whether to collect tumour or a surrogate tissue, whether to retrieve archival tumour samples from pathology laboratories or obtain recent fresh biopsies, how best to design and power the trial to test both the effect of the drug and the possible predictive value of the biomarker. Surrogate samples, such as blood and serum, while being much more accessible carry more risk because the tissue may be representative of tumour exposure but not of the tumour biology. In this sense, some biomarkers are present from the very early tumourigenic process (like K-Ras mutations in colon cancer) but some others are closely related in time to late-stage changes (like PI3K and p53 mutations and PTEN deletions in colon cancer, c-MET mutations in NSCLC and secondary c-KIT mutations in GIST). The latter examples would definitely favour the acquisition of a recent tumour sample in order to guarantee that the possible findings in the tumour correlate with the current dysregulated situation of the disease. On the other hand, retrospective biomarker analysis has the advantage that patient enrolment is not compromised, the assay does not need to be ready prior to study commencement, and multiple biomarkers can be evaluated. Additionally the quality and volume of tissue in the archival sample may limit the success of a selected biomarker analyses. Therefore, the decision on whether archival tissue − usually coming from the primary tumour resection-, or recently fresh biopsied tissue or circulant tumour cells or DNA material is needed for the consecution and success of the biomarker should be made taking in consideration all the previously mentioned aspects. The development of biomarkers in phase II development holds great promise but also creates new challenges. Further actions are needed in order to implement the development of tumour biomarkers in this setting. These include among others the need for greater information among patients, patients' coalitions and advocate groups, institutional review boards, local Health Administrations, Regulatory Agencies, clinicians, pathologists and other physicians involved in the acquisition of good quality tumour samples. The ultimate goal of this biomarker development process from tumour biopsies or circulant tumour cells/DNA will be to facilitate oncology drugs development and to identify which patients are most likely to benefit.

338 Mutations in OXPHOS and Krebs cycle genes and tumour development

M. Sobrinho Simoes[1]. [1]*IPATIMUP – Institute of Molecular Pathol, Pathology, Porto, Portugal*

It was recently shown using a mitochondrial proteomics analysis in yeast that Sdh5, a gene required for flavination of succinate dehydrogenese (SDH), is mutated in human paragangliomas. This is just another example of the increasing number of mutations in OXPHOS and Krebs cycle genes associated to tumourigenesis. Sdh5 interacts with the catalytic subunit of the SDH complex, a component of both the OXPHOS chain and the Krebs cycle. Similar findings have been reported with regard to mutations in mitochondrial and nuclear genes exclusively affecting the electron transport chain (eg. ND1, ND6 and GRIM19, respectively) and in (nuclear) genes affecting the Krebs cycle (eg. FH and IDH). The observation that germline loss-of-function mutations in some of the aforementioned genes segregate with disease in families with hereditary tumours and the demonstration of the tumourigenic effect of mutations in mitochondrial OXPHOS genes have contributed decisively to the renaissance of the interest on tumour metabolism in general, and on the Warburg effect in particular. This renewed interest has been reinforced by an ever-growing number of reports revealing that several oncogenic alterations causing tumour development directly affect glycolysis, cellular response to hypoxia and angiogenesis. Understanding cancer abnormal cellular metabolism and Warburg effect − Why and how do cancer cells activate glycolysis in the presence of oxygen? − has become a major objective for those who think that targeting mitochondria and the peculiarities of tumour cell metabolism may prove therapeutically successful. Some of these issues will be discussed taking together the results of observational studies in several types of human tumours with those obtained in experimental models with an emphasis on the role played by mitochondrial alterations.

339 Breast cancer genomic landscapes – a first step towards personalised cancer medicine

No abstract received.

340 Delivering on the promise of personalized medicines

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Please see abstract **608** of Educational Lecture: Systems approach to personalized molecular medicine.

Monday 28 June 2010 14:35–16:35

Symposium
Maintenance of genome stability

341 The DNA damage response machinery as an anti-cancer barrier and determinant of treatment response

No abstract received.

