on cell motility, invasion and apoptosis. Gene expression profiles were studied by cDNA microarrays.
Results: We confirmed that P-cadherin overexpressing tumours often do not loose E-cadherin. Interestingly, tumours co-expressing both cadherins showed a more aggressive behavior and were related with the worst patient survival. *In vitro*, we showed that cadherins co-expressing breast cancer cells demonstrated increased cell invasion and migration capacities, when compared with the ones expressing only one cadherin. In addition, P-cadherin silencing led to increased levels of apoptosis. Microarrays of breast cancer cells, after E- and/or P-cadherin silencing, generated a large amount of data, which is now being analyzed and validated. However, it was already possible to conclude that both these molecules are important in the regulation of different signaling pathways. As an example, the apoptotic pathway was enriched in cells with P-cadherin silencing, confirming the *in vitro* results obtained.
Conclusions: E- and P-cadherin co-expression has an invasion promoter role in breast cancer cells and is a poor patient prognostic biomarker. In addition, P-cadherin overexpression constitutes a cancer cell survival signal. It was still proven that the role of each cadherin alone is completely distinct from when these are co-expressed in the same cell, conferring different transcriptional programs.

515 Number of stem-like cells and the genetic susceptibility to mammary carcinogenesis in rats

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Background: The rat mammary tumour model has been used for the study on the biology of human breast cancer. Genetic predisposition for mammary carcinogenesis plays a significant role in the rats. Female Sprague-Dawley and Wistar-Furth (WF) rats are sensitive to DMBA- and MNU-induced mammary carcinogenesis, but Copenhagen (COP) rats are completely resistant. F1 hybrids (WF x COP) show resistance, suggesting a dominant tumour suppressive trait of the COP background. The underlying genetic components are complex and not completely understood.
Stem cells and their immediate progeny are considered as the targets for malignant transformation. To elucidate the cellular basis for resistance to mammary carcinogenesis in COP rats, we performed transplantation assays to examine the number of stem-like cells, previously referred to as clonogens, and their response to cancer promoting condition (glucocorticoid deficiency and high prolactin) in comparison with susceptible WF rats.
Materials and Methods: Young-adult female WF and COP rats and their F1 hybrids (WF x COP) were used. The number of stem-like cells was determined by a transplantation assay. Two types of donor rats were used: untreated rats and adrenalectomized, pituitary-transplanted rats. Serially diluted monodispersed mammary epithelial cells from donor rats were transplanted into the interscapular fat pad of recipient F1 rats grafted with mammotrophic pituitary tumour cells. Three weeks after transplantation, the fat pads were removed and examined for the presence of alveolar units (AUs) and branching ductal units (DUs) developed at graft sites. The total number of AU- or DU-forming cells per mammary gland was calculated.
Results: The total number of AU-forming cells per normal female mammary gland was much smaller in COP than WF, being coincided with tumour susceptibility. However, the number of AU-forming cells of F1 rats was comparable to those of WF, which failed to account for the differential tumour susceptibility between WF and F1. On the other hand, the number of DU-forming cells of F1 was one third of those of WF, in good agreement with their tumour susceptibility. More importantly, DU-forming cells in COP and F1 were not stimulated to expand by glucocorticoid deficiency and high level of prolactin, in contrast with the marked response by the WF cells.
Conclusion: The DU-forming cells may be the targets for chemically induced mammary carcinomas.

516 Isolation and functional characterization of tumour-initiating cells using a let-7c micro RNA cellular reporter system

