Project 13: **Regulation of tumor-associated macrophage and myeloid-derived suppressor cell activation and its neutralization in transgenic mouse melanoma model**

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Short Summary

Melanoma immunotherapy is not satisfactory due to the accumulation of chronic inflammatory factors and immunosuppressive myeloid cells such as tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC) in tumor lesions. The goal of the project is to better understand the molecular mechanisms underlying the inhibition of anti-tumor immune responses mediated by TAM and MDSC, and to design novel therapeutic strategies targeting these myeloid cells in melanoma. We will study the role of signaling molecules (p38 MAPK and S100A8/A9) and microRNA in the capacity of TAM and MDSC to inhibit anti-tumor reactivity of T cells. Results of the project will help to develop novel efficient human melanoma treatments neutralizing immunosuppression in the tumor microenvironment.

3 State of the Art

3.1 State of knowledge in the field

Despite the intrinsic melanoma immunogenicity, immunotherapeutic trials were not satisfactory due to the formation of a complex immunosuppressive network mediated by chronic inflammation developing in the tumor microenvironment. Such microenvironment (represented by various cytokines, chemokines and growth factors) can induce a recruitment and expansion of suppressive immune cells such as TAM and MDSC to the tumor site. It has been shown that the expansion and activation of MDSC and TAM requires several signaling pathways (p38 MAPK as well as S100 calcium-binding protein A8 and A9). Small non-coding RNAs designated as microRNAs (miR) has been recently shown to significantly regulate the accumulation and function of TAM and MDSC. Thus, miR-494 can be induced in MDSC by tumor-derived TGF-β supporting immunosuppression and metastasis. On the other side, miR-511-3p was reported to modulate genetic program of TAM limiting their protumoral functions. Transgenic mice overexpressing human receptor tyrosine kinase Ret in melanocytes spontaneously develop skin melanoma with metastases in lymph nodes, lungs, liver, brain, and bone marrow and can be used for studying melanoma progression *in vivo*.

3.2 Previous/preliminary work by the participants

To address the role of MDSC in melanoma progression in clinically relevant conditions, we used the ret transgenic mouse model, which mimics human melanoma with respect to clinical development ensuring natural tumor-stroma interactions. Analyzing Gr1+CD11b+ MDSC in melanoma lesions and lymphatic organs revealed a remarkable elevation of MDSC frequencies. Moreover, we found increasing concentrations of IL-1β, VEGF, IL-6 and GM-CSF in tumors during their progression. MDSC enrichment was accompanied by a decrease in TCR ζ-chain expression in tumor-infiltrating T cells. Therapy of melanoma-bearing mice with the phosphodiesterase-5 inhibitor sildenafil led to decreased numbers and immunosuppressive function of MDSC as reflected by the restoration of ζ-chain expression and significantly increased mouse survival. In addition, we identified in subcutaneous murine melanomas TAM that eco-express not only stabilin-1 and LYVE-1 but also the novel surface marker MS4A8A, a molecule involved in differentiation processes. *In vitro*, these TAM were selectively induced via activation of the p38 MAPK and

glucocorticoid signaling pathways, indicating an important role of the p38 MAPK pathway also for the regulation of TAM activity.

4 Project Plan

4.1 Specific Aims

Aim 1: Investigating signaling molecules and miR involved in the recruitment, expansion and activation of TAM and MDSC during melanoma progression

Aim 2: Studying the neutralization of immunosuppression induced by TAM and MDSC using the modulation of their relevant signaling pathways and miR

4.2 Experimental program

1. We will test the expression and activation (phosphorylation) of such signaling molecules as S100A8/A9 and p38 MAPK in F4/80^{high}CD11b^{+}Gr1^{-} TAM and CD11b^{+}Gr1^{+} MDSC in melanoma lesions and lymphoid organs using FACS. Differentially up- and down-regulated miR in TAM and MDSC will be studied by Affymetrix Microarray analysis and real-time quantitative PCR. Functional relevance of identified miR will be analyzed using lentiviral constructs. TAM and MDSC will be characterized by the expression of arginase-1 and inducible nitric oxide synthase. The activity of these myeloid cells will be determined by the inhibition of T cell proliferation upon the co-culture with TAM and/or MDSC. Inflammatory factors (VEGF, IL-1β, IL-6, TNF-α, TGF-β, etc.) will be detected in melanoma lesions by multiplex technology. Tumor-infiltrating T cells will be validated measuring the ζ-chain expression by FACS.
2. We will suppress signaling pathways involved in the expansion and activation of MDSC and TAM with specific inhibitors p38 MAPK (SB203580 and RO3201195). Activating miR will be blocked by the sponge preventing the interaction of this miRNA with its targets. TAM or MDSC isolated from melanoma lesions will be treated with these inhibitors followed by co-incubation with activated syngeneic T cells. T-cell proliferation and ζ-chain expression will be tested by FACS.

4.3 Collaborations with other projects in the RTG

For the evaluation of the recruitment and activation of TAM and MDSC in the melanoma microenvironment, we will closely collaborate with project 11 (CCR6/CCL20 interaction) and project 12 (proinflammatory dendritic cells).

5 References

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