8 SUMMARY

FUNCTIONAL MOLECULAR IMAGING OF CANCER DEVELOPMENT AND STEM CELL REGENERATION IN THE NERVOUS SYSTEM

Brain tumors are devastating diseases that have a great impact on patients. Glioblastomas are high-grade gliomas with poor prognosis while medulloblastoma patients have a better outcome. Despite this, cognitive effects as a consequence of the treatment significantly reduce medulloblastoma patient quality of life. While lower back pain is considered a pathology that has significantly less impact on patients compared to brain tumors, a relatively high incidence makes it a disease with great socio-economic impact.

It is hypothesized that brain tumors may arise from aberrant stem cells and clonal selection, resulting in heterogeneous tumor development. Tumor heterogeneity and clonal selection remains to be one of the foremost challenges in developing adequate therapies. While in brain tumors, stem cells may prove to be the cause of progressive disease, stem cell transplantation might also be beneficial in tissue regeneration in, for example, degenerated intervertebral disc.

In an effort to develop a method to study tumor heterogeneity we developed a series of functional bioluminescent reporters that can be used in multiplex. Therefore, we fused epitope tags to the naturally secreted functional bioluminescent reporter *Gaussia* luciferase. We then used a tag-specific antibody binding assay to separately measure each secreted reporter from cell culture medium or blood. We showed that by using this method we were able to follow multiple brain tumor cell lines, each expressing a reporter with different tag, in co-culture *in vitro* and *in vivo*. We then used this method to study the function of putative tumor suppressors in glioblastomas.

First, we compiled a comprehensive list of putative tumor suppressor genes and compared this list with gene expression data of medulloblastoma to identify putative tumor suppressor genes that are down regulated. Here we identified DAB2IP, a RAS-GTPase activating protein that is suppressed by EZH2-induced methylation. We also showed that DAP2IP expression in medulloblastoma is a positive marker for patient survival. We then used this list to identify down regulated putative tumor suppressor genes in glioblastoma. We then created neural precursor cell lines expressing short hairpin RNA against selected putative tumor suppressor genes and expressing a specific tagged reporter. We then co-injected these cells in the mouse striatum and analyzed tumor development. We found that RASAL1 might act as a tumor suppressor in neural precursor cells and that knock down can possibly result in tumor induction.

Then, we used luciferase reporters to image adipose derived mesenchymal stem cells over prolonged periods of time inside large mammal intervertebral discs to study stem cell regeneration. We therefore injected luciferase-expressing adipose derived mesenchymal stem cells in intervertebral discs and cultured them over time under simulated loading conditions to mimic physical load. We were able to do bioluminescent imaging of stem cells in goat intervertebral discs, despite confounding

factors that might induce interference. We also found that *Gaussia* luciferase is more suited to image cells in intervertebral discs compared to *Firefly* luciferase.

This research may provide new insights into brain tumor development and heterogeneity and in stem cell biology, providing novel approaches for therapeutic modalities.