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.

Material and Methods: We used the EpCAM expressing squamous cell carcinoma cell lines Kyse 30 and 520 as in vitro model. To measure the potential effects of loss of EpCAM expression, we used the lentiviral pGIPZ shRNAmir system with two different sh-RNAs directed against EpCAM and one control shRNA vector. The EpCAM-suppression of the transduced cells was tested by quantitative RT-PCR and immunoblotting. Those cell lines with at least 80% reduction of EpCAM expression were further analysed. We used the "Fence-assay"® to investigate the migration. The tumour cell invasion was assessed with a commercially available Matrigel-coated Transwell system®. Transcriptome profiling of shRNA-transduced cell lines and control-vector transduced cell lines, respectively, was done with Agilents "*whole genome Array*".
Results: The migration of EpCAM-shRNA-transduced squamous cell carcinoma cells was reduced by 30−50% compared to tumour cells transduced with the control-vector. A 3−4% reduction of the invasion was observed. Both, the reduction in migration and invasion were statistically significant. Changes in the transcriptome expression were noted in shRNA-transduced cell lines compared to control-vector transduced cell lines. The differentially expressed genes fell in the categories cell structure, cell movement and developmental processes.
Conclusion: Our data indicate an active biological role of EpCAM in oesophageal squamous cell carcinoma progression and makes it a promising therapeutic target for this entity. However, the exact mechanisms of action warrant further investigation.

519 Impact of ECM on phenotype and EGFR inhibition in colorectal cancer cell lines

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Background: 3D tumour cell cultures grown in extracellular matrix (ECM) are considered to reflect human tumours more realistic than monolayers grown on plastic. ECM has not only drastic effects on phenotype but also on response to targeted therapies, as recently demonstrated in breast cancer cell lines. Here, we systematically investigated the impact of ECM on phenotype and on EGFR inhibition in commonly used colorectal cancer (CRC) cell lines.
Material and Methods: On-top matrigel assays were performed with SW480, HT29, DLD-1, LOVO, CACO, Colo 205 and Colo 206F cell lines. The phenotype of the 3D culture was assessed and compared to conventional 2D cell culture. Expression of genes involved in proliferation and cell adhesion was analysed on the transcriptional (quantitative RT-PCR) and on the protein level (immunoblotting and confocal imaging). Invasive capacity of the cell lines was assessed via Matrigel®-Boyden chamber assay. EGRF inhibition was achieved using tyrphostin AG 1478.
Results: A specific spheroid growth pattern was observed for all investigated CRC-cell lines. DLD-1 and CACO showed a clear solid tumour cell formation, HT29, SW480 and LOVO exhibited budding structures, while Colo 205 and Colo 206F showed grape-like structures. The 3D culture phenotype of the cell lines was not correlated to their invasive/migratory capacity. A significant reduction of the gene expression was noted for most investigated genes in 3D culture. In contrast, E-cadherin was up-regulated in several cell lines. Effects of EGFR inhibition was noted in 2D and 3D culture of sensitive cell lines.
Conclusion: The observed differences between the cell culture models corroborate the influence of ECM for cancer growth. Compared to conventional 2D cell culture, the 3D cell culture model (Matrigel® on-top assay) offers the opportunity to investigate potential molecular targets under more realistic conditions.

520 Effect of selenium on rat liver cell proliferation after partial hepatectomy

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Background: Studies on tumour cell lines, animal studies and human trials have demonstrated a tumour preventive effect of selenium (Se). Selenium-treatment in tumour preventive doses inhibit liver cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. The selenium dependent redox-enzyme thioredoxin reductase (TxR1) was over expressed in proliferating nodular liver lesions in the model. In this work we have studied the effect of selenium on regenerative cell proliferation and on the expression of TrxR1 in rats after 2/3 partial hepatectomy (PH).
Material and Methods: Fischer344 male rats were given 5ppm sodium selenite in the drinking water one week prior to PH, and until sacrificed. Non-treated hepatectomised and non-treated sham operated rats were used as controls. Bodyweights and relative liver weight were monitored. Cell proliferation, mitotic figures and occurrence of TrxR1 were determined immunohistochemically (IHC). TrxR1 enzyme activity, mRNA expression, and protein levels were analysed using TrxR1-assay, real-time PCR and western blot.
Results: No differences in bodyweights, relative liver weights and regeneration of liver mass were shown between groups. The peak of the S-phase marker Mib5 coincided while the peak of the mitotic figures was slightly delayed in treated rats. IHC staining for TrxR1 revealed a zonal, periportal increase of enzyme expression at 24h post PH, corresponding to the zone of Mib 5 positive cells and mitotic figures. After PH the TrxR1 enzyme activity increased from 8 hours with a peak at 48 hours post PH in Se-treated animals. In non-treated animals a similar but lower induction of the activity was shown between 8−72 h post PH. The TrxR1 activity was not changed over time after sham-surgery. TrxR1 mRNA increase at 4 hours post PH was seen in all groups.
Conclusions: We have concluded that, although a slight delay of cell division was shown, the gain of liver mass and regeneration of the liver function after PH is not affected by selenite. The increase of thioredoxin reductase correlated with cell proliferation and was further induced by selenium.

