levels is observed only after 24hr combined stimulation of both Heparan Sulfate and PDGF compared to individual stimulation.
Conclusions: PDGF has a direct effect in hepatocyte exosome release whenever TLR4 signaling is intact and appears to be associated with the HMGB1 activation in vitro. Future studies will be directed to evidence the downstream HMGB1 pathway associated with PDGF-independent platelet stimulation.
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