

# **TITLE:** Redirecting AAV vectors for delivery of therapeutic molecules to human basal-like breast cancer cells

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## Project Overview/Abstract

Despite novel therapeutic strategies and numerous newly developed anticancer agents, breast cancer still is the leading cause of cancer-related mortality among women in industrialized countries. Surgical resection remains the mainstay of treatment of early breast cancer, but undetected micrometastases or residual tumor cells at the primary site often result in recurrence of the disease even after a latency of several years (1, 2). Clinical management of advanced breast cancer is limited to palliative therapy in which resistance to chemotherapeutic agents is the major drawback. Therefore, supplementing conventional systemic treatment with gene therapy as an unrelated approach to eradicate malignant cells might be beneficial to cancer patients at all stages of the disease. However, vectors that sufficiently and specifically transduce this cell type are scarce.

Recombinant adeno-associated (rAAV) vectors have become very popular as potential gene therapy vectors because of their ability to efficiently transduce mammalian cells and render stable transgene expression. Moreover, together with its replication defective nature, rAAV has good biological safety profile thus permitting its use for gene transfer in vivo, and as potential gene therapy vehicle (3). One drawback is the fact that rAAV vectors have a very wide tropism therefore making pseudotyping necessary towards fitting the needs of the application.

AAV belongs to the *Parvoviridae* family and Dependovirus genus, whose members require co-infection with a helper virus such as adenovirus to promote replication. Virions are composed of a 25 nm icosahedral capsid encompassing a 4.9 kb single-stranded DNA genome with two open reading frames: *rep* and *cap*. The non-structural *rep* gene encodes four regulatory proteins essential for viral replication, whereas *cap* encodes three structural proteins (VP1–3) that assemble into a 60-mer capsid shell. This viral capsid mediates the ability of AAV vectors to overcome many of the biological barriers of viral transduction—including cell surface receptor binding, endocytosis, intracellular trafficking, and unpackaging in the nucleus. In addition, the capsid diversity found among natural AAV variants, or serotypes, isolated from human and nonhuman primate tissues accounts for its diverse tropisms.

Recent advances in rAAV engineering techniques have vastly expanded the ability to develop novel AAV serotypes. Direct evolution strategies have demonstrated the power of mutagenesis and DNA shuffling methods to investigate and enhance preexisting functions of (or generate novel functions) in proteins without underlying mechanistic knowledge (4).

When combined with directed evolution which allow specific characteristics to be selected without *a priori* knowledge of the physical determinants, novel vectors can be identified that exhibit the desired, specific tropisms. Previous peptide-based AAV2 capsid engineering studies have predominantly manipulated the surface loop containing the HSPG-

binding site (5), however, recent studies of the AAV2 capsid have highlighted other regions that significantly influence the transduction properties of AAV2.

The aim of this project is to make use of AAV vectors displaying surface random peptide libraries to identify cell-specific homing peptides towards malignant breast cancer cells.

In a first phase, the student will use an AAV-display library containing randomly mutagenized amino acids in surface loops present in the AAV capsid for biopanning against basal-like breast cancer cells allowing for the selection of specific AAV binders. The mutagenized capsid from the AAV binders will be sequenced to determine the surface loop composition. Vectors expressing a reporter gene (e.g. GFP) will be developed and used to generate recombinant AAV containing the identified binding capsid. Capsid modified rAAV vectors expressing the reporter gene will be produced and characterized in terms of transduction properties and gene delivery ability in order to select for ideal AAV candidates for gene delivery to basal-like breast cancer cells.

The **student** should be familiar with Molecular Biology Techniques, Genetics and Virology, and have good knowledge of the English language.

**Techniques to be used:** Molecular biology, Viral Vector Development and Production, Mammalian Cell Culture, Microscopy and FACS.

References:

1. **Michelfelder, S. and M. Trepel.** 2009. Adeno-associated viral vectors and their redirection to cell-type specific receptors. *Adv Genet* 67:29-60.
2. **Daya, S. and K.I. Berns.** 2008. Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev* 21:583-593.
3. **Da Silva, L., C. Clarke, and S.R. Lakhani.** 2007. Demystifying basal-like breast carcinomas. *Journal of clinical pathology* 60:1328-1332.
4. **Korsching, E., S.S. Jeffrey, W. Meinerz, T. Decker, W. Boecker, and H. Buerger.** 2008. Basal carcinoma of the breast revisited: an old entity with new interpretations. *Journal of clinical pathology* 61:553-560.
5. **Summerford, C., and Samulski, R.J.** 1998. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *J Virol* 72, 1438-1445.