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Tubular exosomes in renal ECM microenvironment promotes fibrosis progression by activating latent TGF-β1

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Background and Aims: Chronic kidney disease (CKD) is characterized with progressive fibrosis caused by excessive extracellular matrix (ECM) deposition. Tubular epithelial cells secreted increasing exosomes into extracellular space under pathological conditions with integrin as the prominent constituent membrane cargo. Due to the critical role of integrin in activation of TGF-β1 signalling, this study aims to investigate the performance of tubular-derived exosomes in the ECM microenvironment, exploring the potential mechanism of exosomal integrin mediated activation of latent TGF-β1.

Method: A fibrosis mouse model was established with unilateral ureteral obstruction (UUO) and an ITGβ6 overexpression cell line were established to observe the effect of ITGβ6+ exosomes in vitro. Elastic or stress-relaxing hydrogels based on Polymethyl Methacrylate (PMMA) and acrylic acid-WGG[KA]3-heparin were constructed to mimic the in situ ECM under normal and fibrotic conditions with different stiffness (2kPa, 50kPa). Exosomes in hydrogels were tracked by single particle tracking to investigate their mobility and retention capability. Purified exosomal integrin β6 and inhibitor of exosome secretion, GW4869 was applied to clarify the role of exosomal integrin in latent TGF-β1 activation.

Results: In UUO model, immunofluorescence staining showed that latent TGF-β1 localized to ECM space and stored in the renal tubular interstitial which was activated with fibrotic progression. Additionally, we observed integrin β6 expression in tubule or interstitial increased with fibrotic progression. Isolated exosomes derived from cultured tubule cells under serum-free condition or renal tissues of UUO mice showed higher proportion of integrin β6+ subpopulation, as demonstrated by single exosome analysis by Nanoimaging (Oxford). Interestingly, these exosomes diffused through hydrogels, while maintaining their original morphology and particle size. It turned out that stress-relaxing hydrogels with lower stiffness exhibited better retention of exosomes. Then, transwell experiment and single-particle tracking both revealed increased exosome diffusion rate in ECM with higher stiffness. Due to the critical role of integrin in the activation of latent TGF-β1, we propose integrin β6+ exosome may exert such effect in ECM microenvironment. Impressively, purified ITGβ6+ exosomes significantly increased TGF-β1 activation in TGF-β1 overexpressed HK-2 cells, while inhibiting exosome secretion via GW4869 treatment reversed such activation remarkably.

Conclusion: We demonstrated that tubular epithelial cell derived exosomal integrin β6 diffused in ECM space and promoted the activation of latent TGF-β1. Higher ECM stiffness correlates with accelerated movement of exosome which enhanced TGF-β1 activation. Exosomal integrin β6 may represent a novel manner of TGF-β1 activation with signal-spreading potential which may become a new therapeutic target for suppression of TGF-β1 activation in CKD treatment.