Influenza A gutless vector: new approach against lung cancer

Francisco Latorre Pérez
Biotechnology Degree, Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain

INTRODUCTION AND OBJECTIVES

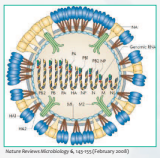
- **Lung cancer** is the most common cause of cancer-related death in men and women worldwide. The actual treatments for this disease are chemotherapy, radiography and surgery; the main problem with them is that they are not always effective and can lead to bad secondary effects. Thus, there is an urgent need in finding an effective and harmless alternative for lung cancer therapeutics.
- The aim of this project is to establish a different treatment against lung cancer, as a part of a **final project report**, based on one of the most promising new therapies nowadays: **virotherapy**. Although the use of engineered virus has been reported in the consulted literature before, no satisfactory results have been achieved. Thus, this work proposes the usage of a gutless engineered **Influenza A/Aichi/2/68 H3N2**, which has a natural tropism for lung, with the gene coding for the **thymidine kinase** protein to infect a culture of human lung malignant cells and test the killing rate in presence of **ganciclovir** in media culture.

METHODOLOGY

- As a basis for the development of this work, many information sources such as paper reviews, patents, books, on-line official web pages, on-line catalogue were consulted. Also, an interview with a specialist in virus-mediated gene therapy from the Institut Català d'Oncologia (ICO).

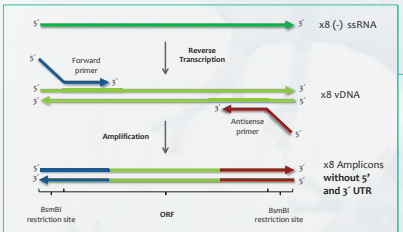
MATERIALS AND METHODS

In vitro experiments



Genome extraction

vRNA



RT-PCR

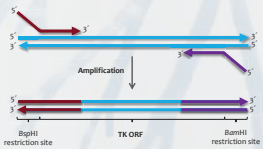
- In this case, the 5´ and 3´UTRs are eliminated in each amplicon due to the need to obtain viral genes without the possibilities of being packed in the viral capsids that they can form.

Digestion with *Bsp*BI and *Bam*H1 + Ligation

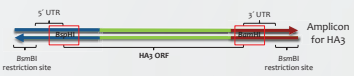
Digestion with *Bsm*BI

Construction of suicide gene

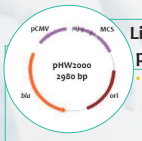
- First, it is needed to do an amplification by PCR of the **thymidine kinase** gene (TK); adding two restriction sites for *Bsp*HI and *Bam*HI as shown below.



- Second, another RT-PCR for the virus Hemagglutinin 3 (HA) gene is performed. In this case, it is amplified the whole gene, with 5´ and 3´UTRs. The result is shown below.

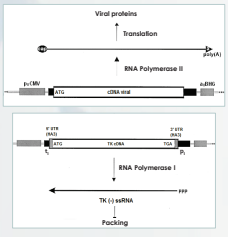


Ligation in pHW2000



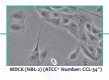
- Two types of molecules are synthetized after ligation:

1. By RNA pol II: mRNA,
2. By RNA pol I: (-) ssRNA (only TK plasmid has the UTRs necessary to be packed).

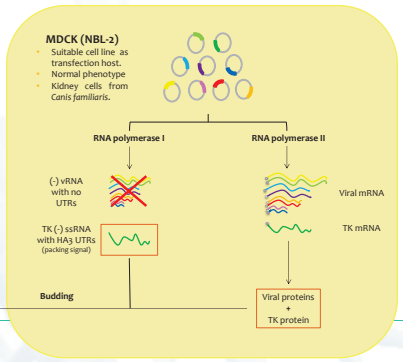


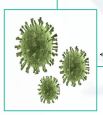
Ex vivo experiments

Transfection into packing cells

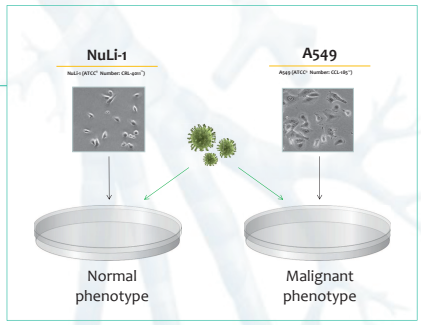


Transfection into MDCK



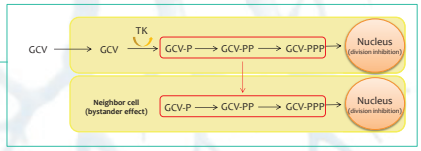


Infection with therapeutic virus



Thymidine kinase production

Ganciclovir killing ratio



- In order to see if the infection is efficient the killing ratio by ganciclovir (GCV) is tested. Also, to test whether there is a differences between normal and malignant phenotypes, two cell lines are used.
- Level of formazan, product from tetrazolium salts reduction, in media culture is required for this toxicity assay since it is only present in living, metabolically active cells.

DISCUSSION AND CONCLUSION

- Since there are no results, only conjectures can be made. Probably, gutless Influenza A vector with TK gene would be obtained, but the main problem of this whole procedure is that the efficiency is very low, which means that in order to achieve a good level of therapeutic virus, high input is required. Then, optimization is obligatory. In addition, counting the optimization time, this project was thought to be done in 3 years as a thesis project.
- In conclusion, despite the fact that some modifications of the process have to be done, this work shows a first attempt in the development of an Influenza A gutless vector that hopefully could be used as a complementary treatment against lung cancer in the future.