Project 5: **Function of the mucin-like glycoprotein podoplanin in squamous cell carcinoma progression**

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Short Summary

The mucin-like glycoprotein podoplanin (PDPN) represents a tumor-associated protein in a variety of tumors including squamous cell carcinoma of the skin in mouse and human and correlates with poor prognosis and metastatic risk. Overexpression of PDPN in pancreatic cancer promoted tumor cell invasion and affected migration of glioblastoma and keratinocyte cell lines via modulation of the cytoskeleton pointing to a fundamental role of PDPN in tumor cell migration and invasion. We will i) measure transcriptional control of PDPN by altered cell death pathways promoting SCC formation and ii) define the *in vivo* function of PDPN in SCC. Here, we will apply loss-of-function approaches in cultured human SCC cells with metastatic potential and measuring parameters of cell invasion and actin cytoskeleton. Importantly, we will make use of floxed PDPN mice recently generated in our lab to specifically delete this gene in keratinocytes via K14-Cre transgenic mice. To unequivocally define the function of PDPN in tumor development and progression, the well established chemically induced *in vivo* skin carcinogenesis protocol will be applied to such mice and tumor cell proliferation, migration and invasion will be determined.

3 State of the Art

3.1 State of knowledge in the field

Non-melanoma skin cancer, such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) is a very common malignancy. SCC as a solid tumour is composed of transformed epidermal keratinocytes with a highly invasive growth and tendency to metastasize. Both *in vitro* and *in vivo* model systems demonstrated that malignant transfor-mation of epidermal cells is a multistage process, in which stepwise accumulation of genetic and epigenetic events determines the transition from normal to malignant cellular state. However, the onset and the order of genetic alterations that lead to development of most sporadic cancers remain undefined. Mouse skin carcinogenesis has been an important tool for developing the current concepts regarding human neoplasia and the multistage nature of tumour development and progression. In fact, some types of mutation in oncogenes and tumour suppressor genes identified in mouse skin models also occur in human epithelial cancers. One of the best-defined experimental *in vivo* systems for epithelial cancer develop-ment is the chemically induced tumour model of mouse back skin. Treatment of the skin with the carcinogen 7,12-dimethylbenz-[a]-anthracene (DMBA) and the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) result in the formation of benign papillomas (PAPs) and malignant tumours (SCCs). Using this model, the timing of genetic and chromosomal alterations, as well as the cellular crosstalk between epithelial cells and cells of the microenvironment (e.g. fibroblasts, endothelial cells and immune cells) that take place during the different stages of tumour development and progression can be studied.

3.2 Previous work by the participants

Using the DMBA/TPA model the central contribution of signal transduction pathways funnelling into transcription factor AP-1 (Fos/Jun) to premalignant conversion and malignant progression of epidermal cells was described. Using gene expression profiling from specimens of TPA-treated back skin and benign and malignant tumours derived from the DMBA/ TPA model we have identified novel TPA-inducible genes in mouse skin including the mucin-like glycoprotein podoplanin (PDPN). We identified *pdpn* as a direct c-Fos target gene being part of a Fos-dependent genetic program in both the DMBA/TPA and the genetic *K5-SOS-F* transgenic skin tumor model exhibiting expression in tumor cells, particularly at the tumor-stroma border. Despite the i) correlation between *pdpn* expression and tumor cell invasion, malignant progression and

metastasis in mouse tumor models, as well as poor prognosis and metastatic risk in human cancer, and ii) accelerated cell motility and invasion *in vitro* and induced tumor growth in a xenograft model upon ectopic Pdpn, the role of PDPN in SCC formation and progression have not been addressed.

4 Project Plan

4.1 Specific Aims

This project will apply a loss-of-function approach to define the function of PDPN in SCC formation and progression *in vitro* and *in vivo*.

- apply siRNA and CRISPR/Cas9 technologies to abolish PDPN expression in SCC cell lines to measure cell proliferation, migration and invasion
- generate mice lacking PDPN expression in keratinocytes to define the impact of *pdpn* deletion on skin homeostasis, hyperplasia and tumor development and progression

4.2 Experimental program

1. We will use well-known HaCaT cell lines harbouring additional Ras mutations, which exhibit high invasive capacity *in vitro* in 3D skin equivalent models (organotypic cultures). We will use this system to introduce both a *pdpn* siRNA producing lentiviral vector (available in the lab) and CRISPR/Cas9-mediated mutagenesis leading to significant reduction and complete loss of PDPN expression, respectively, in the tumor cells. PDPN compromised cells will be analyzed for i) cell proliferation, ii) (trans) migration and iii) invasion through matrigel matrix and in 3D organotypic cultures
2. We have recently generated floxed *pdpn* mice, in which we already confirmed efficient deletion of *pdpn* sequences via Cre recombinase technology. Crossing these mice with K14-cre mice, applying full thickness wound healing conditions (which provoke massive expression of pdpn in epithelial cells at the leading edge) Pdpn expression is completely abolished *in vivo* in keratinocytes. In the present project, we will apply short-term TPA treatment on mouse skin (known to strongly induce *pdpn* expression in basal layer keratinocytes) to evaluate the role of Pdpn in skin hyperplasia. In addition, we will apply the DMBA/TPA protocol of chronic TPA treatment to induce papillomas and subsequently SCC in WT and PDPN KO mice. Both tumor incidence and tumor volume will be determined. Tumors will be harvested and characterized by indicative immuno-histochemical analysis to define the nature of the tumor cells (particularly at the tumor-stroma border) including their ability to execute EMT.

4.3 Collaborations with other Projects in the RTG

We will provide expertise of the chemical- induced skin carcinogenesis model to projects 3 and 9 and collaborate with both projects on transcriptional control of PDPN by the crucial SCC regulator Wnt (project 3) and by Ripoptosome-associated cell death pathways (project 9).

5 References

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