Project 12: **Tumor-directed cytotoxicity of proinflammatory human dendritic cells and natural killer cells in malignant melanoma (MM)**

Principal Investigator:

Prof. Dr. Knut Schäkel, Hautklinik, Universitätsklinikum Heidelberg;

PD Dr. Adelheid Cerwenka; Boveri Research Group Innate Immunity, German Cancer Research Center

London Project Partner:

Prof. Frederic Geissmann, Center for Molecular and Cellular Biology of Inflammation, King's College, London

Short Summary:

There is good evidence that dendritic cells (DC) and natural killer (NK) cells collectively mount a strong anti-tumor immune responses. However, we have a limited understanding of how these populations crosstalk with each other and how we can exploit the NK/DC interaction for the therapy of malignant melanoma. In addition, it is unclear which subtypes of DC are most relevant for inducing anti-tumor effector functions in the presence and absence of NK cells. A recent study from the Cerwenka lab demonstrated that the activation of NK cells with inflammatory cytokines such as IL12/15/18 greatly increases the anti-tumor activity of mouse and human NK cells. The subset of DC that produces the highest levels of IL-12 was identified $CD11c^+$ slan (6-sulfo LacNAc^+) DCs by the Schäkel lab. These slanDCs are equipped with an outstanding capacity to induce pro-inflammatory immune defence functions. slanDC were shown to exert a strong antibody-dependent (ADCC) and an antibody-independent tumor-directed cytotoxicity. They efficiently enhance the NK-directed anti-tumor cytotoxicity but the underlying mechanisms are poorly understood. We hypothesize that the NK/slanDC crosstalk is highly relevant for executing cytotoxic anti-tumor responses in the skin. In the proposed project, we plan to analyze the functional consequences and mechanisms of the NK/slanDC crosstalk *in vitro* and in a xenograft mouse model of human melanoma *in vivo*. In addition, we will analyse the presence of slanDC and NK cells within biopsies of melanoma patients after treatment with inflammatory agents such as application of the TLR-7 ligand Imiquimod. The results gained in this project could lead to novel therapeutic strategies for the treatment of MM based on the exploitation of the slanDC/NK crosstalk.

3 State of the Art

3.1 State of knowledge in the field

The number, the type and the activation status of tumor-associated DCs and NK cells have been shown to be of direct prognostic value. Malignant melanoma cells often express low levels of MHC class I and high levels of activating NK cell ligands, and are therefore very efficiently killed by NK cells. In general, low numbers of NK cells are found in solid tumors but it has been shown that inflammatory agents such as the application of the TLR ligand CpG can facilitate NK cell infiltration into mouse solid tumors. DCs can mount a potent direct cytotoxic anti-tumor response. Cytotoxic DCs were shown to take the lead in inducing a cytotoxic anti-tumor response when MM were treated with the TLR7-ligand imiquimod. Furthermore, the antibody-dependent cell-mediated cellular cytotoxicity (ADCC) of DCs and NK cells contributes to the natural tumor surveillance and may significantly enhance tumor destruction in monoclonal antibody-based immunotherapies with e.g. trastuzumab.

3.2 Preliminary work by the participants

Schäkel lab : We identified the population of slan (6-sulfo LacNAc^+) DCs in humans (Schäkel et al., 2002). slanDCs are a population of proinflammatory DCs that stand out by their high level production of IL-12, IL-23, TNF-α and IL-1ß in response to TLR7 and TLR8 ligation (Hänsel et al. 2011 and 2012). slanDCs have a marked tumor-directed ADCC, and strongly enhance the cytotoxic capacity of NK cells (Schmitz et al., 2005). The role of proinflammatory or cytotoxic slanDCs in tumor tissue and their local interplay with NK cells has not been investigated so far. *Cerwenka lab:* Our previous study (Ni et al, JEM 2012) revealed that adoptive transfer of NK cells that were preactivated with IL12/15/18 resulted in greatly increased anti-tumor activity in mouse

