536 Modeling the human breast stem cell and the breast cancer niche

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Stem cell/niche interactions are essential for tissue homeostasis by controlling stem cell quiescence and stem cell activation. Recent evidence implies that aberrant stem cell/niche interactions are involved in tumourigenesis and tumour progression. Experiments that functionally describe the cellular compartments responsible for self renewal and quiescence of breast stem- and progenitor cells have not been reported. The nature and role of the breast cancer niche in tumourigenesis, tumour maintenance and metastasis remain speculative.

We established methods that allow for the isolation and propagation of different cellular compartments from normal primary breast and tumour tissue. Co-culture of epithelial and mesenchymal precursors from the human breast enables the assembly of structures displaying functional properties of human breast stem cell niches. We successfully defined novel *ex vivo* three dimensional cell culture conditions that allow for long term maintenance of differentiation and proliferation potential of mammary epithelial stem/progenitor cells.

We are currently validating our culture system *in vivo.* We are also performing gain and loss of function experiments with mammary oncogenes and tumour suppressor genes to assess how oncogenic transformation alters stem cell/niche interactions.

Our experiments should identify molecular pathways involved in aberrant stem cell/niche interactions and breast tumourigenesis and ultimately provide novel therapeutic targets.

537 The role of microRNA molecules in the regulation of aggressive features in melanoma

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Melanoma is a high-grade, poorly differentiated malignant tumour of pigment-producing cells (melanocytes), accounting for more than 70% of the skin cancer related deaths. Unfortunately, the currently available treatments are still mostly ineffective. MicroRNAs (miRs) are short non-coding RNA molecules that function as specific epigenetic regulators of the transcriptome. MiRs regulate various cancer-related functions with clinical importance and significance. Our main objective is to identify a miR pattern involved in the regulation of aggressive features in melanoma and delineate the underlying molecular mechanisms.

We employed qPCR-based miR microarray for analysis of two sublines of a single melanoma cell line, which differ in tumourigenicity and related characteristics. Downregulation of 66 mature miRs was identified in the highly aggressive (HAG) cells. Candidate miRs were analyzed informatically using computational algorithm databases that enabled miR prioritization according to predicted target genes (aggressive phenotype). Selected miRs were cloned into pQCXIP mammalian expression vector and introduced into HAG cells. The transfectants were analyzed functionally in proliferation, invasion and vasculogenic mimicry (VM) formation assays.

Here we describe the roles of two exemplar miRs in melanoma, miR-184 and miR-34a. Over expression of miR-184, which underwent shutdown in the HAG subline, caused a 33% decrement in net cell proliferation and 22% decrement in invasion ability, as compared to mock transfecion. Moreover, overexpression of miR-184 impaired the ability of HAG cells to form VM structures. Over expression of miR-34a, which underwent 11-fold downregulation in the HAG subline, caused a 46% decrement in net cell proliferation as compared to mock transfecion and severely impaired the ability of HAG cells to form VM structures. MiR-34a transfected HAG cells displayed substantially reduced tumourigenicity when injected S.C. to SCID mice. Indeed, 60% of the mice did not develop any tumour, while the rest of the mice developed 10-fold smaller tumour masses.

MiRs provide a strong platform for delineation of cancer mechanisms. Studying miR-mediated regulation of aggressive and tumour related features is expected to provide novel mechanistic insights that may pave the way for new diagnostic tools as well as new molecular targets for future therapy.

538 Autocrine vascular endothelial growth factor signaling has no influence on the apoptosis induction by cisplatin in non-small cell lung cancer cell lines

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Background: Therapeutic agents blocking cellular pathways are a current promise. Vascular endothelial growth factor (VEGF) is a major protein upregulated in hypoxia and known to promote angiogenesis and tumour cell survival. VEGF blocking antibodies (bevacizumab) are approved for use in non-small cell lung cancer (NSCLC) patients. Recently, it has been postulated that blocking the VEGF pathway could be more proapoptotic than antiangiogenic, by acting on the auto- or paracrine loop of VEGF. The aim of this study was to analyse, whether such a loop exists in NSCLC cell lines and wether its blocking has an effect on chemotherapy and the apoptotic cell death in normoxia and hypoxia.

