by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Senescence-associated heterochromatic foci (SAHFs) are enriched in heterochromatin markers and they have been proposed to enforce cellular senescence by suppressing the expression of proliferative genes.
We will discuss our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome and the regulation of DDR in stem cells and its impact on their proliferation and viability.

22 DNA repair and cancer

H.E. Krokan[1], B. Kavli[1], M. Otterlei[1], M. Akbari[1], G. Slupphaug[1], P.A. Aas[1]. [1]*Norwegian University of Science and Technology, Department of Cancer Research and Molecular Medicine, Trondheim, Norway*

DNA is continuously being damaged by spontaneous decay and exposure to carcinogens. Such damage is cytotoxic and mutagenic. Spontaneous depurination alone accounts for more than 10,000 events per human cell per day, whereas some 100–200 DNA-cytosines are deaminated to mutagenic U:G mismatches per day. DNA repair processes eliminate cytotoxicity that would otherwise kill the organism, probably within few days, while preventing mutations is important to avoid cancer development. Inherited DNA repair deficiency is associated with strongly increased cancer risk, e.g. rare syndromes like Xeroderma pigmentosum and ataxia teleangiectasia and more common forms of cancer e.g. early onset breast cancer and hereditary nonpolyposis colorectal cancer (HNPCC). In addition, there is evidence that more common single nucleotide polymorphisms (SNPs) in DNA repair genes may increase cancer risk, e.g. lung cancer development, although relative risk increases are generally low. The degree of contribution of DNA repair deficiency arising in the life of somatic cells is less clear, but there is evidence that mutations, epigenetic silencing and imbalanced expression of DNA repair proteins may increase cancer risk. Using mice with targeted mutations in DNA repair genes, defects in each of the excision repair pathways have been found to increase cancer risk. However, in some repair pathways, e.g. base excision repair (BER) some defects do not increase cancer risk possibly due to overlapping functions of some of the repair proteins. Importantly, many DNA repair proteins, such as uracil-DNA glycosylase (UNG) and mismatch repair proteins are also essential for the adaptive immune responses somatic hypermutation (SHM) and class switch recombination (CSR) in B-cells. In mice, UNG-deficiency increases the risk of developing B-cell lymphoma ~20-fold. SHM is essential to generate high affinity antibodies. However, it is a risky process and dysregulated SHM may be an important contributor to B-cell lymphoma. In conclusion, DNA repair proteins may contribute to cancer prevention both via DNA repair and adaptive immunity.

23 Aging and tumour suppression: the double-edged sword of cellular senescence

J. Campisi[1]. [1]*The Buck Institute for Age Research, Lawrence Berkeley National Laboratory, Novato California, USA*

Background: Aging is the largest single risk factor for a host of chronic diseases, most of which are degenerative in nature. These degenerative diseases include cardiac failure, vascular degeneration, macular degeneration, sarcopenia, type II diabetes-associated disability, osteoporosis and others. The exception is hyperproliferative disease, of which cancer is the most important. Cancer is indubitably an age-related disease, but hardly degenerative in nature. For many mammalian species, including humans, both hyperproliferative diseases and degenerative diseases increase with approximately exponential kinetics after about the mid-point of the life span. Is there a common biology that links cancer to the other diseases of aging?
Results: Our research suggests the answer to this question is yes. Aging is most likely driven by somatic damage, which is also a major cause of cancer. Damage occurs to virtually all cellular components, but the genome is particularly vulnerable. Cells respond to severe genomic damage by undergoing cell death or permanent loss of proliferative capacity (cell senescence). These responses are tumour suppressive, and are required to prevent the development of cancer in young mammalian organisms. We find that genomic damage, when severe enough to cause cell senescence, also induces the secretion of a large number of cytokines and other proteins that promote inflammation. Inflammation underlies virtually all age-related diseases, including cancer. We now have molecular evidence to suggest a model by which somatic damage elicits an inflammatory response that drives many age-related pathologies, both degenerative and hyperproliferative.
Conclusions: Our findings not only provide insights into how diverse age-related pathologies might arise, but also provide strategies for rational interventions into the basic aging process, and hence multiple age-related diseases.

Sunday 27 June 2010 10:20–12:20

Symposium
Noncoding RNA

24 Cancerous microRNAs and regulatory RNA binding proteins

R. Agami[1], M. Kedde[1], M. van Kouwenhove[1], W. Zwart[1], J. Oude Vrielink[1]. [1]*The Netherlands Cancer Institute, Department of Gene Regulation, Amsterdam, The Netherlands*

MicroRNAs (miRNAs) are genes involved in normal development and cancer. They inhibit gene expression through interaction with 3′-Untranslated regions (3′UTRs) of messenger RNAs (mRNAs), and are thought to regulate a large proportion of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumourigenesis. We performed in the past genetic screens to identify cancer functions of miRNAs. Using a library of vectors expressing human miRNAs and we identified miRNAs that cooperate with oncogenes in cellular transformation, which stimulate cellular migration, invasion and metastasis, as well as key regulators of tumour suppressor genes.
In recent years, it is becoming apparent that the miRNAs themselves are subjected to intense regulation at various levels. miRNA biogenesis and activity can be kept in pace by RNA-binding proteins (RBPs). We show that interplay between RBPs and miRNA exists that affects gene expression and processes such as development and cancer.

