Characterization of AAV serotype 2 and derived *in vivo*-selected AAV peptide display capsid variants for targeting hepatocellular carcinoma and the liver

Abstract

Adeno-associated viral (AAV) vectors are extensively used in gene therapeutic approaches. AAV vectors have been applied with great clinical success demonstrating efficient therapeutic long-term effects and an excellent safety profile. Like other viruses, AAV and derived vectors possess a broad tropism. While this is advantageous for ex vivo application, it is a challenge for AAV's use in vivo as high vector doses are required to compensate for viral vector loss in off-target organs. Furthermore, off-target expression may induce side effects such as toxicity or immune responses. To overcome this challenge viral vector capsids are engineered to direct tropism towards pre-defined target cell types. A promising strategy in this regard represents the insertion of short receptor-binding peptides into the viral capsid that mediate targeted cell entry and transduction. In order to identify such peptides AAV peptide display library selections are performed. Therefore, capsid variants displaying random 7-mer peptide insertions at capsid protrusions are screened in a high-throughput fashion for candidates that efficiently transduce the target tissue or cell type. Such directed evolutionary approaches are particularly useful to overcome lack of knowledge about suitable target cell-specific receptor-ligand interactions that in the capsid context would mediate efficient and safe gene transfer to desired target tissues.

The liver is one of the main target organs in gene therapy and represents the focus of this study. This relevance results from the fact that a number of inherited as well as acquired diseases are either caused by defects within the liver tissue or could be treated by overexpression and secretion of therapeutic factors from hepatocytes. Therefore, novel gene-based strategies are currently developed and the above mentioned AAV vectors have emerged as promising platform due to their natural tropism for liver and their non-pathogenicity. However, as already outlined above, none of the natural occurring serotypes show a restricted liver tropism. In addition, cellular barriers towards hepatocyte transduction reduce efficacy of this vector system.

Here, we aimed to improve the AAV vector system with regard to efficiency and selectivity of in vivo gene transfer into hepatocytes and its malignant counterpart, hepatocellular carcinoma cells (HCC). Specifically, for the first time a high-throughput in vivo selection of the AAV peptide display library was performed within an orthotopic bitransgenic HCC mouse model. We identified three promising HCC-directed AAV candidates that were characterized compared to AAV2wt, the parental serotype. In vitro experiments on hepatoma cell lines revealed distinct and superior transduction efficiencies of rHCC capsid variants compared to rAAV2wt. The advantage of in vitro

performance of HCC capsid variants could be interpreted due to – depending on the variant and cell line – higher cell entry rates and more efficient vector genome accessibility for transcription. In vivo characterization in the bitransgenic HCC mouse model showed promising results for the rHCC capsid variants with, as of yet, capsid

variant HCC3 exhibiting the best in vivo performance. The rHCC3 variant transduced HCC nodules more efficiently than AAV2wt or the other two candidates. In addition, rHCC3 was found to be superior regarding expression efficiency, i.e. transcripts per vector copy, arguing for a more efficient intracellular processing again compared to AAV2wt or the two further candidates. Interestingly, the HCC capsid variants were also able to efficiently transduce hepatocytes. In this context, the capsid variants were more efficient than AAV2wt. This may argue towards a gain in features that allows to overcome an as of yet not identified barrier towards AAV2 transduction in non-malignant hepatocytes. Notably, AAV2wt – being investigated for the first time in a syngeneic genetically-engineered HCC mouse model – revealed an as of yet unknown natural tropism for HCC that can be employed for further therapeutic strategies. Owing to the heterogeneity of the mouse model and the shortage in animals that could be included into this study, results need to be confirmed in larger cohorts.

The challenging in vivo selection was performed in target cells, which were derived from hepatocytes and thus share common features. Exploiting this aspect resulted in the identification of two liver-directed AAV capsid variants. In vivo characterization showed greatly improved transduction efficiencies compared to the parental AAV2wt and was indicated to be achieved by significantly higher cell entry rates. Notably, the novel capsid variants were shown to exhibit comparable liver tropism and transduction efficiencies to the current gold standard, the hepatotropic AAV serotype 8. The liver-directed variants show great potential to broaden gene therapeutic options in liver disease.