Malignant Glioma is the most common brain cancer in adults, and it has seen very limited therapeutic advances over the last decades. This is largely due to: i) limitation to surgical resection, ii) our limited understanding of brain tissue homeostasis, and of the molecular mechanisms of tumor initiation and maintenance, which could be exploited to design targeted therapies.
Genetic evidences showed that Bmi1 - a transcriptional regulator that belong to the Polycomb group Proteins – is essential to adult stem cells maintenance and to tissue-related neoplasia.
The aim of this project as outlined in the original application is the elucidation of molecular mechanisms required for adult neural progenitor and malignant glioma cells self-renewal. To this end, we dissected the regulatory network of Bmi1 by using cutting-edge high-throughput technologies to identify Bmi1 downstream genes, and to ablate their function in vivo, in a mouse model for glioma.
During this project, we have analyzed mouse in primary mouse and human neural progenitor (NPCs) and glioblastoma "stem-like" cells (GSCs), and we identified mediators of Bmi1 activity by combining high-throughput chromatin-immunoprecipitation with in vivo RNAi screening. This experimental approach led us to conclude that:
1) Bmi1 is important to coordinate the appropriate transcriptional response in adult mouse neural progenitor cells exposed to critical regulators of brain homeostasis such as Tgf-β/BMP agonists, or to general factors inducing adult stem cell differentiation such as those contained in the bovine serum.
2) Bmi1 direct target gene Atf3 is tumor suppressor gene in brain tumors, and directly inhibits key oncogenic pathways in glioblastoma stem cells.

The technological advance and its implications for GBM biology as per this study are outlined:

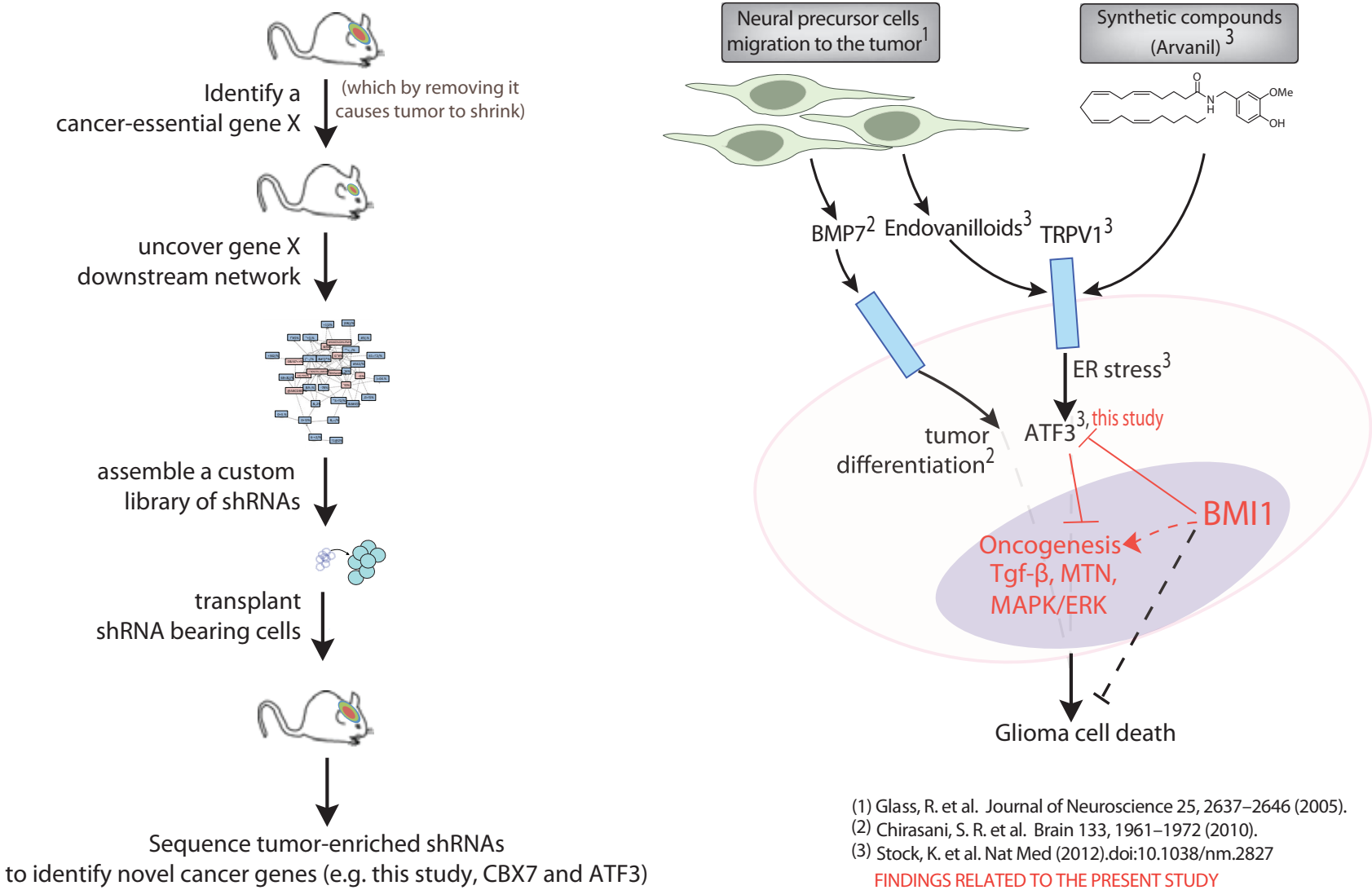
i. Towards the functional identification of critical players in GBM, this study adds on to the rational combination of in vivo HT screenings for epigenetically-silenced tumor suppressor genes as tumor genotype-specific loss-of-function screens are made now possible by next generation deep sequencing. Along with the recent clarification of the GBM genome and transcriptome, these types of screens pose a strong new basis for identifying biomarkers and targets with higher likelihood of therapeutic efficacy.
ii. In order to enable functional studies in line with current limitation of the field of in vivo screens (i.e. not all mouse genes can be tested in one experiment), we exploited the Polycomb pathway –a well-characterized epigenetic regulator of neural progenitor and GBM cell identity in mouse and human cells – to select for candidate cancer genes that could be reliably tested for their impact on in vivo Gliomagenesis.
iii. For the appropriate translation of this study to the human pathology, in this project, we applied technological advances in genome-wide analyses and in vivo shRNA screening to state-of-the-art models of human GBM in mice. Experiments were conducted in validated mouse models for glioma, and implemented using primary mouse and human GBM stem-like cell lines that faithfully represent specific human GBM subtypes. Furthermore, the use of mouse tumor-initiating cells facilitated the

