Application of an infectivity-selective adenoviral CRISPR/Cas9 vector into cancer stem cells as a gene therapy to treat colorectal cancer

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Final Bachelor's Degree Project
Research project

Biochemistry Bsc, 2017-2021
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INTRODUCTION

- **Colorectal cancer (CRC)** is the 3rd leading cause of cancer related deaths for both ♀ and ♂ in industrialized countries. Although there has been much progress in diagnosis and treatment, research gaps are identified in CRC etiology due to an increase in its incidence.
- Current anticancer treatments are developed to target the bulk of the tumour mass, but sometimes they are unlikely to result in long-term remissions if **cancer stem cells (CSCs)** are also not targeted. CSCs are defined with different markers→ **CD44** (transmembrane glycoprotein) and **Nanog** (pluripotency factor).
- **Adenoviral vectors** are one of the most efficient gene delivery systems, as they present high genetic stability and gene transduction. They are a good choice to treat cancer cells, as high but transient gene expression is required and also cellular toxicity and immunogenicity may enhance anti-tumour effects.

HYPOTHESIS AND OBJECTIVES

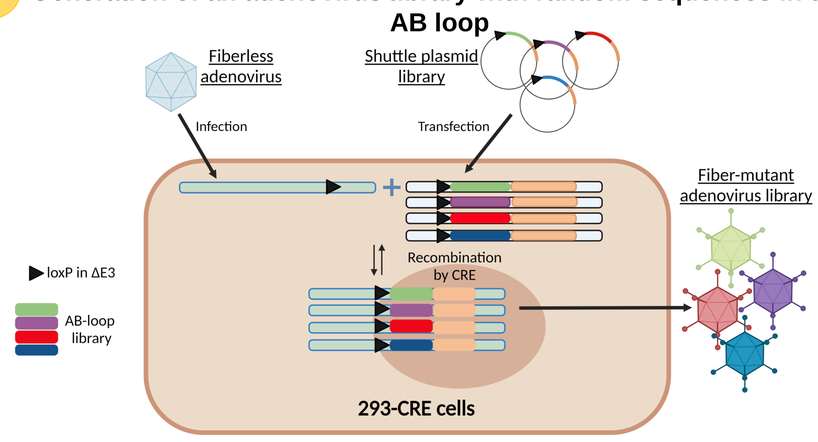
The application of a **second generation adenoviral vector** that specifically targets the **CD44** receptor overexpressed on colorectal **CSCs**, and which is used as a vehicle to carry the **CRISPR/Cas9** technology to produce a deletion of the **Nanog** gene upregulated in these cells, could show promising results in *in vitro* assays.

- Obtainment of a fiber protein that specifically targets CD44 receptor.
- Design of an efficient CRISPR/Cas9 plasmid to delete Nanog gene.
- Production of second generation adenoviral vectors to selectively infect CD44+ colorectal CSCs.
- Test if the gene therapy developed reduces the tumour capacity of colorectal CSCs *in vitro*.