521 Enhanced pulmonary tumourigenesis by N-nitrosobis (2-hydroxypropyl) amine after thoracic irradiation with X-rays in new born, juvenile and adult Wistar rats

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Background: The possibility that combined exposures to environmental pollutants and ionizing radiation could increase the risk of lung cancer of the general public is a matter of great concern. We investigated the combined effects of radiation and a chemical carcinogen on pulmonary tumourigenesis in rats exposed at different age in well-defined exposure conditions.
Material and Methods: Female 1-, 5- and 22-week-old Wistar rats were irradiated locally on the thorax with X-rays (3.18 Gy), and/or were given *N*-nitrosobis (2-hydroxypropyl) amine (BHP; 1 g/kg body weight) intraperitoneally 1 week after thoracic irradiation.
Results: Non-irradiated and non-BHP-injected control rats survived to 90 weeks of age when all rats were sacrificed, but administration of BHP with or without irradiation resulted in survival reduction due to kidney, brain, liver and ovarian tumours. The incidences of lung tumours including adenomas and adenocarcinomas in rats irradiated alone at 1, 5 and 22 weeks were 8.7, 15.0 and 20.0%, respectively. On the other hand, the incidences in the rats administered with BHP alone at 2, 6 and 23 weeks were 60.9, 25.0 and 30.0%, respectively. When a combination of irradiation and BHP was used, the incidences in the rats treated at 1−2, 5−6 and 22−23 weeks were 61.9, 65.0 and 55.0%, respectively. The incidence of adenocarcinomas in the rats treated at 5−6 weeks was significantly increased compared to rats exposed to either X-rays or BHP alone.
Conclusion: The combined effects are age-dependent and administration of BHP after X-ray irradiation synergistically enhances induction of lung adenocarcinomas in juvenile rats. These results indicate that Wistar rats exposed to X-rays and BHP are a suitable animal model to study the risk and the mechanisms of the combined effects of radiation and chemicals on pulmonary tumourigenesis.

522 Selenium homeostasis and induction of thioredoxin reductase upon long term selenium supplementation in the rat

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Background: Selenium is an essential micronutrient for human and animals. Selenium treatment in supranutritional but subtoxic doses of 1 ppm and 5 ppm have shown to inhibit cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. Selenium tumour prevention in chronic liver diseases requires long-term selenium supplementation and there is still quiet limited knowledge on selenium long term effects. Thioredoxin reductase (TrxR1) is a selenoenzyme essential for maintaining intracellular redox status and avoid oxidative stress. TrxR1 is overexpressed in proliferating liver nodules in the rat liver model. In this work we have studied selenium homeostasis in serum and liver as well as TrxR1 induction after long term selenium supplementation in the rat.
Materials and Methods: The kinetics of selenium uptake and accumulation and TrxR1 induction after treatment with sodium selenite in the drinking water in doses of 1 ppm and 5 ppm for 10 weeks have been studied in male Fisher rats. After withdrawal of selenium treatment the selenium status and TrxR1 induction were studied at 3 and 6 months of the experiment.
Results: Long term selenite exposure via the drinking water cause a dose dependent increase of blood and liver levels of selenium. This increase levels out at 6 weeks at the same level of selenium regardless of treatment and dose. Thus, there is no accumulation of selenium in blood and liver over time at chronic exposure. The same effect was seen on the induction of TrxR1 activity, while the induction of TrxR1 mRNA was only seen during the first two days of treatment. Discontinuation of selenite exposure did not result in