understanding grows, inflammatory mediators and microRNAs will provide opportunities to develop novel diagnostic and therapeutic strategies.

27 Causes and consequences of microRNA dysregulation in cancer

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During the past several years it has become clear that alterations in the expression of microRNA genes contribute to the pathogenesis of most, perhaps all, human malignancies. These alterations can be caused by a variety of mechanisms, including deletions, amplifications or mutations involving microRNA loci, by epigenetic silencing or by dysregulation of transcription factors targeting specific microRNAs. Since malignant cells show dependence on the dysregulated expression of microRNA genes, which in turn control or are controlled by dysregulation of multiple protein coding oncogenes or tumour suppressor genes, these small RNAs provide important opportunities for development of future microRNA based therapies.

Sunday 27 June 2010 10:20–12:20

Symposium
Tumour microenvironment interaction

28 Ultraviolet B-induced inflammatory microenvironment promotes melanocyte survival and melanoma susceptibility

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Ultraviolet radiation (UV) is a major risk factor for melanomagenesis, but the underlying mechanisms are not well understood. We have generated a novel genetically engineered mouse model that expresses green fluorescent protein (GFP) in melanocytes specifically in a doxycycline-regulated manner, allowing us to study melanocytes within their natural microenvironment. Using this mouse model we have shown that neonatal UVB irradiation, but not UVA, induces melanocyte activation resulting in proliferation and migration towards the epidermis. Skin melanocytes were isolated through fluorescence-activated cell sorting 1 day and 6 days after in vivo irradiation of one day-old neonatal mice. Microarray analysis showed upregulation of a distinct interferon-induced gene expression signature in melanocytes at 6 days post UVB irradiation only, but not from any post UVA irradiation time points. Antibody-mediated blockage of interferon-gamma (Ifng) eliminated the UVB-induced melanocyte activation, but blockage of Type I interferons had no effect. The source of Ifng was found to be a subset of macrophages that infiltrate the skin after UVB, but not UVA, irradiation. These macrophages enhanced the growth of tumours when admixed with a mouse melanoma cell line and transplanted subcutaneously into syngeneic FVB/N mice. The admixed tumours showed significantly less apoptosis than the control tumours, indicating activation of survival pathways in melanocytes. Notably, the same macrophage infiltration and upregulated Ifng gene signature were detected in melanocytes at the catagen phase of the hair cycle, when the follicles degenerate and only a fraction of melanocytes, including stem cells, survives extensive dermal remodeling. Finally, a human melanoma tissue microarray demonstrated the presence of Ifng-secreting macrophages in 70% of tumours. We conclude that melanocytes actively participate in UVB-induced pro-tumourigenic skin inflammation through macrophage crosstalk, featuring Ifng as a critical signaling component promoting melanocytic survival and immunoevasion.

29 Tumour:stroma interactions in breast cancer and glioma

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Background: Invasion of cancers into surrounding tissues is accompanied by recruitment of stromal cells and the deposition of an altered extracellular matrix. Our laboratory has been investigated the recruitment, activation and function of cancer-associated fibroblasts (CAFs). This talk will focus on the interplay between tumour cells and CAFs in the deposition and remodeling of collagens within the extracellular matrix.

Materials and Methods: The methods employed in these studies include 2D and 3D *in vitro* cell culture experiments, tumour xenografts, bone marrow reconstitution assays, immunohistochemistry, confocal microscopy, biochemistry and molecular/pathological analysis of human tumour.

Results: Results will be presented on (a) the recruitment and activation of CAFs in breast cancers, (b) a comparison of collagen deposition and remodeling in glioma and breast tumours, (c) the role of the collagen internalization receptor Endo180 (MRC2, uPARAP) in these events, and (d) CAFs in primary and metastatic disease.

Conclusions: The major conclusions from these studies address issues of tumour heterogeneity, the balance between extracellular matrix deposition and degradation during tumour cell invasion and the interplay between tumour cells and CAFs in these events.

30 Neuroblastoma and melanoma metastasis: regulation by the tumour microenvironment

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It is well established that interactions between the tumour and its microenvironment (TME) drives tumour progression towards metastasis.

Here we report on interactions of melanoma and neuroblastoma cells with the microenvironment of the specific organ sites that these tumour cells metastasize to.

We recently developed human to mouse xenograft models for melanoma & neuroblastoma metastasis. These models are being utilized to: establish a molecular signature of site-specific metastasis (tumour cells & non-tumour cells alike); identify cancer genes controlled by the site-specific microenvironment and candidates for site-specific therapy targets.

The working hypothesis of our melanoma studies is that interactions of melanoma cells with the brain microenvironment regulate site specific metastasis to this organ. Brain metastasizing and local, cutaneous melanoma cells have a differential gene expression profile, express a different malignancy phenotype and interact differently with brain microenvironmental factors.

The human to mouse xenograft model for neuroblastoma indicated that these tumour cells when inoculated orthotopically into the adrenal gland of nude mice develop metastasis in the lungs. We now report that this metastatic site contains, in addition to macro-metastasis also micrometastasis. Lung macro and micro metastatic cells express a different malignancy phenotype and interact differently with lung microenvironmental factors.

Put together, these studies indicate that molecules involved in, or induced by the interaction of tumour cells metastasizing to specific organ sites with the microenvironment of these sites could serve as specific biomarkers and therapy targets.

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31 The epidermal niche: a regulator for normal and skin tumour stem cells

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While in mouse skin the bulge region of the hair follicle is well established as the epidermal stem cell niche, in human skin the interfollicular epidermis (IFE) prevails but here the stem cells are still difficult to identify, mainly due to lack of definitive markers and the inability to label human beings for label retaining cells (LRCs). Furthermore, organoytpic cultures (collagen-based OTCs) only allowed short-term growth of human keratinocytes suggesting that "air-lift cultures" promote terminal differentiation but not retention of stem cells. Having established a novel-type of scaffold-based OTCs, we now show that the same keratinocytes that are unable to survive >2 weeks in OTCs without fibroblasts, form a perfect epidermis with tissue homeostasis being maintained for >12 weeks − a period spanning several rounds of epidermal regeneration. Stem cells (<1%) establish in the basal layer of these cultures and can be identified as LRCs. Interestingly, LRCs also establish in short-term OTCs, however, long-term survival is hindered by degradation of the basement membrane and dermal matrix while growth factor expression of the dermal fibroblast remains largely unaltered. Thus, failure of long-term growth is not a consequence of lack of stem cells and/or their induction for terminal differentiation but a matter of destruction of the stem cell niche by inadequate expression of metalloproteinases. Applying the scaffold-based OTC model to cells representing different stages of skin carcinogenesis, we can show that also these cells are able to develop a stem cell hierarchy and that the niche determines superficial *versus* invasive growth. Thus, our findings indicate that, as important as the stem cell itself, the niche with its cellular components but in particular also matrix and basement membrane components determines stem cell survival and function, including long-term tissue regeneration and tumour cell invasion.