BIOME UNIVERSITÄT DUISBURG ESSEN

Offen im Denken

Immunology

Tumour Signalling

3rd Annual BIOME Retreat
25th November 2014
Unperfekthaus Essen

Program

Schedule

Abstracts

Schedule

9.00 h Introduction: **Delia Cosgrove**

1st Session Chairs: Stephanie Rost & Sebastian Hönes

9.15 h **Hon. Prof. Dr. Andrea Musacchio**
Max Planck Institute of Molecular Physiology, Dortmund

10.00 h **Nico Ullrich**

10.20 h **Katrin Moses**

10.40 h – 11.10 h COFFEE BREAK

2nd Session Chairs: Sören Lattayer & Julia Badziong

11.10 h **Judith Hönes**

11.30 h **Chranjeevi Chebrolu**

11.50 h **Johanna Klein**

12.10 h – 13.30 h LUNCH

3rd Session Chairs: Julia Badziong & Robert Petri

13.30 h **PD Dr. med. Philipp A. Lang**
Department for Gastroenterology, University Hospital Düsseldorf

14.15 h **Gabriele Büchel**

14.35 h **Lina Spaan**

14.55 h – 15.30 h COFFEE BREAK

4th Session Chairs Robert Petri & Gina-Eva Görtz

15.30 h **Prof. Dr. rer. nat. Carsten Watzl**
Leibniz Research Centre for Working Environment and Human Factors, Dortmund

16.15 h **Vishal Khairnar**

16.35 h **Marija Kovacevic**

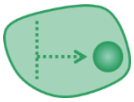
16.55 h **Tina Danielzik**

17.15 h Good Bye: **PD Dr. Annette Paschen**

18.00 h – 21.00 h DINNER & BEER/WINE

Abstracts

1st session:



Nico Ullrich: **Size matters: solely CEACAM1-3S isoform expression improves overall survival and enhances immunogenicity in melanoma**

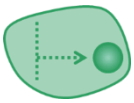
Malignant melanoma is the most aggressive skin cancer characterized by a high metastatic potential. Deregulation of cell adhesion molecules, like CEACAM1, has been shown to be essential for metastatic spread the leading course of death. We could show that CEACAM1 splice variants CEACAM1-4L, CEACAM1-4S, CEACAM1-3L and CEACAM1-3S were expressed in melanoma cell lines and biopsies. CEACAM1-4L and -4S could be found in biopsies of all stages whereas CEACAM1-3S and -3L were only detected in late stage (III/IV) biopsies. Strikingly, a significant correlation of CEACAM1-3S expression with the clinical stage and patient overall survival was detected. *In-vitro* analysis revealed the inhibitory impact of CEACAM1-3S on melanoma cell migration and invasion whereas all other CEACAM1 variants promoted these cellular processes. Interestingly, CEACAM1-3S enhances melanoma cell immunogenicity by modulating the surface expression of NKG2DLs. Contrarily, CEACAM1-4L reduced the surface concentration of these ligands via enhanced shedding, thus, supporting a tumor progressive phenotype. These results highlight the significance of the variant-specific immunomodulatory and cell biological functions of CEACAM1 isoforms in melanoma pathogenesis.

Katrin Moses: **Impact of polymorphonuclear cells (PMN) in head and neck cancer (HNC) progression – a mouse model to investigate time dependent functions of PMN**

We could previously show that high PMN infiltration in HNC tissues correlated with poor survival of patients with advanced disease. Further *in vitro* studies showed that human PMN are manipulated by tumor cells to acquire a protumoral phenotype. We developed mouse models of HNC to further characterize the processes of PMN mediated tumor progression.Two-photon microscopy of malignant tissue and classical immunohistochemistry demonstrated the presence of tumor-associated neutrophils (TAN). *In vitro* analyses showed an immunosuppressive function of neutrophils from tumor bearing mice. The tumor microenvironment induced an upregulation of protumoral factors in TAN that is not seen in the periphery. Depletion of PMN at early time points diminished tumor growth and was associated with aberrant tumor angiogenesis as well as enhanced T cell infiltration. Our results suggest that PMN inhibit anti-tumor immune responses and promote the formation of a structural “niche” in the early phases of tumor growth.

