Marc-Andrea Bärtsch
Dr. med.

MicroRNA-Mediated Multi-Tissue Detargeting of Oncolytic Measles Virus

Fach/Einrichtung: NCT (Nationales Centrum für Tumorerkrankungen)
Doktorvater: Prof. Dr. med. Christof von Kalle

The ability of oncolytic viruses to lyse cancer cells and elicit anti-cancer immune responses is based on their preferential infection of and replication in cancer cells. Oncolytic measles virus (MV) developed from vaccine strains enters host cells predominantly via the complement regulatory protein CD46 that is typically overexpressed on cancer cells but is found on the surface of all nucleated cells of the human body. Defective antiviral response pathways in many cancer cells then allow for enhanced MV replication. Recently, a targeting system based on the insertion of synthetic microRNA target sites (miRTS) into the MV genome has been established and demonstrated to increase cancer specificity. Thereby viral replication is placed under the control of host cell microRNAs, many of which are overexpressed in normal compared to cancer cells.

The hypothesis of this study was that miRTS for multiple different microRNAs can be combined to increase tumor specificity of oncolytic MV by reducing replication in multiple off-target tissues without compromising oncolytic efficacy. microRNAs overexpressed in tissues at risk for toxicity during systemic virotherapy of pancreatic cancer were identified. miR-122 was selected for liver detargeting, miR-7 for detargeting of neuronal and neuroendocrine tissues and miR-148a and miR-375 were selected for detargeting of the pancreas. miRTS-boxes were designed and inserted into the viral *F* and *H* genes. A multi-tissue detargeted MV (MV-EGFP^{mtd}) carrying a miRTS-box for miR-148a in the *F* gene 3'UTR and miRTS-boxes for miR-122 and miR-7 in tandem in the *H* gene 3'UTR was generated and characterized.

microRNA-sensitivity of MV-EGFP^{mtd} was analyzed *in vitro* in the MV producer cell line Vero transfected with cognate pre-microRNAs. Quantitative real-time PCR showed that miR-122 levels in Vero cells transfected with pre-miR-122 were in the same range as in primary human hepatocytes. Viral spread and progeny production were analyzed by fluorescence microscopy and titration of virus progeny particles. Attenuation of MV-EGFP^{mtd} was

observed in Vero cells transfected with pre-microRNAs but not in mock transfected cells as compared to control MVs either harboring a reverse complementary or no miRTS-box. Immunoblotting revealed reduced protein expression of genes harboring miRTS-boxes in presence of the cognate microRNAs. Sensitivity to endogenous microRNA was analyzed *ex vivo* in primary human hepatocytes and primary human liver slices, which are known to express high levels of miR-122. Attenuation of MV-EGFP^{mtd} as compared to both control viruses was observed by fluorescence microscopy and titration of virus progeny particles. The extent of attenuation differed between microRNAs but also between different positions of miRTS for the same microRNA used in this and in other studies. In future studies, candidate microRNAs and different miRTS positions in the MV genome should therefore be evaluated systematically to maximize attenuation in off-target tissues. On the other hand, emphasis should be placed on investigation of microRNA detargeting in relevant toxicity models such as animal models and primary human cells and tissues to define the biologically relevant degree of attenuation.

Oncolytic efficacy of MV-EGFP^{mtd} was analyzed *in vitro* in pancreatic cancer (PC) cell lines (BxPC-3, MIA PaCa-2 and T3M4) and *in vivo* in a murine xenograft model of PC. Cytotoxicity of MV-EGFP^{mtd} and both control viruses was comparable in PC cell lines as measured by XTT cell viability assays. Multi-step growth curves in BxPC-3 cells revealed comparable growth kinetics of MV-EGFP^{mtd} and both control viruses. Finally, immunodeficient *NOD-SCID* mice harboring subcutaneous BxPC-3 xenograft tumors were treated with intratumoral injections of MV-EGFP^{mtd}, a control virus or carrier medium only. Both groups with virus treatment demonstrated significantly delayed tumor growth and prolonged overall survival. No statistically significant differences between groups treated with MV-EGFP^{mtd} and control virus were observed. Thus, MV-EGFP^{mtd} mediated oncolysis was comparable to the parental MV-EGFP in models of PC *in vitro* and *in vivo*.

The work presented here is proof of concept that favorable expression profiles of multiple microRNAs can be exploited simultaneously to protect off-target tissues without compromising oncolytic efficacy of MV. By establishment of additional miRTS positions in the MV genome and combination with other targeting modalities cancer-specificity of oncolytic MV can be further increased. The concept of microRNA-mediated multi-tissue detargeting of MV established in this work is one element for future clinical development of safe and effective oncolytic virotherapy.