342 The role of chromatin in genome instability; identification of novel therapeutic targets

N.J. Gardner[1], A.L. Chambers[1], S.J. Dukes[1], J.A. Downs[1]. [1]*University of Sussex, MRC Genome Damage and Stability Centre, Brighton, United Kingdom*

High mobility group box (HMGB) proteins are abundant, chromatin-associated proteins with essential roles in gene expression and development. The HMG box is a DNA binding domain that binds the minor groove of DNA and in doing so, creates a bend in the DNA. In addition, these proteins bind preferentially to distorted DNA structures, such as DNA lesions created by cisplatin exposure and ultraviolet (UV) irradiation. In budding yeast, Hmo1 appears to be the most abundant HMG box containing protein and has been shown to influence global chromatin structure. We investigated the potential role of Hmo1 in DNA damage responses, and found that Hmo1 mediates the cytotoxic effects of the alkylating agents methane methylsulfonate (MMS) and N-methyl-N-nitrosourea (MNU) *in vivo*. These alkylating agents both methylate DNA at the N7 position of guanine and the N3 position of adenine. We investigated the potential mechanism by which this occurs and find Hmo1 binds to MMS- and MNU-treated DNA preferentially over unmethylated DNA *in vitro*. Unlike other HMGB proteins, Hmo1 does not appear to have any significant influence on the cytotoxic effects of cisplatin *in vivo*, and does not display preferential binding to cisplatin-treated DNA *in vitro*. We determined the domain that mediates this effect and used this to identify a mammalian homologue. The identification of this novel preference for methylated DNA has important implications for the use of alkylating agents as chemotherapeutics.

343 Synthetic lethal approaches to cancer therapy

A. Ashworth[1]. [1]*The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom*

A critical link exists between genomic instability and cancer development. This instability can manifest as small changes at the nucleotide level or as gross chromosomal alterations. Mutations in the genes that encode DNA damage response proteins are responsible for a variety of genomic instability syndromes including Hereditary Non-Polyposis Colorectal Carcinoma, Bloom syndrome, Ataxia-telangiectasia, *BRCA1* and *BRCA2* mutated breast and ovarian cancers and Fanconi anaemia. Similarly epigenetic silencing of genes associated with the maintenance of genomic stability have also been implicated in the pathogenesis of cancer. Here, I discuss how different tumours may be classified not only by tumour site but also by the type of underlying genetic instability. This type of classification may assist in the optimization of treatment regimens as well as informing the development of new therapeutic approaches in particular based on "synthetic lethality".

344 The ATM-mediated DNA damage response: back to DNA repair

Y. Shiloh[1]. [1]*Tel Aviv University, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv, Israel*

The DNA damage response (DDR) is emerging as an extremely complex system, involving DNA repair and affecting cell cycle progression, gene expression, RNA metabolism, and protein modifications, transport and turnover. The response to double strand breaks (DSBs), which vigorously activate this network, is mobilized by the nuclear protein kinase ATM that phosphorylates key players in its various branches. ATM loss or inactivation leads to the genomic instability syndrome ataxia-telangiectasia (A-T), characterized by neuronal degeneration, immunodeficiency, genomic instability, extreme radiation sensitivity, and cancer predisposition. The extreme radiation sensitivity of A-T patients has been attributed to a subtle defect in the repair of double-strand breaks (DSBs). The ATM-dependent component of DSB repair indeed involves a small but distinct fraction of DSBs. Interestingly, the dependence of DSB repair on ATM is more pronounced in Purkinje cells, which are badly affected by ATM loss in humans. One way ATM directly affects the repair process is by phosphorylating repair proteins and modulating their activity. We demonstrate one such process: ATM-dependent phosphorylation of the repair enzyme polynucleotide kinase phosphatase (PNKP). ATM also regulates DSB repair by inducing genome-wide and local chromatin reorganization. Chromatin is now recognized to be an active player in the DDR, and modulation of its organization an essential arm of the

