Mechanisms of cell adhesion and migration

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Adhesion to the extracellular matrix (ECM) or to neighboring cells regulates multiple cellular processes such as cell migration, morphogenesis, proliferation, gene expression and survival. Activation of these responses involves specific interaction of membrane-bound adhesion receptors with the external surface, sensing its chemical, topographic and mechanical features, activation of specific signaling networks and assembly of multi-protein adhesion complexes at the contact site. Topics addressed by different members of the group, include:

Molecular diversity of adhesion complexes: Integrin-mediated adhesions are highly complex structures, consisting of multiple extracellular and cellular components, and associated with the actin cytoskeleton (Fig. 1, top and center panels). Based on their molecular composition and morphology, these adhesions fall into several categories, namely focal adhesions, focal complexes, fibrillar adhesions and podosomes. To determine the roles of individual components, *Sabina Winograd-Katz* is knocking-down individual proteins, using a siRNA transfection approach, and is determining the consequent effects of this treatment on adhesion site development and dynamics. *Tova Volberg* is mapping different states of integrins in stressed and relaxed adhesions, using conformation-specific antibodies, and *Ronen Zaidel-Bar* explores the phosphorylation of specific focal adhesion molecules (e.g. paxillin) and determines the involvement of this modification in regulating adhesion formation. A particular form of integrin-mediated adhesions, namely podosomes, found in osteoclasts and macrophages are investigated by *Chen Luxenburg* (in collaboration with *Lia Addadi*). These, dot-like adhesions were shown to undergo transformation from single contacts to large "sealing zone-like structures" that participate in bone resorption. This process is tightly regulated by the cytoplasmic kinase src, whose target, cortactin, regulates actin dynamics in the adhesion site (Fig 1, middle panel, Right image). The role of cortactin in cell protrusion, as well as in cell-to-cell adhesion, is further investigated by *Elad Landoy* (in collaboration with *A. Bershadsky*), focusing on different molecular partners, as well as modulators of this protein.

The roles of mechanical force in adhesion development: Studies in collaboration with *A. Bershadsky* demonstrated that application of force to focal adhesions induces their growth and/or apparent migration. These observations stimulated the development of several theoretical models, based on different physical considerations (in collaboration with *S. Safran* (WIS), and *M. Kozlov* (TAU)). *Masha Khoutorsky* is currently studying the role of formins in this process (in collaboration with *A. Bershadsky*). *Ronen Zaidel-Bar* showed that exposure of cells to shear stress induces increase in the size of "upstream adhesions", stimulation of local dephosphorylation of paxillin and Cas, and local inactivation of Rac1, leading to cell polarization. Modulation of cell-generated contractile forces is also investigated by Ilana Sabanay (in collaboration with P. Kaufman, U. Wisconsin) in conjunction with the regulation of intra-ocular pressure, and the possibile use of that principle for glaucoma therapy.

Role of phosphorylation in regulating cell adhesion and migration: Focal adhesions are known to be highly tyrosine phosphorylated structures (Fig 1, top). *Ronen Zaidel-Bar* demonstrated that the phosphorylation of at least two components of focal adhesions (paxillin and Cas) is highly sensitive to external forces (shear stress), and might be involved in a local regulation of cell polarization, driven by the small G-protein Rac1. He also demonstrated that tyrosine phosphorylation of specific plaque components has a profound effect on focal adhesion assembly and disassembly, and the consequent inter-conversion of the different forms of integrin adhesions.

