PROJECT 2 (optional)

DoS: Alessandro Sessa

Title: Generation and validation of artificial epigenetic silencers to reduce brain metastases

Curriculum: BAIO

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Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):
Both primary brain tumors and metastases from tumors of other organs are highly impacting the health systems because of the high mortality rate and/or the quality life impoverishment in millions of patients worldwide. It has been proposed that a subpopulation of tumor cells with radio/chemotherapy-resistant properties may have a role in driving tumor initiation, progression, resistance to treatment, and relapse. Due to their abilities of self-renewal, proliferation, and differentiation are named cancer stem cells (CSCs) or metastatic cancer stem cells (MCSCs), and are held responsible for either carcinogenesis or metastatic invasion (Peitzsch et al., 2017). CSCs display molecular features of embryonic progenitors including a plethora of transcription factors that are probably key factors for induce genetic programs that act in maintaining their properties as well as malignancy. This project stems from the concept that blocking the downstream expression cascade of tumorigenic-specific transcription factors (TFs) might be considered as a trojan horse to target and silence its endogenous targets, hopefully blocking the malignant properties of the cells (e.g. proliferative capacity, migration, invasion). We will choose a short list of TFs that are important for stem cell state as activators of their own target genes (unpublished data). We planned to crack the system modifying the candidate protein to obtain a repressive factor. However, the new artificial factor should retain the binding profile on the genome of the original TF and thus, may repress its own targets, resulting detrimental for metastasis invasion and growth.

Skills to be acquired by the student:

To accomplish this project the student will modify the tumorigenic TFs with molecular cloning to revert their transcriptional activity and transform a molecular activator in an epigenetic silencer. After validation of the construct validity (transduction on cell line followed by immunochemistry and Western blot), she/he will use the modified factor(s) in vitro to evaluate the efficacy of the procedure. In fact, after viral transduction of CSCs and various cancer lines, she/he well test their proliferation (growth curve and immunochemistry with proliferation markers), the eventual repression of the targets of the TF (RT-qPCR and RNA-seq); then using ChIP-seq she/he will identify its binding at genome-wide level, as well as the epigenetic features that are predicted to be affected. Then she/he will take care of the in vivo studies. The first protocol will be the use of cells from metastatic cancers (e.g. breast cancer, melanoma, lung cancer) already transduced with the engineered factor(s) (vs mock treated control) to make xenograft in immunocompromised mice. The student will evaluate number and size of brain metastases at fixed time points. Then, the student will perform the experiment with naive metastatic cancer cells to induce brain metastases and then treat them with the artificial factor in vivo using AAV tools. Subsequently, the student will evaluate the properties of treated and

un-treated brain metastases as well as the brain parenchyma both at histochemical as well as molecular level (e.g. RNA-seq, Chip-seq).

References (max. 3)

Peitzsch, C. et al., Cancer stem cells: The root of tumor recurrence and metastases. Seminars in Cancer Biology 44,10-24 (2017).