Project 10: **IAP antagonists: A novel therapeutic option to overcome cell death resistance in malignant melanoma?**

Principal Investigator:

Dr. Peter Geserick, Prof. Dr. Martin Leverkus, Dept. of Dermatology, Medical Faculty Mannheim, Heidelberg University

London Project Partner:

Prof. Henning Walczak, Cell Death and Inflammation Laboratory, Cancer Institute, University College London

Short Summary

Acquisition of cell death resistance is a critical step during skin tumorigenesis. Inhibitor of apoptosis proteins (IAPs) are negative regulators of caspase-dependent apoptotic (e.g. X-linked IAP) and receptor interacting protein 1 (RIP1)-dependent necroptotic (e.g. cIAPs) cell death induced by death receptors. The caspase-8 inhibitor cFLIP is upregulated during tumor progression in malignant melanoma, and protects tumor cells from receptor-mediated cell death. Notably, the short isoform of cFLIP ($cFLIP_S$) protects cells from apoptotic but not necroptotic cell death. This project will study how different IAPs regulate the diverse forms of cell death in malignant melanoma. Therefore, we will investigate (1) how cIAPs regulate the quality and quantity of cell death in cultured malignant melanoma cells; (2) the function of different components of intracellular death pathways and the impact of cFLIP isoform expression on apoptotic and necroptotic cell death and; (3) the potential of IAP antagonists for tumor suppression in xenograft mouse models. These studies will functionally validate the role of cFLIP isoforms in conferring resistance to different forms of cell death, and will elucidate how this relates to the overall therapeutic response in malignant melanoma.

3 State of the Art

3.1 State of knowledge in the field

Activation of death receptor (DR)-induced apoptosis transmitted by cytokines such as TRAIL and CD95L is a required mechanism for elimination of unwanted and transformed cells. This process is highly controlled by antiapoptotic proteins that control caspase activity initiated either by DRs (cFLIP inhibits Caspase 8) or intracellularly (e.g. XIAP blocks caspase-9 and 3). In contrast, cIAPs inhibit necroptosis by regulation of a RIP1-controlled signalling platform. Suppression of such cell death responses mediated by upregulation of cell death inhibitory proteins during tumor progression may confer therapeutic resistance in malignant melanoma. Melanoma cells are known to be highly resistant against death ligand-mediated cell death. Inhibition of this cell death resistance by combined activation of DRs and suppression of IAP activity using the respective antagonists could represent a promising novel anti-cancer strategy for malignant melanoma. IAP antagonists suppress the caspase-inhibitory function of XIAP and additionally promote rapid degradation of cIAP1 and 2. The consequences of these activities of IAP antagonists are either increased apoptosis induction by effector caspase-3 activity, or promotion of apoptosis or necroptosis initiated by activation of the RIP1/RIP3 signalling machinery. These interesting molecular processes could be of critical relevance to overcome cell death resistances in melanomas and therefore for tumor suppression.

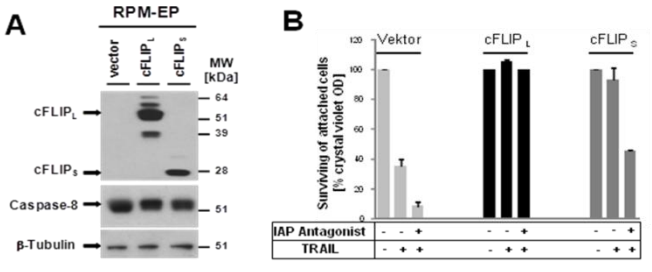

Figure 1. A-B. Overexpression of $cFLIP_L$ but not of $cFLIP_S$ (A; western blot) protect cells against IAP antagonist induced necroptosis (B). B. Melanoma cell transduced by retroviruses containing either $cFLIP_L$ or $cFLIP_S$ cDNA were stimulated for 1 h with IAP antagonist and/or treated with 50 ng/ml TRAIL for 18-24 h. Viability of cells were analyzed by crystal violet assay.