Signaling from the ECM: Interactions with the ECM induce a wide variety of cellular responses, whose nature, extent and duration are regulated by multiple features of the surface. These include the specificity of the adhesive ligand, its local density as well as surface topography and stiffness. In collaboration with the group of *J. Spatz*, from U. Heidelberg, nano-patterned surfaces presenting RGD ligands at variable spacings (ranging from 20-200 nm) are tested for their effect on focal adhesion formation, cell spreading and migration,

as well as other adhesion-dependent processes. An example of a nano-patterned surface with a spacing of 58 nm is shown in Fig 2, top left panel (insert shows long range order in the nano-dots). Another form of ECM consists of fibronectin (FN) fibrils. *Jens Ulmer* is characterizing FN fibrilliogenesis, using an elastic topographic micro-pattern, and following the stretched fibers using scanning EM and fluorescence microscopy (insert, Fig 2, top right panel). The organization and stability of the FN matrix is also studied by *Tova Volberg*, who characterized the de-stabilization of FN network, using a FN fragment, known as anastellin (Fig 2, bottom). Another insight into the matrix, associated with the cell surface, was obtained by *Miriam Cohen* (in collaboration with *Lia Addadi* and *Derk Joster*), who studied the properties of surface-bound hyaluronan, and cell adhesion mediated by the hyaluronan-based pericellular matrix. These studies demonstrated that early stages of cell-surface interaction, preceding the integrin-dependent adhesion, are mediated by a hyaluronan gel.

Cell adhesion and migration in cancer: Wide screens for genes involved in the regulation of cell migration are carried by *Suha Naffar Abu-Amara*, using high-throughput microscopy (see below). In these screens, stationary cells were plated on a monolayer of microbeads, after being infected with genes derived from a highly metastatic breast carcinoma cell line (MDA231-MB) or after expressing in the non-migrating cells full-length cDNA encoding a variety of cancer related proteins. Cell migratory activity was monitored and quantified by analyzing the phagokinetic tracks formed by the cells. Candidate genes are now being evaluated for their role in cancer invasion and metastasis. Variations in cell adhesion and migration were also investigated in multiple myeloma. *Liat Nadav* (in collaboration with B. Katz, Tel Aviv-Sourasky Medical Center) discovered that cultured plasma cells (line ARH-77) form heterogeneous cultures, containing an adhesive (type A) and non-adhesive (type F) sub-populations. Type A and F cells were shown to differ also in their migratory activity,

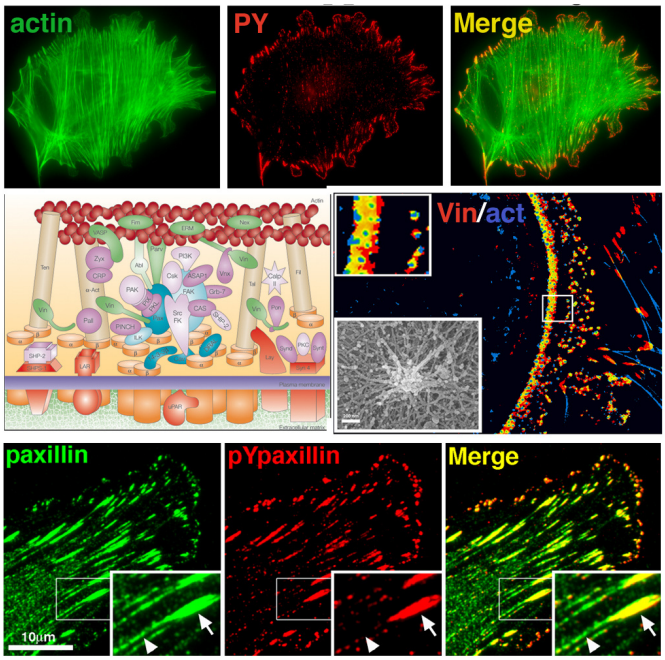

Fig. 1 *Diversity of integrin adhesions. Top panel –Porcine aortic endothelial cells, double-labeled for actin (green) and phospho-tyrosine (red). Notice focal complexes at the cell edge and focal adhesions at the ends of actin cables. Middle panel- Left: A scheme showing the molecular complexity of integrin adhesions (Nature Reviews, Molecular Cell Biology 2:793-805 (2001)); Right: Ratio image of an osteoclast, labeled for actin (blue) and vinculin (red). Overlaps between the two are represented by a spectral scale. Bottom panel- Porcine aortic endothelial cells, double labeled for paxillin (green) and phospho-paxillin (red). Notice the exclusion of the phosphorylated form from fibrillar adhesions (arrowheads).*

