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Altering Lentiviral Tropism: Design and Implications of a Targeted Drug-Delivery System

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A significant challenge in modern clinical medicine is that despite the existence of genetic therapies, lack of techniques to deliver them to the body remains a major roadblock. Viral vectors, benign vehicles for therapeutic delivery produced by removing the reproductive and virulence capacities of wild-type viruses, address this obstacle [1]. Unfortunately, if there is no naturally-occurring virus which infects a particular tissue it is nearly impossible to systematically deliver a therapy to that site. Moreover, the tropism of naturally-occurring viruses is often too broad or too narrow for delivery to both be effective and avoid off-target effects [2].

A solution arises from a potent technique called pseudotyping, commonly used with lentiviral (HIV-1 based) vectors, which alters vectors' tropism by anchoring exogenous proteins into their lipid envelopes [3]. This study analyzed the viability of manipulating vectors' tropism through pseudotyping, and designed an efficient protocol for doing this with diverse proteins. Replication-deficient lentiviral vectors were manufactured by using a packaging cell line. It provides the virulent signals necessary for viral production that cannot be inserted into the vector, on a separate plasmid from all that actually gets packaged into the vector [4].

A common pseudotyping protein is vesicular stomatitis virus glycoprotein (VSVg). It is known for conferring broad tropism, particle stability and high titer [4]. This project studied expanding viral tropism and infection efficacy through VSVg pseudotyping. Data showed a significant ($p < 0.05$) increase in infection efficacy in VSVg pseudotyped vs. non-pseudotyped vectors.

Pseudotyping was also applied to create a vector specific to cancer cells in order to develop a targeted therapy and avoid detrimental effects to healthy tissues. Unique and overexpressed receptors on cancer cells were exploited as markers based on which to direct the vector selectively to malignant cells [5]. Pancreatic cancer was used as a model due to unique expression of the cholecystokinin 1 and 2 (CCK1 and CCK2) receptors which bind cholecystokinin-8 (CCK8) and gastrin-17 (GAST17) [6]. The presence of these receptors was confirmed by fluorescence microscopy and it was hypothesized that pseudotyping a lentiviral vector with GAST17 and/or CCK8 would specifically target it to pancreatic cancer cells.

A protocol was designed for anchoring the non-membrane bound proteins CCK8 and GAST17 into the lipid envelope of a lentiviral vector. This challenge was addressed using the pDisplay vector which fuses any protein of interest to the naturally membrane-bound platelet derived growth factor transmembrane domain. Computer algorithms were used to generate protein sequences optimal for translation in human cells to make pseudotyping more effective [7].

This study establishes a concept for selectively targeting organs and cancers with personalized therapeutic-delivery tools and makes it clinically practical through an innovative protocol.

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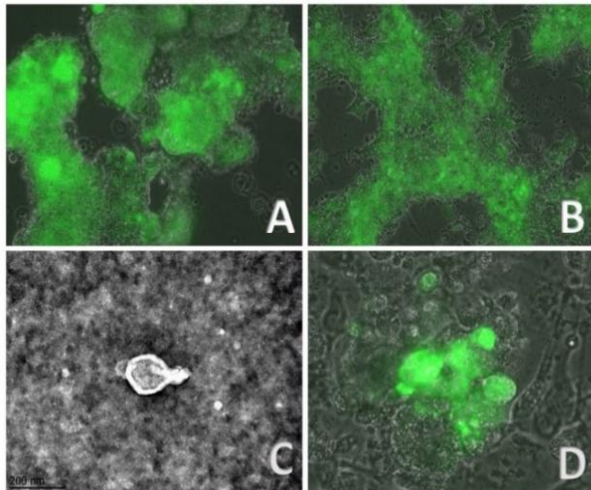

Figure 1: A) B) Packaging cell lines producing non-pseudotyped and VSVg pseudotyped vectors respectively **C)** Vector imaged on the TEM **D)** HeLa cells infected

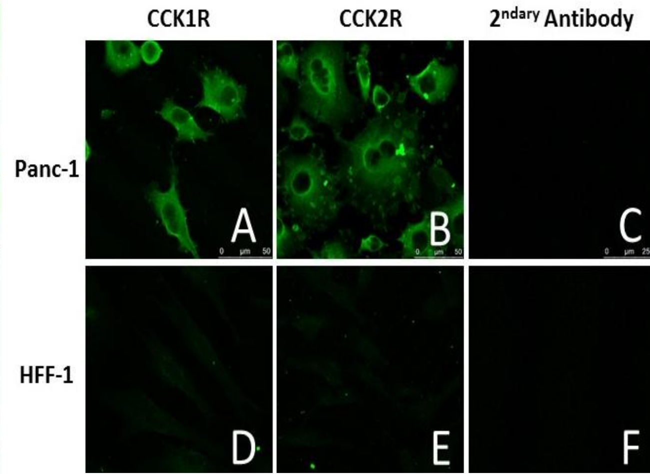

Figure 2: Laser scanning confocal microscope images of pancreatic cells stained against the CCK1 and 2 receptors.

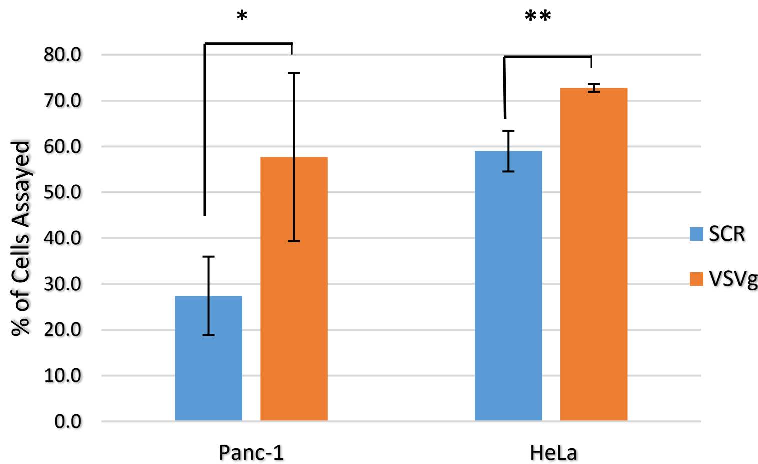

Figure 3: Infection efficacy with non-pseudotyped vs. VSVg pseudotyped vectors. Bars are averages +/- STDEV. Asterisk indicate statistically significant difference as compares to control p<.0.05* and p<0.01**.

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