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

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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?

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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

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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,

c-Fos functions in epidermal development, homeostasis and tumourigenesis are not yet fully understood.
Material and Methods: Gain of function studies are performed using an inducible, epithelial-specific transgenic mouse model for ectopic *c-fos* expression. The carcinogen 7,12-dimethylbenz[α]anthracene (DMBA), inducing activating Ras mutations, is used as a tumour initiator. Protein expression is evaluated by immunohistochemistry using frozen and paraffin-sections and by immunoblotting. RNA expression analyses are performed using qRT-PCR and cytokine levels are measured by ELISA.
Results: Ectopic epidermal-specific *c-fos* expression in adult mice induces epidermal hyperplasia. Moreover, *c-fos* expression in combination with the carcinogen DMBA, is sufficient to promote the formation of highly invasive Squamous Cell Carcinomas (SCC) of the Achantolytic subtype. We also demonstrate the presence of an immune cell infiltrate mainly composed of $CD4^+$ and $CD8^+$ T lymphocytes as well as $F4/80^+$ macrophages, both in the hyperplastic skin and in the stroma of the SCCs. Interestingly, serum levels of IL-6 are increased both in transgenic c-Fos DMBA-free or DMBA-treated mice. Finally, immunohistochemical analyses indicate that human SCCs express high levels of c-Fos.
Conclusions: These results show that c-Fos can induce epidermal hyperplasia and that in combination with DMBA, it is sufficient for the development of SCCs. Expression of c-Fos in human SCCs suggests that inhibition of Fos/AP-1 might be a viable therapeutic option.

351 Identification and analysis of two novel Mdm2-interacting proteins involved in the regulation of cellular stress response pathways

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Background: Tumour suppressor p53 is a key regulator of cellular responses to stress stimuli such as DNA damage, ribosomal stress or hypoxia. Oncoprotein Mdm2, which is often found overexpressed in cancers, serves as a ubiquitin ligase for p53 and promotes p53 degradation via 26S proteasome. In addition to the N-terminal p53-binding domain and the C-terminal RING finger domain, the Mdm2 protein contains a centrally located domain rich in acidic amino acids whose function hasnt been fully explained yet. It has been shown that this part of the Mdm2 protein is required for efficient ubiquitylation and degradation of p53. Another important role of the central region of Mdm2 is the binding of numerous Mdm2 regulators such as YY1, p300, p14Arf, etc. The aim of our project is to identify and characterize new Mdm2-interacting proteins that might regulate Mdm2 function in tumour cells by binding to the central part of Mdm2 oncoprotein.
Methods and Material: To identify novel binding partners for Mdm2 we used tandem affinity tag purification of cellular complexes containing Mdm2, followed by mass spectrometry analysis. Co-immunoprecipitations and immunofluorescence were used to confirm the interactions between Mdm2 and selected candidate proteins. The function of the binding partners was further analysed in various functional assays (e.g. degradation, ubiquitylation, and promoter activity assays) in human cancer cell lines.
Results: We have identified basal transcription factor TFII-I and ubiqutin-specific protease USP48 as new binding partners for Mdm2. Our data indicate that TFII-I promotes Mdm2-mediated p53 ubiquitylation and could be also involved in the regulation of Mdm2 protein levels. On the other hand, TFII-I has been implicated in cellular responses to certain types of the stress and we show that Mdm2 can inhibits the transcriptional activity of TFII-I, suggesting that Mdm2 might take part in the regulation of TFII-I-mediated stress responses.
Our results show that the second identified Mdm2-interacting partner USP48 can also modulate the levels of p53 ubiquitylation. In addition to that, USP48 is able to stabilize Mdm2. However, our data suggest that rather than simply catalyzing Mdm2 deubiquitylation, the role of USP48 in the regulation of Mdm2 protein levels could be more complicated.
Conclusion: We have identified two novel Mdm2-interacting proteins, general transcriptional factor TFII-I and ubiqutin-specific protease USP48. Both proteins can regulate the extent of tumour suppressor p53 ubiquitylation and the cellular levels of oncoprotein Mdm2. In addition to that, the overexpression of Mdm2 found in some types of cancer could influence TFII-I-directed responses to certain stress stimuli.
This work was supported by Cancer Research UK and the Czech Science Foundation grant No. 301/09/1324.