2nd session:

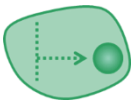
Judith Hönes: **Dose dependent role of Gfi1 in human MDS and AML**



We investigated the role of the myeloid transcription factor Growth factor independence 1 (GFI1) in Acute myeloid Leukemia (AML) development. Based on expression array we observed that Gfi1 is expressed at lower level in human AML blast cells than in CD34 pos. progenitors. Furthermore, low level of Gfi1 expression in blast cells was associated with an inferior prognosis of AML patients. Also AML patients with deletion of the Gfi1 allele on chromosome 1 were characterized by a higher number of blasts and an inferior prognosis. To explain these observations, we used different AML and mouse models mimicking human AML. Knock down of Gfi1 as well as the deletion of one Gfi1 allele significantly accelerates AML development compared to WT mice. Forced overexpression of Gfi1 impeded leukemia progression and induced terminal differentiation of leukemic cells. Finally complete loss of Gfi1 inhibited AML development and progression. On the molecular level we found that Gfi1 has two functions: It promotes differentiation of cells and inhibits the pro-apoptotic protein p53. Thus at high levels Gfi1 induces differentiation of leukemic cells, at low level differentiation is blocked but p53 is still inhibited and absence of Gfi1 induces massive apoptosis in leukemic cells. We are currently investigating how altering Gfi1 level could serve as a therapeutic approach in human patients.

Chranjeevi Chebrolu: **Role of TLRs involved in inflammation induced either by sterile DAMPs or bacterial PAMPs**

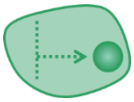
Immune stimulatory pathogen associated molecular patterns (PAMPs) are major drivers of infection pathology. Infections with Gram-negative bacteria or negatively polar and single stranded RNA influenza virus are prominent causes of morbidity and mortality. Toll-like receptor (TLR) 4 is a major host sensor for both of these infections. In order to inhibit TLR4 driven immune activation we have recently developed synthetic tetraacylated lipid A mimetics based on a conformationally restricted bGlcN(1↔1)aGlcN disaccharide scaffold (DA-compounds) that antagonized ectopically overexpressed murine and human TLR4/MD-2 complexes. Our results indicate biological species specificity of LPS antagonism by variably tetraacylated lipid A mimetics and validate three out of six DA-antagonists as promising candidates for development of therapeutically applicable anti-inflammatory compounds. TLRs mediate recognition not only of pathogen associated molecular patterns (PAMPs) but also of danger associated molecular patterns (DAMPs) indicating both infection driven and sterile tissue injury. According to the endosymbiont theory the origin of mitochondria is prokaryotic. Thus, mitochondrial constituents must resemble bacterial PAMPs and specific ones are immune stimulatory DAMPs. we observed an immune stimulatory capacity that depended on TLR3, TLR7, and TLR8. Surprisingly, a 16S mitochondrial ribosome (mr) RNA segment carrying two uracils (Us) in each of its termini as well as the TLR13 ligand core motif GGAAAGA lacked murine macrophage activation property. In contrast, the macrolide lyncosamin streptomycin (MLS) antibiotic and TLR13 binding epitope of bacterial 23S rRNA ortholog, namely 16S mrRNA segment named “mtPTLbl” activated TLR7 and TLR8 due to a UAU motif carried in its 3’ terminus. It lacked, however, a TLR13 stimulatory capacity due to a double residue conversion in its core region (GAGAAGA). Our results implicate a specific 16S mrRNA segment as TLR7 and TLR8 stimulatory, which distinguishes it from its bacterial ortholog while qualifying it as immune stimulatory DAMP.



Johanna Klein: **Induction of immunomodulatory cell death by synthetic RNA mimetics**

Synthetic TLR ligands are used for cancer therapy with varying success. The aim of this study was to assess mechanisms and consequences of tumor cell death induced by poly(I:C) and imiquimod, ligands for endosomal TLRs.Local in vivo TLR therapy of a human head and neck squamous cell carcinoma (HNSCC) xenograft model showed therapeutic efficacy for poly(I:C), while intratumoral injection of imiquimod did not reduce tumor growth. In vitro studies showed reduced cell viability of human HNSCC cells only after intracellular delivery of poly(I:C). Cell death induced by poly(I:C) resulted in cytokine release, activation of monocytes and recruitment of Th1 cells. In contrast, imiquimod also induced tumor cell death and release of IL-6, but cell death was not associated with cytokine release and monocyte activation. These data elucidate molecular events induced by TLR agonists during cancer immunotherapy.

