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NEW CRISPR/CAS9-BASED MOUSE MODEL FOR THE STUDY OF GLIOMAS

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Cancer is an heterogeneous disease characterized by multiple genetic alterations that impact in the molecular, biochemical and cellular activities. However, the function of most of these events is not yet known. Consequently, animal models will be needed to recreate these alterations that occur in patients which will help to study the biology of cancer and to find new treatment options.

For the study of gliomas, the most common primary central nervous system tumor in adults, a widely-used model is based on the RCAS-Tva system, a somatic gene transfer technique, that closely resemble human gliomas. This model is composed by the replication-competent avian leukosis virus splice-acceptor (RCAS) vectors that target gene expression to specific cell types that have been engineered to express the cell surface receptor Tva under control of the *Nestin* (Ntv-a) or *GFAP* (Gtv-a) promoter in transgenic mice. Moreover, the RCAS vector is quite versatile and allows the incorporation of different genetic tools including shRNA and Cre recombinases. The delivery of the RCAS virus can be controlled in a time and space manner by targeting either astrocytes (Gtv-a) or glioneural progenitors (Ntv-a) either in newborns or adult mice.

The development of the CRISPR/Cas9 gene-editing technology that can target and modify DNA precisely, has achieved an enormous benefit and it is one of the most promising tools for the study of diseases, including cancer.

Here we present a novel murine model combining the RCAS-Tva system with the CRISPR genome editing technology in which we can introduce many genetic alterations to study their roles in gliomas formation.

First of all, we took the *Rosa26-LSL-Cas9-GFP* knockin mice that express Cas9 in a Cre dependent manner. We crossed this mice with the Ntv-a or Gtv-a mice and after crossing with mice expressing Cre under control of *Nestin* or *GFAP* promoter, Cas9 is expressed in a specific subset of cells. We also generated a series of RCAS vectors that allow to subclone any sgRNA of interest. We tested different suppressor genes (TSGs) for gliomagenesis (*Trp53, Cdkn2a* or *Pten)* and successfully knocked-out the expression of them *in vivo* producing high-grade gliomas when co-injected with the driver oncogene Platelet Derived Growth Factor Subunit B (*PDGFB*). Taking advantage of this system, we also generated different genetic rearrangements described in gliomas such as *Bcan-Ntrk1* or *Myb-Qk* by simultaneously delivery of pairs sgRNAs. As a result of these events, we were able to achieve transforming potential both *in vitro* and *in vivo*. Finally, by homology directed repair (HDR) we generated a *BRAF V600E* mutation, frequently observed in different gliomas subtypes. Injection of cells carrying *BRAF V600E* mutation led to high-grade glioma formation.

In summary, we have developed a new RCAS/Tva-CRISPR/Cas9 mouse model combining the advantages of the RCAS/Tva system and the genome editing capacity of the CRISPR/Cas9 that allow to recreate different genetic events that have potential role in gliomagenesis. Furthermore, these different models could be used for pre-clinical studies.