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A growing body of evidence suggests that a few stem cell-like cancer cells, also termed tumour-initiating cells (TICs) or cancer stem cells (CSCs), have the ability to self-renew and continuously produce differentiated cancer cells that make up the bulk of a tumour. Expression of multi-drug resistance (MDR) proteins that pump out toxic substances, enhanced activity of enzymes that confer resistance to toxic agents, increased levels of telomerase that prevent telomere-shortening, as well as the quiescent state of TICs may cause resistance to traditional therapy leading to relapses or metastasis. Importantly, such particular properties of TICs can also be exploited to isolate and characterize them to ultimately uncover potential targeting strategies.
Let-7 micro RNA (miRNA) family members are either not expressed or expressed at low levels in TICs, whilst higher expressed in differentiated normal and cancer cells. We take advantage of this feature by using a reporter system consisting of an expression plasmid in which a fluorescent reporter protein (DsRed) is placed under the control of a CMV promoter and a 3′ untranslated region (3′UTR) harboring a perfect complementary let-7c binding site [1]. Hence, the level of DsRed expression is regulated by the endogenous levels of let-7c, allowing us to isolate strongly fluorescent TICs (which have low levels of endogenous let-7) from cancer cell lines, using flow cytometry.
We are currently using the let-7c miRNA reporter system to isolate stem-like cell populations from different human breast cancer cell lines. Colony assays, stemness and differentiation surface marker expression analyses, cell cycle analyses, and examination of stem-like gene levels (e.g. *Nanog, Oct-4, Sox-2*) at mRNA and protein level will reveal whether our approach selects for TICs. Furthermore, we are evaluating the expression of let-7 targets such as the cell cycle regulators CDC25A and CDK6, growth promoters including RAS and c-Myc, as well as known regulators of stemness and differentiation during embryogenesis including HMGA2 and Lin28(B). Lastly, the DsRed reporter assay approach will be employed in mouse models to assess tumour-initiating potential *in vivo.*

Reference(s)

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517 3D culture of oesophageal cancer cells in extracellular matrix: morphology correlates with invasiveness

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Background: Oesophageal cancer is known for its intrinsic resistance to current systemic therapies. Thus far, no comprehensive in vitro or animal models exist for this cancer. Therefore, we investigated systematically a panel of nine oesophageal cancer cell lines in a 3D culture system to establish a more comprehensive model system to study this cancer in vitro.
Material and Methods: We used the on-top Matrigel®-assay to analyse five oesophageal squamous cell carcinoma cell lines (KYSE-30, KYSE-270, KYSE-410, KYSE-520 and COLO-680N) and four adenocarcinoma cell lines (OE19, OE33, LN1590 and PT1590) for their behaviour in contact with extracellular matrix (ECM) in a 3D culture system. The phenotype was compared with conventional 2D culture. The invasiveness was assessed with Matrigel®-coated Transwell System (Boyden Chamber) whereas the Fence-Assay was applied to evaluate the migration of the oesophageal cell lines. Expression of genes related to proliferation and cell adhesion were investigated via quantitative RT-PCR.
Results: Upon the on-top Matrigel®-assay KYSE-30, OE33, LN1590 showed a round mass growth pattern, KYSE cell lines -270, -410, -520 and COLO-680N exhibited tumour mass like pattern, OE19 a grape-like growth pattern and PT1590 grew in stellate spheroids. Interestingly, the distinct growth pattern of the oesophageal cancer cell lines correlated significantly with the invasive capacity as analysed with the Matrigel®-Invasion Chambers ($p = 0.048$). In contrast, the migratory capacity analysed with the Fence-Assay did not correlate with the phenotype observed in the 3D culture. We also noted an impact of the ECM-culture condition on the expression profile of some of the analysed genes.
Conclusion: Compared to monolayer cell culture grown on plastic, the on-top Matrigel 3D culture provides a more realistic environment and led to a distinct growth pattern of the investigated cell lines. Their observed behaviour upon 3D culture covered a comprehensive range from low- to high-aggressive phenotypes. Thus, these nine cell lines cultured in the on-top Matrigel®-assay seem to provide a suitable model to perform therapeutic in vitro studies in oesophageal cancer.

518 The relevance of the therapeutic target EpCAM (CD326) for the progression of esophageal carcinoma

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Background: Oesophageal cancers frequently express EpCAM (CD326) and its strong expression was correlated to poor prognosis in squamous cell carcinomas. Here we tested in cell-based experiments whether EpCAM expression is an epiphenomenon or whether it actively contributes to the aggressive phenotype this cancer type.