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Soutenance de thèse

The role of ALK1 and ALK5 receptors, and their cognate Smads, in TGFβ1-mediated podosome-formation in aortic endothelial cells.

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Transforming growth factor-β (TGF-β) regulates a wide array of cellular processes and deregulation of TGF-β signalling is associated with various vascular disorders. In the Lab of Dr. Génot it was discovered that TGF-β induces the formation of podosomes organised in superstructures called rosettes, in aortic endothelial cells. Podosomes are transient actin-based structures able to degrade the extracellular matrix (ECM). In this project we have studied TGF-β receptors and associated molecular mechanisms underlying podosome formation in response to TGF-β in primary bovine aortic endothelial cells (BAEc). Two types of TGF-β type I receptors (TβRI), ALK5 and ALK1, regulate TGF-β responses in endothelial cells. ALK5 being an ubiquitous receptor and ALK1 being endothelial cell specific. Both ALK5 and ALK1 receptors control the activation of distinct Smad proteins, and both are responsive to TGF-β stimulation. ALK5 activates Smad 2/3 and ALK1 activates Smad 1/5/8. BMP9 is another ligand for ALK1. ALK1 doesn't activate Smad2/3 in BAEc and ALK1 inhibits TGFβ-induced podosome formation. Smad1/5/8 stimulation by TGF-β treatment is induced through ALK1/ALK5 complex signalling. Using a knockdown approach, at the TβRI level, TGF-β induction of podosomes was inhibited. However, transfection of constitutively active (CA) TβRI showed that CA-ALK5 expression bypassed the TGF-β requirement for podosome induction

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whereas CA-ALK1 expression was ineffective. Looking downstream of TβRI signalling, the involvement of Smad proteins was also analysed in terms of podosome formation. Smad3 depletion completely abolished TGFβ-induced podosome formation whereas depletion of Smad1 or Smad5 proteins enhanced the TGFβ-induced podosome response. When overexpressed, Smad2 or Smad3, to some extent, bypassed TGFβ signals, whereas Smad1 overexpression diminished the TGFβ-induced podosome response. TGF-β also modulated the formation of another type of actin structure named actin-stars. The number of cells presenting actin-stars decreased with TGF-β treatment. However, in Smad3 depleted cells the formation of these actin-stars seemed to be stimulated by TGF-β. In BAEc stiffness and ECM proteins also seemed to modulate podosome and actin star formation. This project establishes that although TGF-β stimulates both ALK5 and ALK1, ALK5 signalling triggers podosome formation and ALK1 mitigates this signal. The canonical pathways through Smad protein regulation are important for TGF-β induced podosome in BAEc.

Key-words: TGF-β, ALK1, ALK5, podosomes, endothelial cells, Smads

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