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Podocyte Exosomal MicroRNA Profiling by Next Generation Sequencing

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Objectives : Exosomes play an important role in cell to cell communication. These contain microRNA, mRNA and protein. MicroRNA profiling can provide a snapshot of cellular process healthy and diseased cells. However, very little is known about microRNA profiling in the human podocyte derived exosome. Puromycin induced podocyte is known as a cell injury model of focal glomerular sclerosis. Here we describe the difference of podocyte exosomal microRNA profiling between healthy- and puromycin treated.

Methods : Podocyte apoptosis were induced by puromycin. Exosomal micro RNAs were extracted using Total exosome isolation kit® and exoRNeasy Maxi Kit®. We analysed the expression of exosomal microRNA in healthy and puromycin treated human podocyte using next-generation sequencing. Exosome isolated from podocyte was cocultured with HK2 cell to investigate if HK2 uptake these exosomes using PHK26 labeling.

Results : Western blots confirmed the presence of the exosomal markers CD 63 and TSG 101 in the cell culture supernatant. Exosome from podocyte was uptake by HK2 cells (Fig 1). RNAseq generated reads for 154 micro RNAs, of which 5 were upregulated in puromycin treated cell derived exosome compared to healthy cell derived exosome. These miRNA preferentially targeted TGB-β pathway.

Conclusions : Puromycin treated podocyted derived exosome have a distinct micro RNA content compared to healthy podocyte exosomal micro RNA. Exosome derived from podocyte may play a role in cell-to-cell communication with HK2 cell.

Keywords : podocyte, exosome, microRNA