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ABSTRACT BOOK

P.B.13.16
Impact of Interleukin-22 on two murine models of lung and breast cancer

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Background: Interleukin-22 (IL-22) is a unique cytokine expressed by several immune cells and acting exclusively on interleukin-22-receptor-1 (IL-22-R1) positive non-hematopoietic cells. Recently, we have demonstrated that expression of IL-22 is found in human small and large cell lung cancer and may lead to a more aggressive disease phenotype. The mechanism, source and the role of IL-22 in lung cancer and other tumor entities like breast cancer remain unaddressed.
Methods: The expression of IL-22 and IL-22-R1 were analyzed by ELISA and qRT-PCR in two different cancer cell lines (4T1 and LCCL1). Activation of the IL-22 pathway was detected by Western blot analysis. Proliferation and migration were investigated by scratch assay and cell titer blue. Tumor tissue IL-22 content was quantified in subcutaneous cell-line derived tumors in Balb/c mice with multicolor flow cytometry.
Results: 4T1 and LCCL1 tumor cells expressed the IL-22-R1 on protein and mRNA level. Stimulation with recombinant IL-22 lead to a time dependent increase in $STAT_3$ phosphorylation on protein level. Stimulation with IL-22 significantly increased proliferation in both cell lines. Remarkably, 4T1 and LCCL1 cells in vitro did not produce or secrete IL-22. However, IL-22 was detected within the tumor microenvironment of subcutaneous tumors in different T, NK and myeloid cell subpopulations.
Conclusions: Our results show a possible role of IL-22 in murine breast and lung cancer. More in vivo data is needed of IL-22 in both models. The identification of IL-22 as a factor in breast and lung cancer progression may open new therapeutic opportunities for these diseases.

P.B.13.17
Establishment and characterization of a new cell line of leukaemia: BML01

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Introduction: Cell line BML01 was established from the bone marrow of a patient with myelodysplastic syndrome (MDS) that ineffectively produces myeloid class of blood cells is formerly known as pre-leukemia. The microscopic finding in bone marrow of the patient shows hypercellularity with hematopoietic components accounting for 40-45% of the marrow spaces and blast cells account for less than 5% mononuclear cells by morphology.
Materials and Methods: We verified the tumor properties including karyotyping, subcutaneously transplanted into NOD-SCID mice and analyzed the marker expression with flowcytometry.
Results: The BML01 cells are of a hematopoietic cell as shown by the expression of CD45 surface marker. The karyotype of BML01 is 46, xy, del (20) (q11 q13). BML01 cells posses characteristics of tumor cells based on the cell are tumorigenic in NOD-SCID mice and reduced serum requirements for in vitro cultivation. The doubling time of BML01 cell is 16 hours, and the cells have been successfully cultured in vitro for more than 50 generations. In addition, FACs analysis shows the BML01 cells also express surface molecules including CD14, CD30, CD44, CD81, CD107a, CD135, CD138, CD146, CD235 and CD274. The result of Liu's stain for the BML01 suggested the cells are mononuclear. Furthermore, the cells can be stimulated with PHA but not LPS, mitomycin or IL-2 in terms of the proliferation assay.
Conclusions: The new cell line provides us an in vitro system for the study of the development of leukemia.

P.B.13.18
Tumor lymphangiogenesis in mouse and human melanoma: New roles in immunomodulation