352 Withdrawn

353 ALK kinase controls an angiogenetic program in lymphoma, lung carcinoma and neuroblastoma

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Background: A portion of haematological and solid cancers, such as Anaplastic Large Cell Lymphoma (ALCL), Non Small Cell Lung Carcinoma (NSCLC) and neuroblastoma (NB), may express constitutive active forms of the Anaplastic Lymphoma Kinase (ALK). Constitutively active ALK, mainly in the form of translocations or point mutations, acts as an oncogene in lymphomas and, potentially, in NSCLC and NB. These cancer need to activate an angiogenetic program to sustain their growth. Thus, we investigated the relation between oncogenic ALK and angiogenesis in these cancers.
Material and Methods: We investigated the existence of an ALK-induced angiogenetic program in ALK transformed cells, in particular the expression of VEGF, Hif-1α and Hif-2α. ALCL cells lines TS and SU-DHL1 that carry an NPM-ALK translocation, H2228 and H3122 NSCLC that carry an EML4-ALK translocation, SH-SY5Y NB that have an ALK activating point mutation were used in the experiments. ALK inhibitors or shRNA specific for ALK or ALK-directed siRNA entrapped into liposomes were used to block ALK kinase activity. Gene expression profiling, microarrays, Western Blots were performed on ALCL, NSCLC and NB cells incubated in normoxia, hypoxia (3% O_2), or treated with deferoxamine (DFX), an hypoxia-mimetic compound. Xenografts in immunodeficient mice from ALK positive ALCL lines were treated with bevacizumab.
Result: In ALCL cell lines incubated in normoxia, the inhibition of ALK tyrosine kinase activity significantly decreased VEGF secretion. In ALCL cells incubated in hypoxia or with DFX, ALK inhibition lead to a dramatic reduction of Hif-2α mRNA and protein levels, whereas Hif-1α was less affected. Comparable reduction of Hif-2α after ALK inhibition were observed in H3122 and in SH-SY5Y cell lines. In ALCL, a specific shRNA against Stat3, a pivotal mediator of ALK transforming activity, induced a decrease of Hif-2α protein levels. Finally, treatment with bevacizumab of xenografts lead to a significant delay in ALCL growth. In a subcutaneous mouse model of NB, intratumoural injection of NB-targeted ALK-siRNA liposomes inhibited blood vessel density.
Conclusion: The tyrosine kinase ALK controls a common, Stat3-mediated angiogenetic program in ALCL, NSCLC and NB cells by regulating secretion of VEGF and Hif-2α protein levels. Inhibition of ALK activity reduces angiogenesis in ALK positive cancers and treatment of ALK tumours with anti-angiogenetic drugs is beneficial in reducing tumour growth.

354 Withdrawn

355 Fibrin and type I collagen 3D matrix differentially regulate sprout angiogenesis of human dermal microvascular endothelial cells

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Angiogenesis is a highly regulated event involves complex, dynamic interactions between microvascular endothelial cells and extracellular matrix (ECM) proteins. Alteration of ECM composition and architecture is a hallmark of wound clot and tumour stroma. However, the role of ECM in regulation of angiogenesis associated with wound healing and tumour growth is not well defined. During angiogenesis, endothelial cell responses to growth factors are modulated by the compositional and mechanical properties of a surrounding three-dimensional (3D) extracellular matrix (ECM) that is dominated by either cross-linked fibrin or type I collagen. In this study, we investigated the correlation of sprout angiogenesis and ECM environment using in vivo and in vitro angiogenesis models. In healing full-thickness cutaneous porcine wounds, the fibrin-rich early granulation tissue in 5 day wounds was filled with newly formed vessels. Then the angiogenic neovessels in early granulation tissue mature and then regress as fibrin was replaced by collagen in the wound space. It suggests that provisional matrix, especially fibrin, is essential for sprout angiogenesis. Using an in vitro 3D microcarrier based sprout angiogenesis system we further demonstrated that fibrin and type I collagen 3-D matrix differentially regulated angiogenic sprout formation of human dermal microvascular endothelial cells (HDEMC). Expression of integrin αvβ3 is one of the hallmark features of sprout angiogenesis. Remarkably, integrin β3 expression was highly up-regulated in vasular endothelial cells found in fibrin rich, but not in collagen rich matrix environment in vivo and in vitro. Echistatin, a disintegrin specific for αvβ3, dose dependently inhibited sprout angiogenesis of HDMEC in fibrin. While blocking antibody to integrin α2β1, receptor for collagen, had no inhibitory effect on sprout angiogenesis in vitro,