response. Recently we identified a novel ATM-mediated pathway that leads to transient, global chromatin relaxation. We found ATM's effector in this pathway to be the KAP-1 protein (TRIM28), which is phosphorylated by ATM at DSB sites and then rapidly conveys the chromatin relaxation signal across the nucleus. However, optimal processing and repair of DSBs require chromatin reorganization at damaged sites as well. Chromatin reorganization associated with DNA transactions such as transcription is intimately coupled to alterations in post-translational modifications (PTMs) of the histone proteins. We found that monoubiquitination of histone H2B (mUbH2B) – a modification previously associated with transcription-coupled nucleosome dynamics – is induced by DSBs and is essential for timely DSB repair. This pathway is dependent on ATM and the responsible ubiquitin E3 ligase – a tight complex of the RING finger proteins RNF20 and RNF40, both ATM targets. Damage-induced mUbH2B is specifically required for the stable accumulation of repair proteins at DSB sites. These pathways demonstrate once again the multi-pronged approach of ATM to the systems it regulates, one that allows tight but fine-tuned control.

Monday 28 June 2010 14:35–16:35

Symposium
Inflammation and cancer

345 Improving cancer immunotherapy by preventing chemokine nitration

A. Viola[1]. [1]*Istituto Clinico Humanitas Mirasole, Rozzano (Milan), Italy*

Background: The tumour microenvironment is not suitable for T lymphocyte functions, and indeed a number of reports indicate that tumour-infiltrating lymphocytes (TILs) have defects in both signal transduction compartment and killing effector systems.
At the tumour site, the reactions of nitric oxide (NO) with oxygen (O_2) or oxygen-related reactive intermediates yield numerous reactive nitrogen and oxygen species (RNOS). One of the most studied reaction implicates NO and superoxide anions and the generation of peroxynitrite ($ONOO^-$), a potent oxidant with pleiotroic activities. In the past, we and others provided data showing that intratumoural RNOS, produced by either myeloid cells or by the very same tumour cells, are involved in tumour-induced immunosuppression. In addition to being dysfunctional, TILs are also unable to reach the core of the tumour mass, and they concentrate at the border of the neoplastic lesions. We speculated that RNOS might affect chemokine biology and contribute to keep TILs distant from the tumour.
Materials and Methods: Chemokine nitration was analyzed by Mass Spectrometry and immunohistochemistry in human prostate and colon cancer as well as in several murine tumours.
For adoptive cell therapy, mice bearing the EG7-OVA tumour were treated or not with our compound (AT38) before receiving OT-I CTLs.
Results: We found that the chemoattractants CXCL12, CCL21 and CCL2 lose their ability to recruit T lymphocytes when exposed to peroxynitrite. However, the modified chemokine CCL2 retains its capacity of recruiting myeloid cells to the tumour site, suggesting that chemokine post-translational modification might represent a way to selectively modify the tumour microenvironment and favor immune dysfunction.
Based on our findings, drugs controlling the *in situ* production of RNOS might be useful to aid immunotherapeutic approaches for the treatment of cancer, by creating a favorable tumour environment for lymphocyte recruitment and activation. We have developed and screened novel small molecules aimed at interfering with multiple, interconnected metabolic pathways leading to RNOS generation within tumour microenvironment. One of these new compounds (AT38) was used to verify *in vivo* the hypothesis that peroxynitrites may restrain T cell access to the tumour.
We found that *in vivo* inhibition of intratumoural RNOS production results in massive TIL infiltration and has a strong impact on the outcome of adoptive T cell therapy.
Conclusions: These data indicate that chemokines are post-translationally modified by RNOS in the tumour microenvironment and identify novel targets for regulating the composition of tumour infiltrate and sustain properly the antitumour immune response.

