The Roll of Toll-like Receptors in the Stem Cell Inflammatory Response

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Background: Toll-like receptors serve as ligands for LPS and other inflammatory mediators. Previous studies have shown that TLR4 is deleterious, while TLR9 is beneficial in the setting of inflammation and ischemia. Umbilical stem cells (USCs) have shown promise in the acute treatment of inflammation but their response to inflammatory mediators has not been fully elucidated. We hypothesized that knockdown of TLR4 in USCs would result in lower levels of IL-6 and VEGF production, while knockdown of TLR9 would produce higher levels of these cytokines when cells were exposed to LPS or hypoxia.

Methods: USCs were cultured in polystyrene flasks in Mesenpro media at 37C in 5% CO_2 in air. Cells were plated into 12-well plates at a concentration of 100,000 cells/well. Cells were transfected for 24h with siRNA to knockdown TLR4 and TLR9, respectively. Knockdown was confirmed by PCR. Experimental groups were: 1) Control, 2) Scramble siRNA for negative control, 3) TLR4 siRNA and 4) TLR9 siRNA. After 24 hours the media was changed and cells were exposed to either LPS (200ng/ml) or 5% oxygen for 24 hours. The supernatant was then collected and analyzed with ELISA for VEGF and IL-6. Data were analyzed by Mann Whitney test and $p<0.05$ was significant.

Results: TLR4 and TLR9 were effectively knocked down by the transfection process. However, no significant levels of VEGF or IL-6 were detected from any of the experimental groups.

Conclusion and Implications: Although no significant levels of VEGF or IL-6 were detected in the ELISA after exposure to inflammatory agents, there is still indication that TLR4 and TLR9 play critical roles in inflammation. The experiment should be run again and tested for more inflammatory cytokines. Positive results from this study can translate to an in vivo model, in which engineered TLR4KD stem cells have the potential to minimize tissue inflammation and beyond standard cell therapy.

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