3.2 Previous work by the participants

Over the past years our group intensively worked on the identification of resistance mechanisms in skin tumors. We identified the critical role of cFLIP for DR-induced cell death in melanoma and the role of IAPs for regulation of necroptotic cell death responses. We further demonstrate necroptosis

induction in transformed, but not primary cells when IAP function is suppressed. Analysis of these signalling pathways in melanoma cells demonstrated a substantial upregulation of cFLIP and IAPs, and concomitant increased resistance to death ligand-mediated cell death in malignant melanoma cells. Intriguingly, in the presence of IAP antagonists, we were able to overcome cell death resistance and increase sensitivity to TRAIL, indicative of the indispensable role of IAPs for cell death resistance in melanoma cells. A more precise role of cFLIP in the regulation of DR-induced cell death was shown by cFLIP overexpression. In line with our previous studies, both cFLIP isoforms were able to block TRAIL-induced apoptosis in melanoma. However, only $cFLIP_S$ promotes necroptotic cell death responses in the presence of IAP antagonists (Figure 1). These data are indicative of the critical but differential role of cFLIP isoforms in cell death regulation.

4 Project Plan

4.1 Specific Aims

The general aim of the project is to study the mechanistic relevance of IAPs and cFLIPs for resistance of DR-induced cell death in melanoma cells and the role of IAP antagonists as a potential therapeutic for melanoma treatment. For this purpose, we will analyze expression of IAPs and cFLIP isoforms and check the sensitivity of cells to TRAIL in the presence or absence of IAP antagonists from in a set of melanomas representing different tumour stages, and in melanoma genetically modified with downregulation or overexpression of IAPs and cFLIPs. The growth and metastasis of melanomas expressing cFLIP isoforms upon xenotransplantation into immune deficient nude mice will be assessed. Finally, the ability of a combination therapy of IAP antagonist/DL to treat established tumors in a xenograft mouse model will be analyzed.

4.2 Experimental program

A repertoire of melanoma cells representing different tumour progression stages will be analysed for expression of cFLIP and IAP proteins (XIAP, cIAP1/2) as well as of their counteracting protein molecules (caspases, RIP1/RIP3, MLKL) using various biochemical approaches (immunoblot, qPCR). The determination of the quality and quantity of cell death initiated by treatment with TRAIL or CD95L will be analyzed using crystal violet and PI/AnnexinV assay, and fluorescence microscopy. The relevance of the respective proteins (cFLIP, IAPs) involved in cell death resistance in melanomas will be investigated by genetic manipulation of the endogenous expression levels (siRNA, lentiviral shRNA, retroviral expression of cFLIP) followed by analysis of the quality and quantity of cell death upon combined IAP antagonist and DL treatment. To investigate the role of cFLIPs and IAPs for tumor growth and cell death resistance, appropriate established and biochemically- characterized melanoma cells will be xenotransplanted into immune deficient mice. Tumor growth and metastasis as well as the effect of IAP antagonists in combination with DL (TRAIL, CD95L) for cell death sensitivity and for tumor suppression will be investigated in mice with established melanoma in vivo following DL and IAP antagonist treatment.

4.3 Collaborations with other Projects in the RTG

Cooperation with project 4 will allow us to perform optimal knockdown conditions in large-scale but also in small scale for specific proteins (cFLIP, IAPs) in melanoma. Cooperation with project 2 will enable us to perform experiments with xenograft mouse models. We will share our expertise in the field of cell death signalling in vitro and in vivo with projects 3, 5, 8, and 11.

5 References

1. Feoktistova M*, **Geserick P***, Kellert B, Panayotova-Dimitrova D, Langlais C, Hupe M, Cain K, MacFarlain M, Häcker G, **Leverkus M.** 2011. cIAPs block Ripoptosome formation, a RIP1/caspase 8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. *Mol Cell* 43:449-63 *(* equal contribution)*
2. **Geserick P**, Hupe M, Moulin M, Wong WW, Feoktistova M, Kellert B, Gollnick H, Silke J, **M Leverkus**. 2009. Cellular IAPs inhibit a cryptic CD95-induced cell death by limiting RIP1 kinase recruitment. *J Cell Biol.* 187:1037-1054.
3. **Geserick P,** Drewniok C, Hupe M, Haas TL, Diessenbacher P, Sprick MR, Schon MP, Henkler F, Gollnick H, Walczak H, **Leverkus M.** 2008. Suppression of cFLIP is sufficient to sensitize human melanoma cells to TRAIL or CD95L-mediated apoptosis. *Oncogene* 27:3211-3220.