GENETIC RE-TARGETING AND DE-TARGETING OF ADENOVIRUS TYPE 5 IN ORDER TO CREATE VECTORS FOR GENE THERAPY

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av

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I. Myhre S, Henning P, Granio O, Tylo AS, Nygren PA, Olofsson S, Boulanger P, Lindholm L. Decreased immune reactivity towards a knobless, affibody-targeted Adenovirus type 5 vector.
Gene Therapy 2007, Feb 14 (4):376-81.

II. Vellinga J, de Vrij J, Myhre S, Uil T, Martineau P, Lindholm L and Hoeben R.C. Efficient incorporation of a functional hyper-stable single-chain antibody fragment protein-IX fusion in the adenovirus capsid.
Gene Therapy 2007 Apr;14(8):664-70.

III. Magnusson MK, Henning P, Myhre S, Wikman M, Uil TG, Friedman M, Andersson KM, Hong SS, Hoeben RC, Habib NA, Ståhl S, Boulanger P and Lindholm L. An Adenovirus 5 vector genetically re-targeted by an Affibody™ molecule with specificity for tumor antigen HER2/neu.
Cancer Gene therapy 2007 May;14(5):468-79.

IV. Myhre S, Henning P, Lindholm L and Magnusson M.K. In vitro and in vivo evaluation of HER2/neu re-targeted and CAR-, $\alpha_v$integrin- and HSPG-binding de-targeted Adenovirus vectors.
Manuscript.

V. Myhre S, Magnusson M.K, Friedholm M, Henning P, Ståhl S and Lindholm L
A re-targeted Adenovirus with dual specificity; evaluation of binding specificities for Affibody® molecules at different positions in the HI-loop of the fiber knob.
Manuscript.

GÖTEBORGS UNIVERSITET

GENETIC RE-TARGETING AND DE-TARGETING OF ADENOVIRUS TYPE 5 IN ORDER TO CREATE VECTORS FOR GENE THERAPY

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Gene therapy has been considered to be a revolutionary development in medicine, whereby the cause and not the symptoms of the disease would be treated. These expectations have as yet not been realized, mainly due to lack of suitable vectors. Adenoviruses type 5 (Ad5) are the most commonly used vectors for gene therapy and have a great potential in this field.

The main aim of this thesis was to generate genetically re-targeted and de-targeted Ad5 in order to create suitable vectors for gene therapy. To re-target the Ad5 vector ligands have been incorporated into the C-terminus at the pIX protein and at different positions in the fiber protein. Ligands that have been used are affibody molecules, with specificity for Taq DNA polymerase and for the tumour antigen HER2/neu, and a hyperstable single chain antibody, scFv, directed against β-galactosidase. In order to generate a virus with double specificity and determine which position is best suited for ligand–target cell interaction a re-targeted Ad5 vector with two different affibody molecules in the same genome was constructed. The re-targeted vectors were evaluated for growth, infectivity and specificity. For de-targeting assessments the neutralizing antibody reactivity in blood donor sera have been tested against a recombinant Ad5 vector with a shortened knobless fiber and a new cell binding ligand and a re-targeted vector with three different de-targeting steps have been evaluated for vector characterizations, tissue distribution and interaction with blood cells.

Ligands that are to be used for re-targeting of Ad must be able to fold correctly and stably in the reducing milieu of the eukaryotic cytoplasm which is not conducive to the formation of disulphide bonds. Both the affibody molecules and the scFv did fulfill these criteria and could be rescued into functional Ad. Incorporation of ligands in the HI-loop of the fiber knob was shown to be superior to ligand insertions into truncated knobless fibers in terms of growth characteristics. It was possible to incorporate the scFv at the pIX protein with retained antigen binding when loaded on Ad5 virions, however transduction experiments could not be performed because a suitable cell line was not avalible. It was shown that generation of a vector with dual specificity was feasible, on the other hand it was important to evaluate which positions is best suited for efficient binding to target cell. The virus with a truncated knobless fiber and a new cellular ligand showed a much-reduced sensitivity to human pre-formed antibodies compared to wild type (WT) Ad5. The re- and de-targeted Ad vectors did not bind to normal tissues in mice as much as WT Ad5 and the association with human blood cells was much decreased for the recombinant vectors when compared to WT.

In conclusion, both the affibody molecules and the scFv evaluated in this thesis can be used for genetic re-targeting of Ad5. Re-targeted viruses with ligands in the fiber often suffer from low growth rate, high infectivity indexes (PP/pfu) and low fiber content. However virus with ligand incorporation in the HI-loop does largely overcome those obstacles and the virus with ligand specificity for the tumour antigen HER2/neu may have relevance as a clinical vector. The adenovirus minor capsid protein IX can function as an anchor protein for relatively large ligand insertions, which is promising for future de-targeting purposes. Generation of recombinant viruses with double specificity may exploit the possibility to target several tumour antigens. The reduced neutralizing activity against the short knobless fiber and the reduced binding to normal tissues and interaction with blood cells of the re-targeted viruses with additional de-targeting steps represents, to this author, an important step towards the construction of “stealth” adenoviruses for gene therapy.

Key words: Adenovirus, vector, gene therapy, re-targeting, de-targeting, fiber, pIX, affibody molecule, single chain antibody.
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