models of RMA-S lymphoma and B16 melanoma compared to NK cells pretreated conventionally with IL15 or IL2. Importantly, human NK cells activated with IL12/15/18 also displayed sustained effector function and higher cell recoveries. To investigate the *in vivo* anti-tumor activity of human NK cells, we have established a mouse xenograft model in which luciferase-expressing human melanoma cells can be imaged *in vivo* after injection into NSG mice. In addition, our lab has a long-standing expertise in the investigation of activating receptors expressed by NK cells such as NKG2D and NKp30 and their ligands expressed by tumor cells (Textor et al, Cancer Res, 2011).

4 Project Plan

4.1 Specific Aims

1. To determine the anti-melanoma activity induced by slanDCs/NK cell crosstalk *in vitro.*
2. To determine the melanoma-directed cytotoxicity induced by slanDCs/NK cell crosstalk in a xenograft mouse model *in vivo.*
3. To determine the presence and the activation state of proinflammatory slanDCs and NK cells in MM biopsies with and without treatment with the TLR7 ligand Imiquimod.

4.2 Experimental program

1. We will establish co-cultures of NK cells and DC with a focus on slanDCs. The crosstalk between these cells types in the absence and presence of TLR ligands will be assessed by cytotoxicity assays using melanoma cell lines that are well established in our laboratory as targets. Further experiments will assess the molecules involved in the NK/slanDC crosstalk (cell surface and soluble factors).
2. Next we will investigate the consequences of the NK/slanDC crosstalk in a xenograft model of MM. Co-cultures will be established *in vitro* and primed NK cells (or DC) will be adoptively transferred into melanoma-bearing mice. Tumor growth and functional parameters of the transferred cell populations will be monitored. In order to fully activate slanDCs, melanomas will be treated with Imiquimod.
3. We will conduct a comprehensive analysis to detect slanDCs and the NK cells by Tissue-FAX in tumor tissues. We will carefully determine the expression of parameters that provide information about the activation status (iNOS, TNF-α) and the maturational stage of slanDCs (CD83 versus CD206). The Heidelberg Tumor Registry will provide us with requisite tissue arrays. We will focus here on melanoma but will compare the obtained data with studies on SCC and basal cell carcinomas. Attention will be paid to spontaneously regressing tumors, and tumors regressing following TLR7-treatment.

4.3 Collaborations with other projects in the RTG

For the evaluation of proinflammatory DC and NK cells in melanoma cells we will closly collaborate with project 13 (on myeloid cell subsets) and project 11 (on cellular recruitment by chemokines).

5 References

1. Ni J, Miller M, Stojanovic A, Garbi N, **Cerwenka A.** 2012. Sustained effector function of IL-12/15/18 preactivated NK cells against established tumors. *J Exp Med* 209:2351-65.
2. Textor S, Fiegler N, Arnold A, Porgador A, Hofmann TG, **Cerwenka A**. 2011. Human NK cells are alerted to induction of p53 in cancer cells by up-regulation of the NKG2D-ligands ULBP1 and ULBP2, *Cancer Res* 71:5998-6009.
3. Hansel A, Gunther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, Meurer M, Rieber EP, **Schäkel K**. 2011. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong T(h)17/T(h)1 T-cell responses. *J. Allergy Clin Immunol* 127:787-794.
4. **Schäkel K**, von Kietzell M, Hänsel A, Ebling A, Schulze L, Haase M, Semmler C, Sarfati M, Barclay AN, Randolph GJ, Meurer M, Rieber EP. 2006. Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. *Immunity* 24:767-777.
5. Schmitz M, Zhao S, Deuse Y, **Schäkel K**, Wehner R, Wohner H, Holig K, Wienforth F, Kiessling A, Bornhauser M, Temme A, Rieger MA, Weigle B, Bachmann M, Rieber EP. 2005. Tumoricidal potential of native blood dendritic cells: direct tumor cell killing and activation of NK cell-mediated cytotoxicity. *J Immunol* 174:4127-4134.