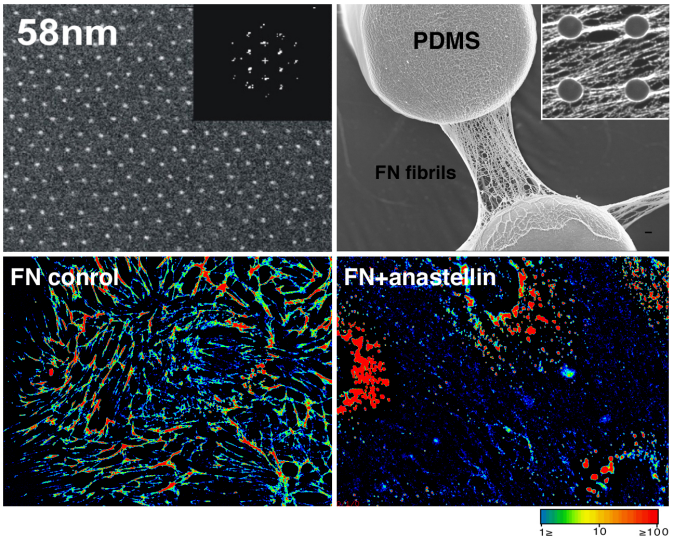

Fig. 2 *Top. left: Nano-pattern of RGD-coupled gold dots, with typical spacing of 58 nm (from J. Spatz); Top, right: Fibrils of bovine serum FN, formed between PDMS posts (10 µm height, diameter of 5 µm and inter-spacing of 3 µm). Samples where either immunostained with polyclonal anti-FN antibody (insert) or critical point dried and examined by SEM. Bottom panel: Effect of anastellin treatment (20µM, 6 hours) on FN fibrils (right), formed by Porcine aortic endothelial cells, compared to untreated control culture (left).*

surface markers, gene expression profiles and tumorigenic activity in mice. Interestingly, the differences between these lines appear to be non-genetic, and each sub-population re-diversify into both types following cultivation under non-selective conditions.

Quantitatibve automated microscopy and high throughput screens: In collaboration with *Z. Kam* an advanced system for automated quantitative microscopy was developed and applied for several screening projects, including discovery of novel adhesion proteins and search for modulators of p53 distribution and action (*Irina Lavelin*, *Victoria Simchaev*), screening for cytoskeleton modulating genes and compounds, and establishment of automated morphometric scoring (*Yael Paran*), migration screens (*Suha Naffar Abu-Amara*), and examination of siRNA effects (*Sabina Winograd-Katz*). In these screens a wide variety of reporter cells are used, and special image processing software is tailored for each project.

Selected publications

Bershadsky A.D., Balaban N.Q., and Geiger B (2003). Adhesion-dependent cell mechanosensitivity. Annu Rev Cell Dev Biol. 19, 677-95.

Zaidel-Bar R., Cohen M., Addadi L. and Geiger B (2004). Hierarchical assembly of cell-matrix adhesion complexes. Biochem Soc.Trans. 32, 416-20.

Cohen M., Kam Z., Addadi L. and Geiger B (2006). Dynamic study of the transition from hyaluronan-mediated to integrin-mediated adhesion in chondrocytes. EMBO J. 25:302-11

Zaidel-Bar R., Kam Z. and Geiger B (2005). Polarized down-regulation of the paxillin-p130CAS-Rac1 pathway induced by shear flow J Cell Sci. 118:3997-4007

Ballestrem C, Erez N, Kirchner J, Kam Z, Bershadsky A, Geiger B (2006). Molecular mapping of tyrosine-phosphorylated proteins in focal adhesions using fluorescence resonance energy transfer. J Cell Sci. 119:866-75.

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