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Tumor-associated lymphangiogenesis is correlated with poor prognosis, increased metastasis, and resistance to treatment of cancer. In the tumor stroma, lymphatic vessels activated by VEGF-C become hyperplastic and secrete many signaling factors that affect other cell types in the tumor microenvironment. Using a lymphangiogenic melanoma model, our lab recently identified the lymphatic endothelium as a novel inhibitor of naive CD8 T cell activation in the sentinel or tumor-draining lymph node (Lund et al. Cell Reports, 2012). Here, we addressed the hypothesis that tumor-induced lymphangiogenesis promotes the development of an immune suppressive microenvironment in the primary tumor itself, driving exhaustion and tolerance of infiltrating lymphocytes. In support of this, we found that anti-lymphangiogenic therapy (in a genetically engineered mouse model of melanoma) decreased immunosuppressive cells in the tumor, including $FoxP3^+$ regulatory T cells, and cytokines, like CCL21, which we previously showed to promote T cell infiltration. Taken together, our results suggest that lymphangiogenic tumors may employ mechanisms of stromal remodeling to decrease the functionality of lymphocytes within the tumor microenvironment. Therapeutic strategies that target the immunosuppressive effect of lymphangiogenesis may be important in combination with therapeutic cancer immunotherapies.

P.B.13.19
B16F10-melanoma cells modulate effector functions of mast cells via the inhibitory gp49B-receptor

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Introduction: Melanoma represents an aggressive skin cancer caused by malignant melanocytes. Interleukin (IL)-9-producing cells - including Th9 and mast cells - were previously described to enable an effective anti-melanoma immune response regarding B16F10-tumor cells and it was also shown that mast cells were recruited to the periphery of melanomas. In addition, mast cell-derived IL-9 production depends on IRF4 and activation of mast cells via the high affinity IgE receptor (FcεRI) can be impaired by crosslinking the inhibitory gp49B-receptor.
Materials and Methods: Both, wild type or gp49B-receptor-deficient mast cells were co-cultured in vitro with B16F10-melanoma cells and production of IL-9 and IL-6 was determined by ELISA. Antibody-mediated crosslinking of the gp49B1-receptor was used to analyze the influence of this inhibitory receptor on mast cell degranulation and cytokine (IL-9, IL-6) production. The anti-melanoma activity of mast cell-derived IL-9 was analyzed using a subcutaneous B16F10-melanoma-model by determination of the tumor burden in wild type mice compared to mice bearing Irf4-deficient mast cells.
Results: We could demonstrate that IL-9 production by mast cells was significantly reduced in the presence of B16F10-tumor cells, while IL-6 production and degranulation were minimally affected. Crosslinking of gp49B1-receptor led to comparable effects suggesting that melanoma suppress mast cell-derived IL-9 via this inhibitory receptor. Employing mast cell-specific IRF4-deficient mice in a subcutaneous B16F10-melanoma model revealed enhanced tumor growth in these mice compared to littermate controls indicating the importance of mast cell-derived IL-9.
Conclusion: This study reveals a potential immune escape mechanism used by melanoma to prevent an IL-9-dependent anti-tumor response.

P.B.13.20
Production of interleukin 17 correlates with radioiodine-induced micronuclei frequency in patients with papillary thyroid cancer

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Introduction. The aim of our study was to analyze micronuclei (MN) frequency and cytokine production in patients with papillary thyroid cancer (PTC) before and 7 days after radioactive iodine (131-I) therapy. Materials and Methods. Study population included 15 patients with PTC. MN frequency was determined in peripheral blood lymphocytes using cytokinesis-block micronucleus (CBMN) assay. The concentrations of cytokines: interferon gamma (IFN-γ), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 13 (IL-13) and interleukin 17 (IL-17A) were measured in supernatants from CBMN assay using multiplex cytokine detection systems for Human Th1/Th2/Th9/Th17/Th22. Results. The mean MN frequency before 131-I therapy was 19.5 ± 5.02 MN/1000 binucleated (BN) cells, while after 131-I therapy was 26.08 ± 6.05 MN/1000 BN cells. There was no correlation of any cytokine tested with MN frequency before as well as after radioactive 131-I therapy. However, the concentration of IL-17 after 131-I therapy positively correlated with the difference in MN frequency (after minus before treatment) (bivariate correlation test, Spearman r = 0.578, p = 0.049). Conclusion. Increase in MN frequency induced by radioactive iodine therapy correlates with the production of IL-17.