PROJECT 1

DoS: Alessandro Sessa

Title: Validation of artificial epigenetic silencers to reduce GBM in immunocompetent mouse model

Curriculum: BAIO

Link to OSR/UniSR personal page: http://www.unisr.it/alessandro-sessa/

Project description (*Number of characters, including spaces*: 2.000 - 3.000):

In the laboratory, we previously generated synthetic factors able to inhibit the growth of GBM cell lines and xenografts. These factors are built using inhibitory domain of chromatin factors (e.g. DNA methylases) and the DNA binding domains of transcription factors commonly found in brain cancers (e.g. SOX2, MYC, etc). Delivered in a gene-therapy-like fashion, their function is to directly inhibit, rather than activate, the molecular network of the original factors used (e.g. SOX2, MYC, etc), in order to both restrain the malignant properties of GBM cells (e.g. proliferation) and to induce cell death, likely in a cell autonomous manner. We have extensive data on human GBM cell lines, patient derived cancer stem cells and their engraftment in immunocompetent animals, however no data have been so far collected on the immune system behavior: how it reacts to the artificial factor delivery itself and to the tumor shrinkage due to the treatment.
Thus, the rationale of the project is the following:
since we cannot exclude a contribution (either positive or negative) of the immune system in our proposed treatment, we will employ a murine immunocompetent model of GBM (based on the destruction of key oncosuppressors) to test our approach based on synthetic factors on both the tumor growth and the immune reaction.

To accomplish this project the student will firstly build, validate and use a lentiviral construct carrying the genetic elements needed to mutate oncosuppressor genes by an Cre-inducible CRISPR. Then, she/he will generate the GBM models, stereotactically inject the lentiviral particles in mice carrying Cre enzyme under either neuron-, or glial-specific promoters. After the validation of the procedure (tumor generation within 1-2 months from the injection) the student will study the dynamic of the tumors appearance in time and space in each model using in immunohistochemistry and scRNAseq.
Whether this genetic model will not meet our expectations (insufficient tumor generation/growth) we will transplant murine GBM cells (e.g. GL261, mGB2) in immunocompetent mice to induce GBM-like tumors.
Subsequently, she/he will use the modified factors in vivo to evaluate the efficacy on generation and growth over time of GBM-like tumors in these new immunocompetent models. Specifically, the student will test the morphometric parameters of the tumors, and the molecular effects on the targets of the TF used (RT-qPCR, RNA-seq, and ChIP-seq).
Finally, she/he will evaluate the properties of treated and un-treated brain tumor niches at cell population level using immunohistochemistry analyses and single cells approaches.
This will allow a detailed characterization of both tumor and immunological niches during the trajectories of either tumor development and time of treatment

Skills to be acquired by the student (*Number of characters, including spaces*: max 600):

The student will acquire abilities in molecular cloning, mouse handling, mouse surgery, histochemical analyses. The project will also foresee many genomic approaches: the student will be able to proficiently generate libraries ready to be sequenced, while bioinformatics analysis will be performed by a skilled bioinformatician in the lab.
The student will apply genomic techniques also at single cell levels to gain molecular understanding of the macro-phenotypes of the animals and ultimately the clinical signs of the cancer progression.

References (max. 15)

Amabile, A. et al. Inheritable Silencing of Endogenous Genes by Hit- and-Run Targeted Epigenetic Editing. Cell 167, 219-32 (2016).

Friedmann-Morvinski et al., Science: Dedifferentiation of Neurons and Astrocytes by Oncogenes Can Induce Gliomas in Mice 338, 1080-1084 (2012).

Suva, M.L. et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell 157, 580-94 (2014).