Secreted Factors from Bioactive Kidney Cells Attenuate NFκB Signaling Pathways: Implications for a Paracrine Mechanism of Immune Regulation and Regenerative Outcomes

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Introduction: Nuclear Factor κB (NFκB) activation is thought to participate in acute and chronic diseases by eliciting robust innate immune responses in injured or diseased tissues; however, recent work suggests that NFκB pathways might also participate in the resolution of inflammation in a model of kidney disease1. The bifunctional nature of this pathway suggests that NFκB may modulate a balance between pro-inflammatory, fibrotic mechanisms of tissue repair and restorative or regenerative outcomes in vivo. We have previously demonstrated that intra-renal delivery of bioactive kidney cells, a Neo Kidney Augment™ (NKA) prototype, can preserve kidney function and tissue integrity in a Lewis rat model of chronic kidney disease2. In this study, we investigated the role of NFκB pathways in the NKA-initiated attenuation of disease progression in the 5/6 nephrectomy model and to identify properties of the bioactive kidney cells that may contribute to regenerative outcomes through direct modulation of NFκB activation.

Materials and Methods: Remnant kidneys were harvested from Lewis rats in which a two-step 5/6 nephrectomy procedure was performed 6 weeks prior to being treated with an NKA prototype. NKA-treated (TX) or untreated (UNTX) tissues were assayed for NFκB activation by immunohistochemistry, RT-PCR, Western blot analysis, and electrophoresis mobility shift assays (EMSA). Conditioned media (CM) collected from ex vivo NKA cell cultures grown in serum- and supplement-free media was used for in vitro functional assays. The human proximal tubule cell line (HK-2) was used as the target cell type for molecular and immunofluorescence-based assay readouts. Vesicular particles shed by cells into the culture media (exosomes) were collected by high-speed centrifugation. Total RNA isolated from exosomes was screened using PCR-based arrays of known microRNA sequences (Qiagen).

Results: Nuclear localization of the NFκB subunit, RelA/p65, was observed in remnant kidneys from 5/6 nephrectomized rats, suggesting activation of inflammatory pathways in UNTX tissues. Preliminary comparison with TX tissues by RT-PCR showed a decrease in RelA gene expression, suggesting that NKA treatment may influence NFκB pathway activation through inhibition of RelA/p65 expression; this hypothesis is supported by the observation that CM attenuates Tumor Necrosis Factor-α (TNFα)-induced NFκB activation in vitro, as evidenced by the reduced nuclear localization of RelA/p65 in CM-exposed HK-2 cells (Figure 1) relative to that seen in response to TNFα alone. Ongoing RT-PCR analyses of NKA exosome microRNAs are investigating whether sequences known to influence NFκB pathways are present.

Discussion and Conclusions: These data suggest that factors secreted from NKA cultures can modulate NFκB pathways, supporting a paracrine mechanism for immune modulation and regeneration in vivo. While it remains uncertain whether these influential factors include microRNAs, we speculate that cell-to-cell transfer of microRNAs via exosomes following NKA treatment may alter NFκB activation states in renal tissue.

References:

Disclosures: Tengion employees disclose stock options.