European Cells and Materials Vol. 2. Suppl. 1, 2001 (page 23) ISSN 1473-2262

INTEGRIN DENSITY IN FOCAL ADHESION SITES: A MEASURE FOR INTRACELLULAR TENSION AND RIGIDITY OF ADHESIVE SURFACES

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INTRODUCTION: Adhesion, spreading and migration of cells on extracellular matrix (ECM) are complex processes that play fundamental roles in development, wound healing and immune response. Heterodimeric transmembrane receptors of the integrin family provide the mechanical link between the extracellular environment and the actin cytoskeleton required for adhesion and migration. Prior to adhesion, integrins diffuse freely in the cell membrane. Upon recognition of ECM ligands, integrins aggregate laterally and get anchored at their cytoplasmic side within a scaffold of cytoskeletal proteins [l]. This protein complex forms an adhesion site in which mechanical forces required for cell motility are exchanged between the actin cytoskeleton and the extracellular environment [2]. It is our aim to understand the architecture and behaviour of integrins within adhesion sites in response to biological surfaces and how this architecture is modulated by changes in intracellular contractile forces during cell spreading and migration.

METHODS: We developed a new tool to directly study integrin behaviour in living cells. Green fluorescent protein (GFP) was fused to the cytoplasmic domain of the mouse β3-integrin subunit (GFP-β3 integrin). Transfected GFP-β3 integrin was expressed on the cell surface and formed fully functional integrin heterodimers with the endogenously expressed αV integrin subunit [3]. We plated stably GFP-β3 integrin expressing cells on ECM coated glass coverslips and studied adhesion site dynamics by time lapse microscopy. Relative fluorescence intensity of integrin adhesion sites was measured using a CCD-camera piloted by the Openlab software. Modification of intracellular contractile forces was either accomplished by transfecting cells with dominant active or dominant negative members of the Rho family of small GTPases (Rac1, CDC42, RhoA) or by pharmacological activation or inhibition of the acto-myosin contractile system.

RESULTS: Dynamic analysis of adhesion sites in migrating cells revealed the existence of two functionally different types of αVβ3 integrin containing adhesion sites. At the cell front, protruding lamellipodia exhibited many stationary, low-density integrin adhesion sites. While the cell moved forward, low-density integrin adhesion sites transformed into high-density αVβ3 integrin containing adhesion sites, which began to slide when located to retracting parts of the cell rear. Integrin densities could vary up to 3-fold between these two types of contacts. Low-density integrin adhesion sites formed after relaxation of intracellular tension or stimulation by dominant active forms of Rac1 and Cdc42. In contrast, high-density integrin adhesion sites formed in a RhoA induced acto-myosin dependent manner.

DISCUSSION & CONCLUSIONS: The integrity of focal adhesion sites is maintained by a scaffold of actin filaments, that is cross-linked by bivalent actin-binding proteins. Integrins are physically linked to this framework by adaptor proteins such as talin. Due to the cytoplasmic anchorage of integrins within this actin lattice, we postulate that the integrin density is a direct reflection of the degree of acto-myosin contraction within an adhesion site. During cell migration, exploring organelles like lamellipodia or filopodia always form low-density integrin contacts that are subsequently transformed into high-density contacts by an acto-myosin dependent process. We propose, based on the ability of integrins to directly link the ECM to the actin cytoskeleton, that intracellular contraction of low-density integrin contacts is a way to probe the nano-rigidity of the encountered ECM surfaces and determine the maximal intracellular force that can be applied to such contacts. By linking different adhesion sites through the actin cytoskeleton, a cell may be able to integrate information of substrate rigidity, and modify its spreading and migratory behaviour accordingly resulting in durotaxis [4].

REFERENCES: [1]M. Hynes, R.O. (1992) *Cell* **69**, 11-25. [2]I.R. Balaban, N.Q. et al (2001) *Nat Cell Biol* **3**, 466-72. [3]Ballestrem, C. et al (2001) (submitted). [4]Lo, C.-M. et al (2000) *Biophys. J.* **79**, 144-152.

ACKNOWLEDGEMENTS: We would like to thank Marie-Claude Jacquier for excellent technical assistance and Patrick Ross for the mouse cDNA of β3 integrin.