Project 11: **Characterization and modulation of CC-chemokine receptor 6 (CCR6) - mediated immunosurveillance in malignant melanoma**

Principal Investigator:

Dr. Anke S. Lonsdorf, Prof. Dr. Alexander H. Enk, Department of Dermatology; Medical Faculty Heidelberg, Ruprecht-Karls-University Heidelberg

London Project Partner:

Prof. Fran Balkwill, Centre for Cancer and Inflammation, Barts Cancer Institute, London

Short Summary

The general objective of this research proposal is to evaluate the distinct functional contribution of CC-chemokine receptor (CCR) 6 interactions with its cognate ligand CCL20 in modulating specific cellular anti-melanoma immune responses. The relevance of the CCR6/CCL20 axis for the immune control of melanoma at the primary tumor site and the skin draining lymph nodes (LNs) will be studied in two established murine models: 1) the B16 transplanted melanoma model in C57BL/6 (WT) and CCR6 knockout (KO) mice and 2) the spontaneous melanoma model in Ret-transgenic mice as well as human melanoma tissue samples. An increased understanding of the functional interplay between CCR6/CCL20-guided pathways in the local control of melanoma and draining LNs may allow for the identification of novel therapeutic strategies on a molecular level.

3 State of the Art

3.1 State of knowledge in the field

Chemokines, a family of small, secreted molecules, and their cognate G-protein-coupled receptors play an essential role in the elicitation of specific immune responses, particularly directed compartment-specific migration of immune and tumor cells (i.e. chemotaxis). CCL20 and the anti-microbial peptide ß-defensin expressed in the epidermis are a potent impetus for the recruitment of subsets of dendritic cell (DC), B-cells and memory T cell subsets expressing CCR6, its exclusive receptor. In addition to its constitutive expression the epidermis, CCL20 and a CCR6- expressing immune cell infiltrate has been detected in several malignancies, including melanoma. Yet, the functional contribution of the CCR6/CCL20 axis in the immune control of melanoma remains controversial: While CCR6/ CCL20 interactions have been found to support anti-melanoma immune responses in a murine model of lung metastasis, tumor-derived CCL20 has been reported to promote tumor growth and immune escape; partially by recruiting subsets of tolerogenic immature DC or regulatory T cells. Analysis of the kinetics and distribution of CCR6-guided immune cell subsets and their functional contribution for the immune control of melanoma comprises the focus of this research proposal. Furthermore, stimuli by which the expression of CCR6 ligands may be modulated at the tumor site and/ or the skin-draining LN are poorly understood and their potential relevance for anti-tumoral immune responses warrants further investigation.

3.2 Preliminary work by the participants

Our group has a long-standing interest in studying mechanisms of compartment-specific trafficking of melanoma and immune cells with a particular focus on the role of chemokine interactions. In previous studies we provided evidence that CCR6 expression on DC subsets and effector T cells vitally contributes to the elicitation of skin modulating immune responses by directing their recruitment to sites of enhanced CCL20 expression (Hedrick and Lonsdorf et al., 2009). We have also demonstrated that small-molecule activators, such as toll-like receptor (TLR)-activating microbial products, support the formation of protective antitumoral immune responses in the skin (Lonsdorf et al., 2003) and that the accumulation of both, epidermal chemokines and CCR-bearing immune cells in skin-draining LNs may be amplified by topically applied immunmodulators *in vivo* (Chien et al., 2009; Huang et al., 2008).

4 Project Plan

4.1 Specific Aims

1. Characterization of CCR6/CCL20-dependent anti-tumor immune responses in primary malignant melanoma and skin-draining LNs in murine models of melanoma.
2. Identification of local immunmodulatory factors with functional relevance for CCR6/CCL20-mediated anti-melanoma immune responses in primary tumors and skin-draining LNs.
3. Correlation of CCR6/CCL20 expression patterns in primary human melanoma and LN metastasis with respect to disease stage and local tumor progression.

