Development of virus-dependent gene expression switches for gene therapy applications

Place of work/: RNA Systems Biology Lab - Gene Expression and Regulation Group, BioISI - FCUL

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Abstract / MSc thesis project proposal

Latent virus infections – namely caused by HIV virus – are a serious health problem that thwarts the implementation of effective cures. This MSc proposal is integrated in a FCT funded research project that aims to address this issue through a gene therapy approach. The global aim is to achieve a highly efficient targeted elimination of cells harboring HIV latent proviral DNA.
The selected student will apply genetic engineering methods to build a "kill-switch" system that is able to respond specifically to the accumulation of HIV proteins. Two alternative approaches have been conceptualized for this purpose that rely on the presence of viral proteins regulate the expression of peptide sequences encoding toxic proteins. These "viral sensors" will be linked to the expression of a dCas9 transcriptional trans-activator able to recognize the presence of latent proviral DNA integrated into the cellular genome and induce its re-activation.
The first approach relies on the design of an intron containing transcript whose expression requires the presence of the HIV-1 Rev protein as a regulator of nuclear export of unspliced (viral) RNAs. The second approach aims to build a vector platform with two independent transcriptional units, one that is constitutively expressed, and the other that requires the presence of the HIV Tat transcription factor.
The work plan will focus on the development and validation of the two viral response elements, requiring the understanding of the underlying gene expression mechanisms and regulatory elements. The first task of the project will focus on the design of sequence elements able to achieve the desired effect, and to determine the best experimental approaches for building these elements. In the second task of the project the actual construction and validation of the DNA constructs will be performed. Fluorescent reporter proteins will be used in flow cytometry assays to assess the efficiency of different constructs to elicit controlled, HIV-dependent expression of the reporter protein only in the presence of Tat or Rev. Assays will be performed in a T cell line that contains a latent infection of a modified HIV virus. Once an efficient construct is built, the reporter protein will be replaced by a toxic construct to evaluate the ability to generate controlled cell death. Finally, the whole system will be built together to address the ability to detect and eliminate latently infected cells.
The project will allow the student to acquire a deep understanding of the mechanisms controlling different steps of the eukaryotic gene expression pathway, develop significant expertise in genetic engineering methods, as well as in the growth, manipulation and analysis of human cell lines.