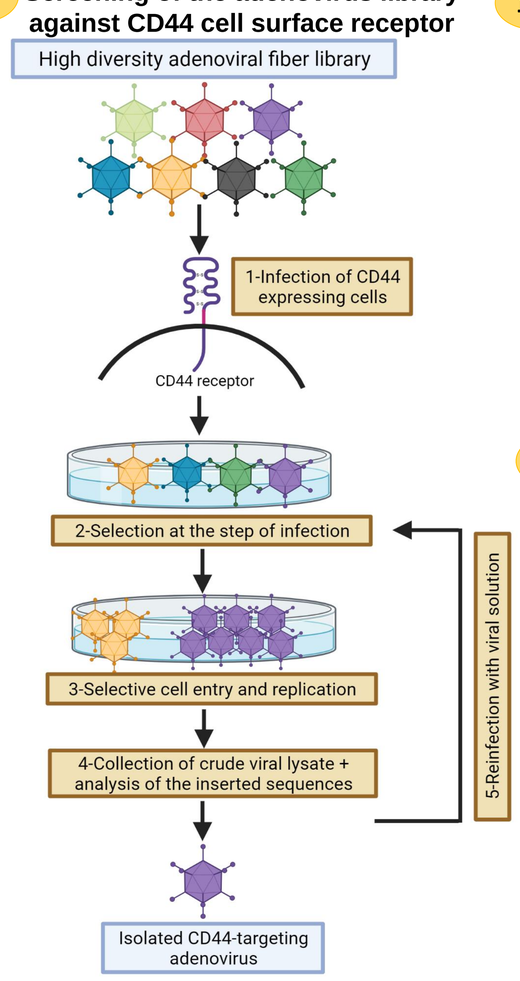
METHODOLOGY

1. Obtainment of a specific CD44-targeting fiber protein

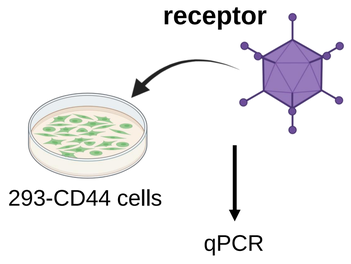
1.1 Generation of an adenovirus library with random sequences in the AB loop



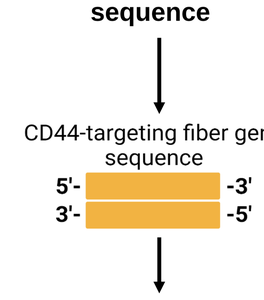
1.2 Screening of the adenovirus library against CD44 cell surface receptor



