Cover Page

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ADDENDUM

SUMMARY
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SUMMARY

Recombinant viral vectors hold great promise in the field of cancer gene therapy. While a plethora of viruses is being evaluated as oncolytic agents, human adenoviruses of serotype 5 (HAdV-5) are among the most popular of viruses to be developed. Although clinical studies have demonstrated safety of cancer gene therapy with HAdV-5-derived vectors, the efficacy still needs further enhancement. Several factors have been identified that limit the anti-tumor efficacy, as reviewed in **Chapter 2** of this thesis. One major bottleneck is the inadequate penetration and spread of the virus within the tumor. This is attributable, at least in part, to the low or heterogeneous expression of the coxsackie and adenovirus receptor (CAR) on the tumor cells. This thesis describes the development and preclinical evaluation of novel tumor-targeted HAdV-5 vectors, through implementing the genetic fusion of capsid proteins (protein IX and fiber) with a variety of tumor-targeting polypeptides.

Chapters 3, 4, and 5 describe the usability of the HAdV-5 minor capsid protein IX as a locale for genetically fusing tumor targeting ligands. At first, proof-of-principle is described of fusing protein IX with hyper-stable single-chain antibody fragments (scFv's) (**Chapter 3**). Hyper-stable scFv's are anticipated to be highly suitable for incorporation in adenovirus capsids, as a result of their ability to fold correctly in the reducing environment of the cytoplasm. A hyper-stable scFv directed against β-galactosidase (13R4) was fused with protein IX. To ensure enhanced protrusion of the scFv at the virus surface, a 75-Ångstrom α-helical spacer was included between protein IX and the 13R4. The protein IX-13R4 fusion proteins were efficiently incorporated in the HAdV-5 capsid and, importantly, 13R4 appeared to preserve its functionality in terms of β-galactosidase binding.

Next, protein IX also appears to be suitable for fusing tumor cell-directed single-chain T cell receptors (scTCRs) (**Chapter 4**). Tumor cell targeting was established via the fusion of protein IX with a scTCR (scTCR$^{HLA\text{-}A1/MAGE\text{-}A1}$) directed against the cancer testis (CT) antigen MAGE-A1, presented on the cell surface in complex with human leukocyte antigens of haplotype A1 (HLA-A1). HAdV-5 vector particles loaded with protein IX-scTCR$^{HLA\text{-}A1/MAGE\text{-}A1}$ fusion proteins transduced melanoma target cell lines with at least 10-fold higher efficiency than the control particles. Importantly, specificity of targeting could be shown as well. These results underscore the potential of using protein IX for targeting HAdV-5, and demonstrate the feasibility of targeting HAdV-5 vectors to intracellularly-derived CT antigens. The highly specific expression profiles of CT antigens, which are expressed in a variety of cancerous tissues and are generally silent in normal tissues (except for the testis), make them interesting target molecules for cancer therapies.

Chapter 5 describes protein IX-mediated tumor targeting of HAdV-5 through fusing Affibody molecules. Affibody molecules might be valuable moieties for virus targeting, because of their relatively small size and high binding affinity. Previous reports, however, showed limited targeting efficacies after fusing 'high-affinity binders', such as Affibody molecules, to protein IX, which has been suggested to be the result of inefficient release of the virions from their targeted receptors in the endosome. Our studies demonstrate that the transduction of tumor cells is augmented by incorporating a cathepsin-cleavage site (CCS) between protein IX and a 'ZH Affibody molecule',

directed against the Human Epidermal growth factor Receptor 2 (HER2). Virus particles harboring the protein IX-CCS-ZH in their capsid transduced HER2 positive SKOV-3 ovarian carcinoma cells with increased efficiency in monolayer cultures, 3-dimensional spheroid cultures, and in SKOV-3 tumors grown on the chorioallantoic membrane of embryonated chicken eggs. These findings further augment the applicability of protein IX as an anchor for coupling tumor-targeting ligands.

An alternative HAdV-5 targeting strategy is described in **Chapter 6**, concerning the genetic fusion of a scTCR with the fiber capsid protein. In the genome of wild-type HAdV-5, the sequence encoding for the fiber knob domain was replaced by sequences encoding a scTCR (identical to the scTCR molecule as described in Chapter 4) and an artificial trimerization domain. The resulting virus was, as anticipated, detargeted from Coxsackie- and Adenovirus Receptor (CAR) binding, and targeted to HLA-A1/MAGE-A1 molecules on tumor cells. Efficient and specific killing of targeted melanoma cell cultures was observed.

Finally, **Chapter 7** provides information on functional consequences of deleting the protein IX gene from HAdV-5 vectors. Various effects of protein IX deletion are reported, including enhanced reporter gene delivery to CAR-negative cell lines and enhanced activation of peripheral blood mononuclear cells. These findings suggest that protein IX can affect the cell tropism of HAdV-5, and may function to dampen the innate immune responses against HAdV particles. This may be of relevance for future development and clinical implementation of protein IX-modified HAdV-5 vectors.