25 Non-coding RNA production by RNA polymerase III is implicated in cancer

R.J. White[1]. [1]*Beatson Institute for Cancer Research, Glasgow, United Kingdom*

RNA polymerase III is responsible for ~10% of nuclear transcription and makes a variety of short non-coding RNAs, including tRNA. Elevated expression of Pol III products has been observed in many types of transformed and tumour cells. This overexpression can be ascribed to three categories of molecular change [1].
(a) Release from repression by tumour suppressors. In untransformed cells the pol III-specific transcription factor TFIIIB is directly repressed by RB and p53 [2,3]. Inactivation of one or both of these tumour suppressors is frequent in cancer and releases TFIIIB from restraint, allowing pol III output to rise.
(b) Activation by oncogene products. Pol III transcription can be stimulated by many oncogene products. Perhaps the most important is c-Myc, which binds to TFIIIB and recruits GCN5 to pol III-transcribed genes [4,5].
(c) Pol III-specific transcription factors are produced at abnormally high levels in some types of tumour, such as prostate and ovarian carcinomas [6].
One of the key pol III products is the initator tRNAMet, which is required for production of new polypeptides. Levels of this tRNA are limiting for translation in fibroblasts. Mild overexpression of initator tRNAMet not only stimulates protein synthesis, but also promotes cell proliferation and oncogenic transformation [7]. Translational induction of c-Myc is implicated in this. Positive feedback may occur, with c-Myc stimulating pol III transcription of tRNA genes and then elevated tRNA selectively promoting translation of mRNA encoding c-Myc.

Reference(s)

[1] Marshall & White (2008) Nature Rev Cancer 8, 911–914.
[2] White et al. (1996) Nature 382, 88−90.
[3] Cairns & White (1998) EMBO J 17, 3112–3123.
[4] Gomez-Roman et al. (2003) Nature 421, 290–294.
[5] Kenneth et al. (2007) PNAS 104, 14917–14922.
[6] Winter et al. (2000) PNAS 97, 12619–12624.
[7] Marshall et al. (2008) Cell 133, 78−89.

26 Interweaving microRNA, inflammatory cytokine and p53 pathways in human cancer

C.C. Harris[1]. [1]*National Cancer Institute, Center for Cancer Research, Bethesda, USA*

We and others have identified specific microRNAs and changes in their expression in human lung, colon, and esophagus cancers that are associated with diagnosis, prognosis, and therapeutic outcome. We have also identified expression profiles of inflammation-related genes that can be combined by COX regression hazard analysis to be prognostic classifies, i.e., inflammatory risk score (IRS). For example, IRS and miR-21 expression are independent predictors of prognosis and together may be clinically useful in identifying patients with early stage cancer at high risk of metastases. As our

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

C.M. Croce[1]. [1]*The Ohio State University, Molecular Virology Immunology and Medical Genetics, Columbus Ohio, USA*

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

P. Meltzer[1], M. Zaidi[2], E. De Fabo[3], S. Davis[1], T. Hornyak[4], E. Fuchs[5], H. Arnheiter[6], G. Trinchieri[7], F. Noonan[3], G. Merlino[2]. [1]*National Institutes of Health NCI, Genetics Branch, Bethesda, USA,* [2]*National Institutes of Health NCI, Laboratory of Cancer Biology and Genetics, Bethesda, USA,* [3]*George Washington University Medical Center, Department of Environmental and Occupational Health, Washington, USA,* [4]*National Institutes of Health NCI, Dermatology Branch, Bethesda, USA,* [5]*Rockefeller University, Laboratory of Mammalian Cell Biology and Development, New York, USA,* [6]*National Institutes of Health NINDS, Mammalian Development Section, Bethesda, USA,* [7]*National Institutes of Health NCI, Cancer and Inflammation Program, Frederick, USA*

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

C.M. Isacke[1], I. Huijbers[1], A. Avgustinova[1], P. Klingbeil[1], C. Jones[2]. [1]*The Institute of Cancer Research, Breakthrough Breast Cancer Research Centre, London, United Kingdom,* [2]*The Institute of Cancer Research, Paediatric Oncology, London, United Kingdom*

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

I. Witz[1], S. Izraely[1], A. Klein[1], L. Edry-Botzer[1], S. Maman[1], O. Sagi-Assif[1], T. Meshel[1], D. Hoon[2]. [1]*Tel Aviv University, Cell Research & Immunology, Tel Aviv, Israel,* [2]*John Wayne Cancer Institute, Molecular Oncology, Santa Monica, USA*

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.

These studies were supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (Needham, MA, USA) and by Bonnie and Steven Stern (New York. NY, USA).

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

P. Boukamp[1]. [1]*Deutsches Krebsforschungszentrum, Genetik der Hautcarcinogenese, Heidelberg, Germany*

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.