1.3 Binding assay of the isolated clone to the CD44 receptor

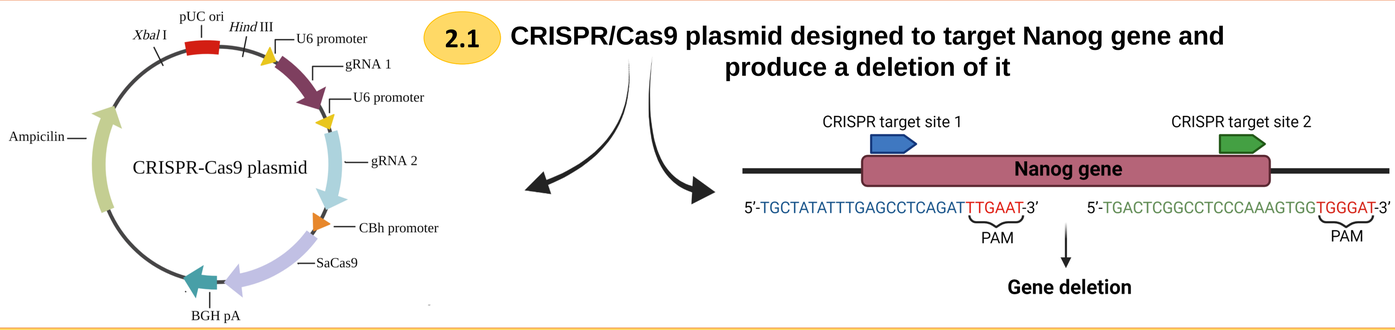


1.4 Synthetic synthesis of the CD44-targeting fiber sequence



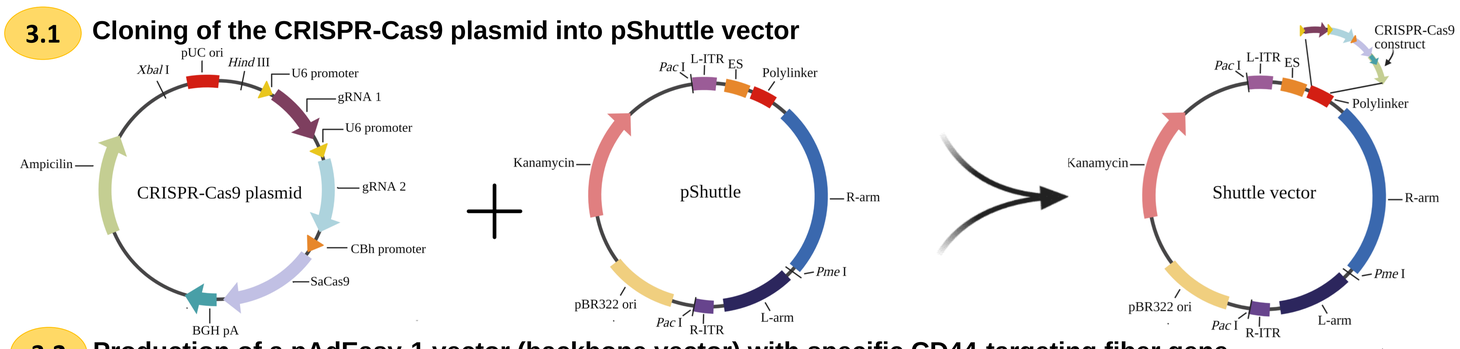
2. Design and production of the CRISPR/Cas9 plasmid

2.1 CRISPR/Cas9 plasmid designed to target Nanog gene and produce a deletion of it

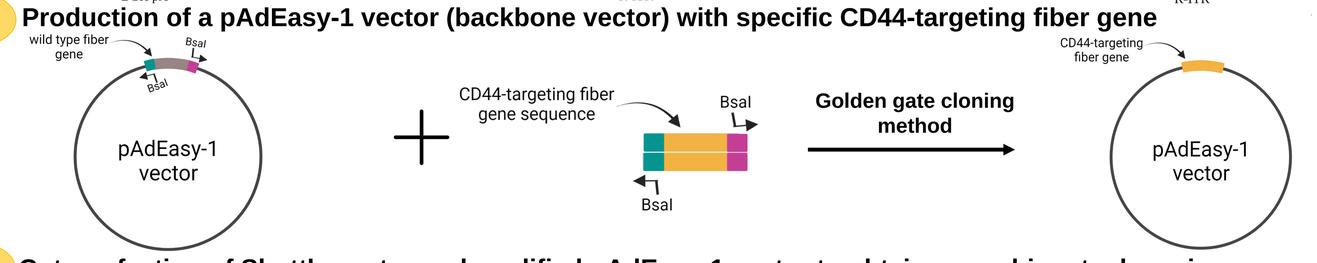


3. Production of second generation adenoviral CRISPR/Cas9 vectors

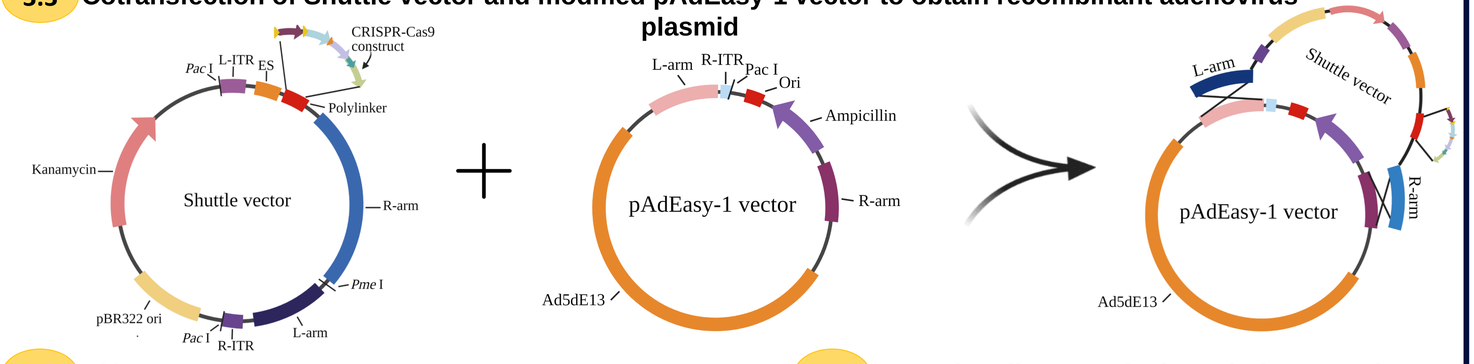
3.1 Cloning of the CRISPR-Cas9 plasmid into pShuttle vector