Material and Methods: A549 and H358 cells were preincubated for 3 days at 1% O2 or ambient O2 in serum starved medium (0.5% FCS) supplied with predefined concentrations of bevacizumab. After preincubation fresh bevacizumab was added and the cells were treated with cisplatin [32 μM] for another 3 days. Apoptosis rate was analysed by measuring active caspase-3 with flow cytometry (PhiPhiLux®). Regulation of VEGF under hypoxia was assessed via qRT-PCR. Expression of VEGF-Receptor 1 and 2 was investigated with qRT-PCR, western blot and immunocytochemistry.

Results: VEGF was expressed in both cell lines but only in hypoxic A549 cells it was significantly upregulated by a factor of 2 ($p = 0.037$) compared to normoxic cells. VEGF-Receptor 1 was found on mRNA- and protein level in both cell lines. In contrast VEGF-Receptor 2 was found only in H358 cells. Cisplatin led to 44.9% (±5.6%) apoptosis rate in A549 cells and 27.9% (±2.4%) in H358 cells in normoxia. In hypoxia, cisplatin induced an apoptosis rate of 7.6% (±3.1%) in A549 cells and 13.56% (±8.8%) in H358 cells. Co-incubating the cells with up to 250 μg/ml bevacizumab led to an apoptosis rate of 48.0% (±4.1%; $p = 0.48$ to cisplatin alone) in normoxic and 8.8% (±3.3%; $p = 0.67$) in hypoxic A549 cells and 24.69% (±2.6%; $p = 0.34$) in normoxic and 12.51% (±9.9%; $p = 0.92$) in hypoxic H358 cells, respectively.

Incubating the cells with up to 250 μg/ml bevacizumab without cisplatin induced no apoptosis in normoxia and hypoxia (data not shown).

Summary and Conclusion: Although the components for an auto- or paracrine loop of VEGF are present in A549 and H358 cells the apoptosis rate of the cells could not be influenced by blocking soluble VEGF with bevacizumab. A combination of cisplatin and bevacizumab had no beneficial effect on apoptosis induction, neither in normoxia nor in hypoxia. Either the auto- or paracrine VEGF loop is not functional in A549 and H358 cells or its blocking has no proapoptotic effect on these cell lines.

539 Elevated IGF-1R signaling induces anti-estrogen resistance and provokes a switch from antagonistic to agonistic effect of tamoxifen in breast cancer cells

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Background: Acquired resistance to anti-estrogens is a consequent setback leading to relapse and poor prognosis of estrogen receptor (ER) positive breast cancers. Emerging data indicate the relevance of altered expression and modification of growth factor receptors and downstream signaling to the anti-estrogen resistance. Here, we have investigated the role of elevated insulin-like growth factor receptor type 1 (IGF1R) in tamoxifen resistance.

Material and Methods: We used human breast cancer MCF7 cells (MCF7/IGF1R) overexpressing human IGF1R by retroviral transduction generated in our laboratory. The cells were exposed to estradiol and various antiestrogens in 96 well plates. Proliferation was measured colometrically with the sulphrhodamine B assay.

Results: Overexpression and phosphorylation of IGF-1R in ER positive human MCF7 breast cancer cells induces acquired resistance to the anti-estrogens tamoxifen and fulvestrant (ICI 182780).

Particularly, increased IGF-1R signaling can convert tamoxifen, but not fulvestrant, from an antagonist into agonist, thereby further enhancing proliferation of MCF7/IGF1R cells. Auto-phosphorylation of tyrosine kinase domains in IGF-1R was fully activated upon exposure to IGF-1, concurrently initiating downstream ERK signaling significantly stronger in MCF/IGF1R cells than that in parental MCF7 cells. By use of IGF-1R inhibitor BMS-536924, MEK/ERK inhibitor U0126 and PI3K inhibitor BEZ235 respectively, it was shown that IGF-1R signaling involves both downstream ERK and PI3K/Akt pathways activation.