Symposium "Molecular Approaches in Cell-Cell Adhesion", January 27, 2001 in Essen (Germany)

Gap junctions and hematopoiesis

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Gap junctions between hematopoietic cells were described by Rosendaal (Microsc Res Tech 31 (1995) 396-407). Up till now, the role of gap junction in the regulation of hematopoiesis is not clear. Therefore, we visualised gap junction proteins in cultured human bone marrow stroma cells by means of confocal immunofluorescence microscopy, transmission electron microscopy and freeze-fracture replica labelling technique. In a first step we could prove by immunoblotting that gap junction protein connexin 43 exists in cells of our model system. Immunofluorescence localisation of connexin 43 with an anti connexin 43 antibody revealed fine punctuate labelling preferably in the contact area between neighbouring cells. With the freeze fracture replica technique we could also demonstrate the binding of anti connexin 43 antibody in the region of gap junctions on the p-face as well as on the e-face. We checked the binding capacity of the antibody conjugated to different gold sizes. On the transmission electron microscopic pictures gap junction often can be seen in combination with other intercellular junctions like tight junctions or desmosomes.

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Peri-implantation expression of CD9 in bovine endometrium

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The cell surface glycoprotein CD9 is a member of the transmembrane 4 superfamily that has been implicated in the promotion of cell motility and cell fusion in several cell systems. Ruminant implantation is characterized by the formation of trophoblast binucleate cells which migrate across the fetomaternal interface to fuse with the luminal epithelial cells of the endometrium. The objectives of the current study were to determine whether CD9 was present at the fetomaternal interface in cattle and distributed in a pattern consistent with a role in implantation. Immunohistochemical localization using polyclonal rabbit anti-CD9 antibody indicated that bovine CD9 was consitutively expressed on the apical surfaces of luminal and glandular epithelial cells of bovine endometrium from non-pregnant and pregnant animals at days 18, 21, 24 and 30 of gestation. The antigen also was expressed in trophoblast, exhibiting a pericellular distribution in binucleate cells prior to their fusion with maternal epithelium. Western blotting of bovine trophoblast with anti-CD9 revealed that the molecular weight of bovine CD9 under reducing conditions is approximately 25 kD. Preliminary immunoprecipitation experiments using antibody to the integrin $\alpha 3$ subunits followed by immunoblotting with anti-CD9 showed that bovine CD9 associates with an $\alpha 3$ integrin in trophoblast. The results suggest that CD9 is involved in binucleate cell migration and/or fusion during bovine implantation.

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Extracellular Matrix and Integrin Receptors during Bovine Placentation

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Binding of extracellular matrix (ECM) to heterodimeric integrins may trigger the transduction of signals into and out of cells via cytoskeletal changes and phosphorylation of various kinases. These signals may have regulatory functions during implantation and trophoblast invasion in the hemochorial human placenta. The epitheliochorial placentation of the cow is characterized by a restricted invasion where trophoblast giant cells (TGC) migrate towards the endometrium and fuse with uterine epithelial cells. To evaluate the role of integrin-ECM interactions during bovine placentation we collected placentomes of 18 cows and assigned them to six gestational ages (days 80, 120, 150, 220, 240, 270 p.i.). Tissue samples were parallely snapfrozen in N_2 or perfusion-fixed with 4 % buffered paraformaldehyde. Antibodies against ECM molecules collagen I (C-I), IV (C-IV), fibronectin (FN), laminin (Lam) and tenascin (T) and integrin subunits α1, α2, α3, α4, α5, α6, αv, β1, β3, β4 were used for indirect immunohistochemistry. Cells of fetal and maternal stroma were immunostained by FN, C-IV and C-I, whereas basement membranes (BM) showed immunoreactivity for FN, C-IV, Lam and T. In the first half of gestation Lam was equally expressed in fetal and maternal BM, thus contrasting later gestation, when the staining for Lam in maternal BM decreased from d 220. Immunostaining for integrin α1, α2, α5, αv, β3, and β4 subunits switched from the stroma of the stem septa in early gestation to the septal tips in late stages. In the same time, the expression of α1 was overall decreasing, while it was increasing for β3. β1, generally staining maternal stroma at d 80, shifted to BM and endothelial cells from d 150. The integrin subunit α6, expressed basally on trophoblast cells, decreased in the course of gestation. In contrast, the whole cytoplasmic membrane of unpolarized TGC was immunostained for α6. These changes indicate the involvement of ECM-integrin interactions during the differentiation of maternal septae and the modulation of cell functions, like the control of TGC migration in the fetal compartment

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Differential Cadherin Expression in Ovarian Cancer Cell Lines is associated with Transmigration through Matrigel

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Cadherins are integral membrane glycoproteins that mediate calcium-dependent cell adhesion in a homotypic manner. The classical cadherins, which include E-, N-, and P-cadherin, play an important role in establishing intercellular junctions and cell polarity as well as in maintaining tissue integrity. Since cell associations are often disorganized in tumors and, clearly, must be compromised in invasive and metastatic tumors, changes in cadherin expression/function have been implicated as important components of tumorigenesis and tumor progression. Ovarian carcinomas are a prevalent malignancy in women and the gynecological cancer with the high mortality. Ovarian cancers are derived predominantly from the ovarian surface epithelium (OSE). We have examined the expression of E- and N-cadherin in five OSE cancer cell lines. Since E- and N-cadherin are expressed in normal human OSE, we asked if expression of either one or both of these cadherins was altered in OSE cells. We also determined if differential cadherin expression in OSE cells was indicative of an invasive phenotype as assessed by cell transmigration through Matrigel. E- and N-cadherin expression in the five cell lines (HEY, SKOV3, CaOV3, OVCAR3, SW626) was evaluated by RT-PCR, Northern, and Western blotting. Cell surface cadherin expression was assessed by immunocytochemistry. OSE cell migration was evaluated in Millipore transwell chambers, containing Matrigel-coated filters, and loaded with ^{3}H-thymidine-labeled OSE cells. The chambers were placed in individual wells of 24 well dishes.

HEY and SKOV3 cells expressed only N-cadherin mRNA and protein while OVCAR3 and SW626 cells expressed only E-cadherin mRNA and protein. CaOV3 cells expressed both cadherins. Immunocytochemistry indicated cell surface cadherin expression. HEY and SKOV3 cells (N-cadherin expressing) showed high Matrigel transmigration (~30% of applied radioactivity recovered from the wells and bottom of the filters). OVCAR3 and SW626 cells had poor transmigration. CaOV3 cells were intermediate. These results indicate that OSE cancer cells can display differential cadherin expression and that either maintained expression of N-cadherin or loss of E-cadherin is associated with an invasive phenotype.