depolymerization and/or recycling mechanisms that could be focused in the cell center. Support for this hypothesis comes from the fact that depactin, the echinoderm analogue of ADF/cofilin, appears concentrated in the center of flowing cells (antibody a gift from Dr. I. Mabuchi). Taken together these results suggest that phosphorylation status has a major impact on actin cytoskeletal structure in the cell periphery and the dynamics of acto-myosin contraction and actin depolymerization in the cell center. Current experiments are concentrating on the identification of proteins hyperphosphorylated by calyculin A treatment. Supported by the NSF, the NIH, and the Whitaker Foundation.

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Centrosome Reorientation in Migrating Tissue Cultured Cells Is Cell-type Specific

Barbara A. Danowski[1], Jonathan Walker[2], Alexey Khodjakov[3], Patricia Wadsworth[2], [1]Biology, Union College, Union Ave, Schenectady, NY 12308, [2]University of Massachusetts, [3]New York State Heath Labs, Wadsworth Center

Prior research showed that in many cell types undergoing directed migration, the microtubule-organizing center (MTOC) or centrosome is positioned in front of the nucleus, towards the protruding edge. We reported previously that when hepatocyte growth factor (HGF) is used to stimulate motility in PtK2 epithelial cells, centrosome reorientation does not occur. To reconcile this finding with previously published work, we report here a comparison of centrosome movement and reorientation in wound-edge PtK2 cells and CHO fibroblasts. Performing both immunofluorescence with a monoclonal antibody to gamma-tubulin and imaging of expressed GFP-gamma-tubulin in live cells, we found no evidence for reorientation of the centrosome in PtK2 cells. After 4 hours post-wounding, only 19% of wound-edge cells showed centrosomes positioned toward the direction of locomotion. Analysis of live cells during locomotion showed that the velocities of the nucleii and centrosomes are either tightly coupled or else the centrosome lags behind, and then 'catches up' in a burst of movement. In contrast, centrosomes in wound-edge CHO cells do orient toward the direction of migration. Four hours post-wounding, 72% of centrosomes were located in front of the nucleus, toward the leading lamellipodia. Analysis of 8 live wound-edge CHO cells showed that during the observation period, the velocities of the centrosomes often surpassed the velocities of the nucleii. Immunofluorescence analysis of a third cell line, CV-1 fibroblasts, indicates that a moderate amount of centrosome reorientation occurred in cells at the wound edge (47% of the centrosomes were positioned toward the direction of migration after 4 hours). These results indicate that centrosome reorientation is cell-type specific. PtK2 cells have a large number of non-centrosomal microtubules, while in CHO cells, the microtubules are primarily centrosomal. We suggest that these differences might influence the extent to which centrosome reorientation takes place.

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Induction of Collagen Expression during Inter-Sertoli Tight Junction (TJ) Assembly *In Vitro*

Michelle Kwan Yee Siu[1], Will M. Lee[2], C. Yan Cheng[3], [1]Center for Biomedical Research, Population Council, 1230 York Avenue New York, NY 10021, Manhattan, New York NY 10021, [2]University of Hong Kong, [3]Population Council

During spermatogenesis, preleptotene spermatocytes (PLS) must translocate across the blood-testis barrier in a timely fashion. However, the mechanism by which the opening and closing of the inter-Sertoli-TJs is regulated is not known. Since Sertoli cells (SC) must anchor onto the basal lamina via the extracellular matrix (ECM), we investigated whether ECM is involved in inter-Sertoli-TJ assembly. When SC were cultured at $1x10^6$cells/cm^2 on Matrigel-coated bicameral units to allow the assembly of inter-Sertoli-TJs, a significant increase in &~alpha3(IV) collagen chain [&~alpha3(IV)chain] expression was detected between days 1-3 coinciding with the assembly of inter-Sertoli-TJs. To assess the effects of collagen on the assembly of inter-Sertoli-TJs, an anti-collagen antibody (0.1-1&~mug IgG/chamber) was added either to the apical- or basal-chamber of the bicameral unit when SC were plated at time 0. In controls, a steady increase in transepithelial-electrical-resistance (TER) across the SC-epithelia between days 1-3 was detected and the TER reached plateau by day 4. The presence of this antibody perturbed the assembly of inter-Sertoli-TJs as manifested by a decline in TER on day 3. This perturbing effect was more pronounce when the anti-collagen antibody was added to the basal chamber possibly it had greater access to SC. These results illustrate ECM may regulate inter-Sertoli-TJ assembly. When the relative level of &~alpha3(IV)chain expression in SC and GC was compared, GC expressed about 80% of &~alpha3(IV)chain of that in SC. Its expression increased steadily in GC during maturation using GC isolated from rats of different ages, which is in contrast to the pattern in developing testis where &~alpha3(IV)chain expression peaked at days 10-20 and declined rapidly. In summary: (i) GC express collagen and contribute significantly to the pool of collagen in the testis, (ii) collagen plays a crucial role in regulating inter-Sertoli-TJ assembly.