3rd session:



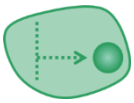
Gabriele Büchel : **Galectin-1 modulates the immune response and angiogenic properties in a transgenic model of neuroblastoma**

Galectin-1 (Gal-1) is a multifunctional protein that enhances tumor aggressiveness by inducing angiogenesis and contributing to the tumor immune escape. Here, we aimed to assess the effect of Gal-1 on the immune system and also on tumorigenesis of neuroblastoma (NB) using modulation of Gal-1 expression in immune effector cells and a transgenic NB model, respectively. We first investigated the tumor incidence in the established TH-MYCN NB mouse model as a function of the Gal-1 gene dosage using Gal-1 knockout mice. Interestingly, the tumor incidence was higher in the double transgenic TH-MYCN, Gal-$1^{-/-}$ mice. Reduced Gal-1 gene dose correlated with reduced tumor angiogenesis as shown by immunohistochemistry. Tumor tissue from TH-MYCN mice differed in lymphocytic infiltration compared to double transgenic TH-MYCN, Gal-$1^{-/-}$ mice. These results confirm a role for Gal-1 in modulating the immune phenotype and angiogenesis in a transgenic NB model. The implications of targeting Galectin-1 in therapeutic settings, as it is currently being developed for other tumor entities, remain to be explored for NB.

Lina Spaan: **Activation of the innate cytosolic receptor RIG-I enhances melanoma immunogenicity independent of autocrine type I interferon signaling**

Malignant melanoma is a highly aggressive cancer. Inefficiency of anti-tumor $CD8^+$ T cell responses can in part be explained by downregulation of MHC class I molecules on melanoma cells. Therefore strategies to enhance melanoma immunogenicity are needed. One appealing strategy is the mimicry of viral infection. Viral nucleic acids are sensed by ubiquitously expressed receptors, including RIG-I. In this study we showed that transfection of melanoma cells with RIG-I agonist 3pRNA directly increased their immunogenicity. We demonstrated an upregulated expression of different components of the antigen processing and presenting machinery (APM). This led to an enhanced expression of HLA class I molecules on the tumor cells that in turn increased their recognition by autologous $CD8^+$ T cells. Although 3pRNA-transfected tumor cells produced type I IFN, we observed an APM upregulation also in the absence of type I IFN signalling. Our findings suggest that synthetic viral RNA mimetics effectively enhances melanoma immunogenicity and therefore have therapeutic potential.

4th session:



Marija Kovacevic: **Effect of PDE δ and Arl2 on mTORC1 signaling via control of Rheb localization**

mTOR pathway is one of the regulators of cell growth, metabolism and survival. Its deregulation leads to tumor angiogenesis or insulin resistance.The central component, mammalian target of rapamycin complex 1 (mTORC1) is a kinase activated upon binding to small GTP-ase Rheb. We have shown the localization of Rheb is maintined via Arl2- PDEδ system, analogous to the KRas spatial cycle. Since the localization of Rheb is essential for proper mTORC1 activity, we focused, as well, on the effect of preturbation of PDEδ on the signaling output.Downregulation of PDEδ or Arl2 leads to delocalization of Rheb, while PDE δ inhibition lowers the phosphorylation of S6 protein.In this work, we present maintainance of Rheb localization via Arl2- PDEδ system, and the effect of its preturbation on mTORC1 signaling. These findings confirm PDEδ and Arl2 as essential factors in localization and targets for inhibition of pathways including all farnesylated Ras proteins.

Tina Danielzik: **Wilms' tumor 1 (WT-1)-specific T lymphocytes as a tool for cellular immunotherapy in acute myeloid leukaemia**

The Wilms' tumor 1 (WT-1) antigen is known to be highly expressed in hematopoietic malignancies such as acute myeloid leukaemia (AML) but only at a low level in healthy tissue and some progenitor cells. We started a study focussing on the identification and functional characterization of WT-1-specific T lymphocytes that may provide a useful tool for cellular immunotherapy. We isolated PBMCs of healthy persons and AML patients pre- and AML patients post-transplantation. ELISpot analysis resulted in low frequencies of WT-1-specific T lymphocytes in all three groups. Fluorescence-coupled ELISpot (Fluorospot) could confirm previous frequencies and showed simultaneous cytokine secretion for WT-1-specific cells analysed from AML patients post-transplantation. Due to these low cell frequencies, expansion of WT-1-specific T cells was considered the crucial step for characterisation.