T cell recognition of breast cancer antigens

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the influence of hypoxia and/or the TAM phenotype. In sum, we have identified a set of novel key molecules that dictate the TAM phenotype during tumor growth.
*†Equal contribution

B-31445

Targeting metastasis in mammary carcinoma: functional analysis of serglycin proteoglycan (and the chymase mMCP-4)

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In hematopoietic cells, serglycin proteoglycans mainly contribute to proper storage and secretion of inflammatory mediators via their negatively charged glycosaminoglycans. In cancer cells serglycin proteoglycans are also expressed in close association with the cell surface marker CD44, where increased expression of serglycin has been linked to poor prognosis. We recently reported that genetic ablation of serglycin completely blocked lung metastasis in the MMTV-PyMT-driven mouse breast cancer model, while serglycin-deficiency did not affect primary tumour growth or number of mammary tumours. In the primary tumours CCL2 expression indicated equal levels of inflammation, whereas numbers of tumour-associated mast cells were reduced in the serglycin-deficient mice. A microarray expression analysis and functional annotation of differentially expressed genes in primary tumour tissue identified several biological pathways where serglycin may be important. Although E-cadherin expression was higher in the serglycin-deficient primary tumour tissue, indicating reduced invasiveness, serglycin-deficient tumour cells were still detected in the circulation. Primary tumour cells ($SG^{+/-}$ and $SG^{-/-}$) have been established in cell culture, where we investigate the role for serglycin in migration and extravasation with CRISPR/Cas9 mediated knockdown. In addition, when the primary mouse tumour cells were injected i.v. in SG-competent and SG-deficient mice injected $SG^{-/-}$ tumour cells failed metastasis to the lung. Our results suggest that serglycin proteoglycans play an essential role in extravasation of metastatic cells, and that serglycin and serglycin-dependent mediators are potential drug targets to prevent metastatic disease/dissemination of cancer. We now aim to identify the serglycin-dependent mediators promoting metastasis and define their role.

B-31458

Microfluidic vascular models for studies of cell extravasation

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Cellular endothelial transmigration is an important process whereby cells exit the circulatory system and invade surrounding tissues, key to normal immune system function and cancer metastasis. During normal leukocyte behaviour, the circulatory system enables leukocytes to patrol the body, searching for infections and physical damage. Upon encountering cues indicative of these situations, leukocytes will exit the circulatory system through the vessel wall and enter the surrounding tissue. Cancer metastasis is the process during which cancer cells leave the primary tumour, move through the surrounding tissue and enter the vascular system. The cancer cells will travel through the vascular system and eventually cross the blood vessel walls and establish new tumours in the surrounding tissue, with detrimental consequences to the patients. The mechanisms involved in these processes are inadequately understood and current knowledge relies heavily on cumbersome animal experiments and non-physiological *in vitro* models. The aim of this project is to adopt, adapt and improve microfluidic blood vessel models for use in our lab in order to create more reliable *in vitro* conditions to study leukocyte and cancer cell extravasation, thus reducing the number of animal experiments needed. So far, we have established a lab for manufacturing and operating microfluidic devices and currently we are testing suitable device designs for our extravasation studies.
*Shared first-authorship

B-31459

T cell recognition of breast cancer antigens

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Recent studies are encouraging research of breast cancer immunogenicity to evaluate the applicability of

immunotherapy as a treatment strategy. The epitope landscape in breast cancer is minimally described, thus it is necessary to identify T cell targets to develop immune mediated therapies.

This project investigates four proteins commonly upregulated in breast cancer and thus probable tumor associated antigens (TAAs). Aromatase, prolactin, NEK3, and PIAS3 contribute to increase growth, survival, and motility of malignant cells. Aspiring to uncover novel epitopes for cytotoxic T cells, a reverse immunology approach is applied. Via *in silico* screening of the protein sequences, 415 peptides were predicted as HLA-A*0201 and HLA-B*0702 binders. Subsequent *in vitro* binding analysis in a MHC ELISA platform confirmed binding for 147 of the 415 predicted binders. The 147 peptides were evaluated for T cell recognition utilizing DNA barcode labeled MHC multimers to screen peripheral blood lymphocytes from breast cancer patients and healthy donor samples. Significantly more TAA specific T cell responses were detected in breast cancer patients than healthy donors for both HLA-A*0201 ($P < 0.0039$) and HLA-B*0702 ($P < 0.001$) restricted peptides. Importantly, several of the identified responses were toward peptides that were predicted as poor or intermediate affinity binders. This is indicative of the importance of inclusion these in the search for epitopes within shared TAAs.

Thus, the inspected proteins indeed contain targets for T cell reactivity. Further research will include functional testing of peptide specific T cell cultures to validate the peptides as true T cell epitopes through demonstration of intracellular processing and presentation at the cell surface.

B-31463

Modification of hematopoietic stem cells using CRISPR/Cas9 system for investigating pathogenesis of arthritis

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Background: The role of specific components of the immune system during initiation and progression of arthritis can be studied using various mouse models. Establishing mouse models with different genetic modifications is a powerful way to test the function of the modified genes, but this the process is costly and time consuming. Enforced expression of Hox homeodomain transcription factors promotes the expansion of hematopoietic stem cells and can be used generate an unlimited source of immune cell precursors. CRISPR/Cas9 can be used to rapidly genetically modify these precursor cells, and the behavior of the modified cells can then be followed as they develop into mature immune cells both *in vitro* and *in vivo*.

Methods: Mouse hematopoietic stem cells were immortalized by retroviral delivery of Hox genes. These precursor cells were selected and transferred to irradiated mice. Reconstitution by the precursor cells was followed for several weeks. Results: A retroviral construct encoding a fusion protein of the estrogen binding domain and several different Hox genes was generated and virus particles were produced. Mouse bone marrow cells were infected with these constructs resulting in conditionally immortalize hematopoietic precursor cells. These cells have potential to differentiate into myeloid and lymphoid cells when transferred to irradiated mice. The next step is to modify these precursor cells using CRISPR/Cas9 and then transfer them to arthritic and wild type mice. This could be used to rapidly test the function of specific genes for the pathogenesis of arthritis.

Conclusion: This is a simple and unique method to immortalize mouse bone marrow cells to generate hematopoietic progenitor cell lines which can be modified and differentiated to committed progenitors upon stimuli.

B-31470

Semaphorin3A re-educates myeloid derived suppressor cells toward a pro-inflammatory phenotype

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Myeloid-derived suppressor cells (MDSCs) are immunosuppressive regulators in the tumor microenvironment and are associated with adverse clinical outcomes in most cancers. Here, we study the effect of the tumor suppressor Semaphorin3A (SEMA3A) on MDSCs by employing lentiviral mediated overexpression of SEMA3A in a mouse mammary carcinoma model. We show a decreased infiltration of gMDSCs in SEMA3A overexpressing tumors compared to controls. Moreover, gMDSCs derived from SEMA3A tumor bearing mice show induced levels of the pro-inflammatory cytokines CCL2, CCL5, CXCL9, CXCL10 and CXCL11 and decreased levels of MDSC induced gene S100A9. Interestingly, by depleting the gMDSCs, we revert the SEMA3A mediated tumor growth inhibition. We have previously shown that SEMA3A, via macrophages, recruits cytotoxic lymphocytes to the tumor microenvironment. Here, our preliminary data show a trend toward a mechanism whereby the more pro-inflammatory gMDSCs in SEMA3A overexpressing tumors