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Reorganization of the Actin Cytoskeleton Induces Gelatinase A Activation Independent of Transcription and Translation

Eric W. Howard[1], Dawn L. Updike[2], Elizabeth C. Bullen[2], James J. Tomasek[2], [1]Dept. of Cell Biology, University of Oklahoma Health Sciences Center, 940 SL Young Blvd., Oklahoma City, Oklahoma 73190, [2]University of Oklahoma Health Sciences Center

Matrix metalloproteinases (MMPs) play a critical role in cell migration and invasion. Fibroblasts can be induced to bind and activate the MMP, gelatinase A, by agents that cause reorganization of the actin cytoskeleton. In this study, cell shape changes induced by concanavalin A, cytochalasin D, or latrunculin B resulted in the appearance of activated gelatinase A at the cell surface concomitant with actin stress fiber and focal adhesion disassembly. Rapid, MMP-dependent activation occurred in the absence of transcription and translation, and could not be blocked by inhibitors of tyrosine or serine/threonine kinases. Western blots of cell lysates demonstrated the constitutive presence of activated MT1-MMP, the primary activator of gelatinase A. These results suggest that MT1-MMP is located on or within cells, but is unable to bind or activate gelatinase A. Upon the loss of stress fibers or focal adhesions, it becomes available for interaction with its target. A novel mechanism for mechanically-mediated gelatinase A activation is suggested. Cell motility involves controlled and localized matrix adhesion and de-adhesion. We show that a mediator of this process, gelatinase A, is regulated by changes in the actin cytoskeleton. The ability of cells to very rapidly activate and localize gelatinase A at the cell surface may provide a mechanism uniquely suited to effect rapid changes in localized matrix adhesion and de-adhesion.

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Laminin-5 mediates human mature thymocyte migration.

Mylene Vivinus[1], Jean-Philippe Breittmayer[2], Patricia Rousselle[3], Alain Bernard[2], [1]U343, INSERM, Hopital de l'Archet, route Saint Antoine de Ginestiere, NICE, Alpes Maritimes 06202 France, Metropolitan, [2]INSERM, [3]CNRS

The thymus is a compartimentalized lymphoepithelial organ and thymocytes interactions with the thymic microenvironment play an important role in T cell maturation. During the differentiation process T cells move through the different compartments and exit to the periphery via imperfectly understood mechanisms. We previously observed that laminin-5 is expressed in the human thymic medulla, the area where mature thymocytes are located before their exit. Moreover, laminin-5 receptors are expressed on human thymocytes: alpha-3 beta-1 and alpha-6 beta-1 integrins are expressed by all thymocytes whereas alpha-6 beta-4 integrin expression parallels CD3 upregulation and cell maturation. As laminin-5 is involved in the migration of various cell types we investigated its potential role in human thymocytes migration. Firstly, we observed that after interaction with laminin-5, mature thymocytes (CD1-, CD4 or CD8 singly-positive sorted cells) exhibited migration structures (uropodia, lamellipodia) and actin reorganization whereas immature doubly-positive thymocytes (CD1+, CD4+CD8+ sorted cells) did not. Secondly, in transwell chambers, laminin-5 induced a significant migration of mature thymocytes. Both emergence of migration structures and migration were blocked by addition of the anti-laminin-5 mAb CM6. Finally, migration was inhibited by the metalloproteinases inhibitors BB94 and TIMP-2. These data show that laminin-5 promotes human mature thymocytes migration in vitro via a mechanism involving metalloproteinases and suggest that laminin-5 might play a role in vivo in the later step of intrathymic migration and/or in the thymic emigration process.

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CD47 facilitates neutrophil transmigration by regulating the rate of cell migration

Yuan Liu[1], Didier Merlin[2], Shawn P. Colgan[2], Fredrick J. Schnell[2], Charles A. Parkos[2], [1]Department of Pathology, Emory University, WMRB 2333, Atlanta, GA 30322, [2]Emory University

Neutrophil (PMN) migration from the peripheral circulation across vascular endothelium, extracellular matrix and epithelia is a key event in the acute inflammatory response. CD47, a cell surface transmembrane glycoprotein universally expressed in most cell types, plays an important role in modulating neutrophil migration since anti-CD47 antibodies inhibit PMN migration across endothelial and epithelial monolayers. However, the mechanism(s) by which CD47 regulates PMN migration is not well defined. Here, using an in vitro model, we analyzed the time course of PMN migration across T84 epithelial monolayers and matrix coated filters towards chemoattractant fMLP in the presence and absence of functionally inhibitory anti-CD47 antibodies (mAbs C5/D5, B6H12). Compared to control mAbs, addition of anti-CD47 mAbs resulted in a significant delay in PMN migration across both T84 monolayers and matrix coated filters. However, despite delayed transmigration, the number of PMN eventually crossing the epithelium or filters in the presence of anti-CD47 mAbs was the same as that of non-inhibitory controls. To evaluate changes in cell surface expression of CD47 during PMN migration, cell surface labeling experiments were