4.2 Experimental program

1. **A)** Luciferase-transduced murine B16 melanoma (B16-luc) will be injected s.c. into syngeneic WT and CCR6 KO mice. Kinetics of skin tumors and LN metastases will be monitored in vivo (calliper, bioluminescence analysis). Additionally, tumor burden and melanoma metastasis will be quantified ex vivo by exploiting a bioluminescence reporter system. .Immune cell infiltrates, tumor vascularisation and the predominant chemokine/CCR and cytokine profiles will be analysed (immunohistochemistry (IHC), FACS, RT-PCR) in primary transplanted B16-luc melanomas and corresponding skin-draining LNs. Results will be validated in spontaneously arising melanomas in Ret-transgenic mice **B)** Subsets of CCR6-expressing immune cells from tumor-bearing WT mice will be adoptively transferred into CCR6 KO mice before/ after B16-luc inoculation. Tumor kinetics and LN metastasis, tumor-homing capabilities and alterations in the tissue microenvironment will be monitored in primary melanomas and LNs as described above and by in vivo multiphoton microscopy.
2. **A)** B16-luc overexpressing CCL20 (B16-luc-L20) and appropriate empty vector control cells will be injected s.c. into WT and CCR6 KO mice to study local immune cell infiltrates, tumor vascularisation and cytokine profiles (IHC, FACS RT-PCR, ELISA). Also, melanoma cells derived from B16-luc-L20 primary melanoma will be analysed for mechanisms of apoptosis resistance **B)** The effect of topical immunmodulators (i.e. TLR7- agonist imiquimod, DNCB) on CCL20/ ß –defensin expression, immune cell recruitment and tumor kinetics will be studied within the experimental setting and methods described above in B16-luc transplanted melanomas and melanomas of Ret-mice. Also, the effects of intratumoral injections of a blocking anti-mCCL20 antibody will be studied in both murine models.
3. Paraffin embedded human primary melanoma and LN metastases will be analyzed by IHC for CCL20 and ß -defensin expression as well as phenotype and distribution of an (CCR6-expressing) immune cell infiltrate. Correlation with pathomorphological patterns, disease stage and tumor progression will be performed.

4.3 Collaborations with other Projects within the RTG

Our project will utilize bioluminescence imaging (Project 7), multiphoton microscopy (Project 6) and the Ret-mouse model system (Project 13) in close collaboration. Mechanisms of apoptosis resistance in B16-luc-L20 melanoma will be studied with partners of project 10.

5 References

1. **Lonsdorf AS**, Kraemer BF, Fahrleitner M, Schoenberger T, Gnerlich S, Ring S, Gehring S, Schneider SW, Kruhlak MJ, Meuth SG, Nieswandt B, Gawaz M, Enk AH, and HF Langer. 2012. Engagement of αIIbβ3 (GPIIb/IIIa) with αvβ3 mediates interaction of melanoma cells with platelets - a connection to hematogeneous metastasis. *J Biol Chem* 287:2168-2178.
2. Chien AJ*, Moore EC**, Lonsdorf AS**, Kulikauskas RM, Rothberg BG, Berger AJ, Major MB, Hwang ST, Rimm DL, Moon RT. 2009. Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. *Proc Natl Acad Sci USA* 106:1193-1198. **equal contribution, alphabetical order*
3. Hedrick MN*, **Lonsdorf AS***, Shirakawa AK, Lee CCR, Liao F, Singh SP, Zhang HH, Love PE, Hwang ST, Farber JM. 2009. CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. *J Clin Invest* 119:2317-2329. **equal contribution, alphabetical order*
4. Huang V, **Lonsdorf AS**, Fang L, Kakinuma T, Lee VC, Cha E, Zhang H, Nagao K, Zaleska M, Olszewski WL, Hwang ST. 2008. Cutting edge: rapid accumulation of epidermal CCL27 in skin-draining lymph nodes following topical application of a contact sensitizer recruits CCR10-expressing T cells. *J Immunol* 180:6462-6466.
5. **Lonsdorf AS**, Kuekrek H, Stern BV, Boehm BO, Lehmann PV, Tary-Lehmann M. 2003. Intratumor CpG injection induces protective antitumor T cell immunity. *J Immunol* 171:3941-3946