3.2 Production of a pAdEasy-1 vector (backbone vector) with specific CD44-targeting fiber gene



3.3 Cotransfection of Shuttle vector and modified pAdEasy-1 vector to obtain recombinant adenovirus plasmid



3.4 Vector characterisation

After purification of **recombinant CRISPR/Cas9 adenoviral vectors**→

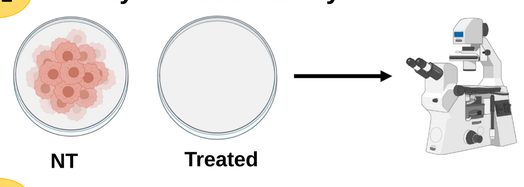
- Determination of **viral particle concentration**.
- Determination of the **infectious virus titer**→ **$TCID_{50}$ assay.**

3.5 Functionality test of adenoviral CRISPR/Cas9 vector

- **RT-PCR** → quantity of total RNA isolated from cells.
- **Western Blotting.**

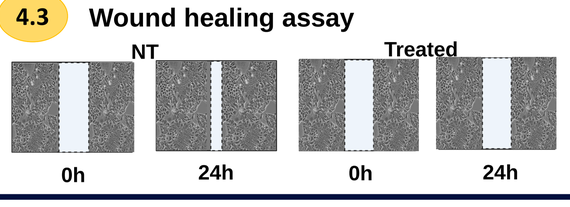
4. Evaluation of the antitumour effects of the gene therapy in colorectal CSCs *in vitro*

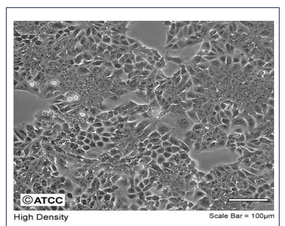
4.1 Colony formation assay



4.2 Cell proliferation assay

4.3 Wound healing assay





HTC-116 cells: CD44+ Nanog+

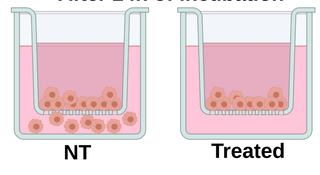
Experimental group

- HTC-116 cells treated with the adenoviral CRISPR/Cas9 vector.

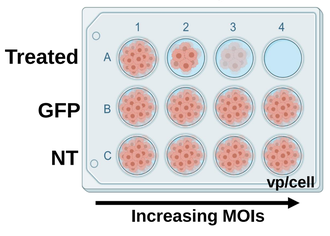
Control groups

- HTC-116 cells treated with adenoviral vector encoding GFP.
- Non-treated HTC-116 cells.

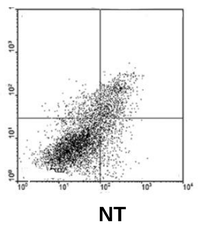
4.4 Transwell cell invasion assay



4.5 Cytopathic effect assay



4.6 Apoptosis assay



CONCLUSIONS

- By deleting Nanog gene upregulated in this cells, it is expected to observe a **decrease in the tumourogenic capacity** of CSCs *in vitro*.
- Production of a second generation adenovirus specific for CD44 allows **disruption** of the receptor signaling pathway + **less toxicity** derived from off-targets + **cytotoxic response** that enhances the **anti-tumour effects**.
- Possible **future drawbacks** in the implementation of this project: low efficiency in vector production, insufficient number of vectors reaching the target tissue.

DIFUSSION PLAN

The dissemination strategy aims to make the main accomplishments of this project visible to the scientific world. It would entail publishing the findings obtained in:

- **Scientific journals and magazines.**
- **National and international conferences.**
- **Congresses and meetings.**

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