ABSTRACT

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Title: ARHGEF10 acts as a GEF for RhoB in controlling capillary endothelial permeability
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Background: Microvascular endothelial cells (ECs) establish a semi-permeable barrier between the blood and tissue, regulating selective exchange of fluid and solutes. Capillary EC contribute to this barrier by forming tight junctions (TJs) that exclude even the smallest solutes. In critical illness, such as sepsis, capillary inter-EC junctions are disrupted in response to inflammatory cytokines, such as TNF, leading to excessive leakage of fluid and solutes into the tissues. Active disassembly of TJs is, in part, mediated by Rho-GTPases, such as RhoA, RhoB and RhoC, which are activated by Rho guanine nucleotide exchange factors (GEFs). The specific GEFs that activate Rho-GTPases in the setting of sepsis inflammation have not been definitively identified.

Methods: Human dermal microvascular ECs (HDMECs), which form tight junctions, were used to replicate the capillary vasculature. Candidate GEFs were identified by whole transcriptome profiling of HDMECs under basal conditions and after TNF stimulation. Function of expressed candidates was assessed by siRNA screening both for modulation of TNF-induced changes in trans-endothelial electrical resistance (TEER) and for TNF-induced disruption of the expression patterns of specific tight junction proteins analyzed by confocal microscopy. Western blotting and qRT-PCR was performed to assess TNF-induced changes in protein and mRNA levels, respectively, of functional GEFs. Finally, activity of GTP-bound Rho-GTPases in cell lysates and direct interaction of immunoprecipitated GEFs with specific Rho proteins were assessed with Rhotekin bead pulldown assays.

Results: Of the 73 known GEFs, 15 were constitutively expressed in HDMEC and up-regulated by TNF stimulation. Of these, siRNA depletion of ArhGEF10 significantly diminished TNF-mediated reductions of TEER of HDMEC monolayers. Concordantly, HDMECs depleted of ArhGEF10 displayed less TNF induced disruption of tight junction molecules claudin-5 and ZO-1. Activity of RhoB in HDMEC cell lysates after TNF stimulation was significantly decreased with ArhGEF10 depletion while activity of RhoA and RhoC was minimally altered. Immunoprecipitated ArhGEF10 incubated with isolated recombinant Rho proteins and GTP markedly and selectively increased RhoB GTP loading compared to RhoA and RhoC.

Conclusions: We demonstrate that ArhGEF10 acts as a GEF for RhoB to promote capillary EC barrier disruption in setting of inflammation.

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