346 From inflammation and regeneration to hepatocarcinogenesis

E. Galun[1]. [1]*Hadassah Hebrew University Hospital, Goldyne Savad Institute of Gene Therapy, Jerusalem, Israel*

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide and is considered to be the outcome of chronic liver inflammation. Currently, the main treatment for HCC is surgical resection. However, survival rates are suboptimal partially because of tumour recurrence in the remaining liver. Our aim was to understand the molecular mechanisms linking liver regeneration under chronic inflammation to hepatic tumourigenesis. Mdr2-KO mice, a model of inflammation-associated cancer, underwent partial hepatectomy (PHx), which led to enhanced hepatocarcinogenesis. Moreover, liver regeneration in these mice was severely attenuated. We demonstrate the activation of the DNA damage-response machinery and increased genomic instability during early liver inflammatory stages resulting in hepatocyte apoptosis, cell-cycle arrest, and senescence and suggest their involvement in tumour growth acceleration subsequent to PHx. We propose that under the regenerative proliferative stress induced by liver resection, the genomic unstable hepatocytes generated during chronic inflammation escape senescence and apoptosis and reenter the cell cycle, triggering the enhanced tumourigenesis. Thus, we clarify the immediate and long-term contributions of the DNA damage response to HCC development and recurrence.

347 The inflammatory tumour microenvironment: tumour-protective or tumour promoting?

K.E. De Visser[1], T. Hau[1], M. Ciampricotti[1], E. Speksnijder[1], C. Doornebal[1], J. Jonkers[1]. [1]*The Netherlands Cancer Institute, Department of Molecular Biology, Amsterdam, The Netherlands*

Whereas it has become generally accepted that chronic activation of innate immune cells contributes to cancer development and/or progression, the role of the adaptive immune system during tumourigenesis is still a matter of debate. Both tumour-protective and tumour-promoting properties of the adaptive immune system have been described in clinical and experimental settings. The overall goal of our research is to address the role and underlying pathways of the adaptive and innate immune system during sporadic breast cancer progression and metastasis formation. We utilize a mouse tumour model that faithfully recapitulates human invasive and metastatic lobular carcinoma, e.g. a conditional mouse breast cancer model based on mammary epithelium-specific deletion of p53 and E-cadherin. Like human breast cancers, mammary carcinomas arising in this mouse model are characterized by abundant presence of innate immune cells, including degranulating mast cells and macrophages, T and B lymphocytes, antibody depositions and increased levels of pro-inflammatory mediators. By genetic elimination and pharmacological inhibition of specific subsets of the adaptive and innate immune system, we are currently investigating their functional significance in a tumour-stage specific manner. Genetic elimination of the adaptive immune system in this mouse model did not alter latency and outgrowth of primary breast cancers, indicating that immunosurveillance does not play a role during sporadic breast cancer development. Importantly, absence of the adaptive immune system resulted in almost complete abrogation of spontaneous metastasis formation. We are currently assessing the underlying mechanisms by which the adaptive immune system promotes metastasis formation of sporadic breast cancer. Ultimately, the outcome of these studies may shift therapeutic focus from a cancer cell intrinsic point of view towards a more combined cancer cell intrinsic and extrinsic point of view.
Research supported by the Dutch Cancer Society, NKB 2006–3715 and NWO/VIDI 91796307.

348 Control of tumour progression and metastasis by inflammatory signals

No abstract received.

Monday 28 June 2010 17:30–18:20

Dentist O. Aase and E. Granqvist Memorial Lecture

349 Targeted therapy – novel anti-HER2 strategies in the therapy of breast cancer

No abstract received.

Monday 28 June 2010 09:45–17:30

Poster Session
Cell and Tumour Biology

350 Role of c-Fos/AP-1 in the progression to squamous cell carcinomas

E.M. Briso[1], L. Bakiri[1], J. Guinea-Viniegra[1], E.F. Wagner[1]. [1]*Centro Nacional de Investigaciones Oncológicas (CNIO), Banco Bilbao Vizcaya Argentaria (BBVA)-Foundation Cancer Cell Biology Programme, Madrid, Spain*

Background: The proto-oncogene *c-fos* is a component of the AP-1 transcription factor complex, which is involved in the regulation of cell proliferation, differentiation and transformation. AP-1 is the effector downstream of many signal transduction pathways and *c-fos* particularly plays important roles in bone, skin and muscle tumourigenesis *in vivo*. However,