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Title: CRITICAL ROLE OF MTORC1 IN ENDOTHELIAL BARRIER REGULATION

Abstract

Introduction: Increased endothelial cell (EC) permeability is an important component of many inflammatory diseases such as sepsis and acute lung injury/acute respiratory distress syndrome (ALI/ARDS). Mechanistic (formerly mammalian) Target of Rapamycin (MTOR) is a central controller of cell growth, metabolism, and cytoskeletal organization, and exists in two distinct multiprotein complexes, MTORC1 and MTORC2, to exert these functions. However, the role of these complexes in regulating EC barrier function under inflammatory conditions is poorly understood. In this study, we addressed the role of MTORC1 in this response by modulating its activity via knockdown of RAPTOR (rapamycin-sensitive regulatory associated protein of MTOR, an essential subunit of MTORC1) or TSC2 (tuberous sclerosis complex 2, a suppressor of MTORC1). Our results show that MTORC1 plays an important role in EC barrier dysfunction induced by thrombin, an edemagenic and proinflammatory agonist whose concentration is elevated in plasma and lavage fluids of patients with ALI/ARDS and sepsis. Methods: Human Pulmonary Artery Endothelial Cells (HPAEC) were transfected with siRNA (siControl) or siRNA targeting TSC2 (siTSC2) to activate MTORC1 or RAPTOR (siRAPTOR) to inactivate MTORC1. Thrombin was used as an agonist for EC barrier dysfunction. EC barrier integrity was evaluated by measuring transendothelial electrical resistance (TEER), FITC-Dextran flux in a transwell assay. VE-cadherin disassembly and actin stress fiber formation were determined by immunofluorescence. Protein levels were analyzed by immunoblotting. Data was analyzed by Student's t-test. $P < 0.05$ was considered statistically significant. Results: Silencing of TSC2 (to activate MTORC1) augmented EC barrier disruption caused by thrombin. This effect was predominantly associated with impaired barrier recovery after it is disrupted in TSC2-silenced cells. Consistent with this, TSC2 knockdown caused a robust and persistent VE-cadherin disassembly at AJs and actin stress fiber formation and thereby gap formation in response to thrombin. Importantly, treatment of TSC2-silenced cells with Br-cAMP, an activator of barrier enhancing Rac1 GTPase, restored the barrier after it was disrupted by thrombin. Br-cAMP treatment also reduced the barrier disruption by thrombin, albeit to a lesser extent. In reciprocal experiments, silencing of RAPTOR (to inactivate MTORC1) attenuated the loss of barrier integrity induced by thrombin. Conclusion: MTORC1 activation promotes whereas its inactivation limits

thrombin-induced EC barrier dysfunction. The effect of MTORC1 activation is mediated, at least in part, by impairing Rac1-dependent recovery of the disrupted barrier. Together, these data indicate a novel role of MTORC1 in regulating EC barrier disruption by suppressing the activity of Rac1. Supported by: NIH (HL148695, GM130463, and T32 HL066988)