Mesenchymal stromal cells (MSC) accelerate epithelial tight junction assembly via the AMP-activated protein kinase (AMPK) pathway, independently of Liver kinase b1 (Lkb1)

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Background. Disruption of epithelial tight junctions (TJ) is one of the earliest hallmarks of acute kidney injury (AKI). Mesenchymal stromal cells (MSC) represent a heterogeneous population of adult fibroblast-like multipotent cells capable of tissue repair properties following AKI. Hence, we hypothesized that MSC may modulate TJ. We focused on the AMP-activated protein kinase (AMPK) pathway since it participates both in energy salvation and TJ maintenance.

Methods. Madin-Darby canine kidney (MDCK) cells were cultured alone or in direct contact with rat bone marrow derived MSC (upon a 3:1 ratio) for 5 days. Next, a Ca2+ switch, i.e. switching cells from [5µM] Ca2+ (for 48h) to [1.8mM] Ca2+ (up to 2h), was performed. TJ formation was assessed upon ZO-1 relocation by immunofluorescence, and AMPK phosphorylation was quantified by immunoblotting. Experiments were repeated using MDCK stably expressing ShRNA against the AMPK kinase, Liver kinase b1 (Lkb1), or against Luciferase (LUC, used as control).

Results. Following Ca2+ switch, ZO-1 relocation occurred significantly faster in MDCK/MSC versus MDCK. Correspondingly, phospho-AMPK/total AMPK ratio was 1.7-fold increased in MDCK/MSC versus MDCK alone (n=4, $p<0.001$). Of note, AMPK was not

detectable in MSC alone. As previously reported, Ca2+-induced ZO-1 relocation to TJ was significantly delayed in Lkb1-ShRNA versus LUC-ShRNA MDCK. However, after 48-hour Ca2+ deprivation, TJ-associated ZO-1 was significantly more abundant in MSC co-culture systems of either ShRNA in comparison to corresponding ShRNA MDCK alone. Following Ca2+ switch, ZO-1 relocation occurred twice faster in ShRNA MDCK/MSC versus ShRNA MDCK alone ($n=4$, $p<0.001$). Phospho-AMPK/total AMPK ratio was 1.5-fold increased following Ca2+ switch in ShRNA MDCK/MSC versus ShRNA MDCK alone ($n=4$, $p<0.001$). No difference in phospho-AMPK/total AMPK ratio was observed between Lkb1-ShRNA versus LUC-ShRNA MDCK following Ca2+ switch.

Conclusions. Our results suggest that MSC may modulate AMPK activation in epithelial cells at the time of Ca2+-induced TJ assembly, independently of Lkb1.