evaluation of candidate cancer genes in a context where tumor initiation is driven only by specific pathways and not confounded by passenger mutations. The functional discovery of GBM genes and their synthetic lethal interactions –in addition to increasing our knowledge of basic mechanisms underlying Gliomagenesis – holds great potential for the possible testing of new combination therapies.

Furthermore, the methodology that we employed to identify Bmi1 downstream genes relevant for glioma genesis and homeostasis has proven to be robust and generally applicable to other cancer-relevant factors, and animal models of disease, providing solid background for future similar research projects. Hence, this study paves the way for several research lines based on a candidate approach to reveal the mechanistical relevance of the herein identified cancer genes in gliomagenesis, but also enables a wider generalization of the herein approach to be extended to additional cancer models, and alternative tumor-relevant pathways.

As a main outcome of our study, we discovered the role for Atf3 and Cbx7 as candidate tumors suppressor genes in the broader scheme of the Tgf-β/BMP and Polycomb pathways. Along with several other genes identified in this study, epigenetically-silenced TSGs may be used as novel prognostic factors as well as driving the rational design of new therapeutic strategies to contrast tumor growth. Importantly, based on our data we have built a model in which Atf3 repression in GBM "stem cells" may be one key component for the crosstalk between Bmi1 and Tgf-β/BMP signaling pathways, ultimately resulting in efficient grafting and explaining Atf3 tumor suppressive function in our system. Therefore, therapeutic strategies aiming at restoring Atf3 expression in glioma cells may be an important step toward the inhibition of cancer cell survival. In fact, our mechanistical dissection of Atf3 pathway revealed several downstream oncogenic and metabolic pathways to be inhibited upon Atf3 de-repression.

The results hereby reported have been submitted for peer-review publication, and the contribution of the Marie Curie Intra-European Fellowship has been acknowledged.



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FINDINGS RELATED TO THE PRESENT STUDY

FCGMOG – Graphical abstract for dissemination.
A refined methodology for cancer genes discovery based on the identification of the gene network associated with validated oncogenes, followed by the pooled library based knockdown of several genes simultaneously (left). In our study, this procedure led to the identification of Atf3 and Cbx7 as tumor suppressor genes in glioma. On the right, the mechanistical dissection of Bmi1 and Atf3 functions in glioma cells. Neural progenitor cells (NPCs) induce glioma cell death via TRPV1 receptors. NPC-derived endovanilloids stimulate TRPV1, leading to increased ATF3 expression and activation of the ER stress pathway via Atf3, resulting in glioma cell death. The synthetic vanilloid arvanil elicits similar tumor-suppressive effects. We found that upon this stimulation, Atf3 directly inhibits oncogenic pathways, and that Bmi1 directly suppress Atf3 expression, leading to increased glioma cell survival. Thus, therapeutic interventions aiming at interfering with Bmi1 and at inducing ER stress may have higher likelihood of success.