SEVE BALLESTEROS FOUNDATION-CNIO BRAIN TUMOUR JUNIOR GROUP

Massimo Squatrito (until September)
Junior Group Leader

Staff Scientist
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Post-Doctoral Fellow
Miguel Jiménez (until July)

Graduate Students
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OVERVIEW

A decade of studies has underlined the complexity of the genetic events that characterise the genomic landscapes of common forms of human cancer, including gliomas. While a few cancer genes are mutated at high frequencies (>20%), the greatest number of cancer genes in most patients appear at intermediate frequencies (2–20%) or lower. Strikingly, the functional significance of the vast majority of these alterations remains elusive. A current high priority in glioma research is to functionally validate candidate genetic alterations in order to identify those that are significant for cancer progression and treatment response.

In our laboratory, we use a combination of genomic analyses, mouse models, and primary tumour cell cultures, with the main goal of identifying the molecular mechanisms that could provide the basis for novel treatments for glioma patients.

"The main focus of our group is to uncover the genetic alterations present in glioma patients that are responsible for the aggressiveness and the poor treatment response of these tumours."

Technicians
Alicia Marie Gaëlle Leblond (until September)

Titulado Superior (Advanced Degree)

Visiting Scientist
Ernesto Mejías (until March) (Ludwig Maximilian University of Munich, Munich, Germany)

RESEARCH HIGHLIGHTS

Point mutations accumulate in the cells of multicellular organisms over cycles of cell divisions. The majority of point mutations that occur in somatic cells are innocuous to the organism. However, some somatic mutations are capable of driving the tumorigenic transformation of cells. To properly recapitulate the high genetic heterogeneity observed in cancer patients, we need flexible and informative genetic models able to recreate not just a handful of genetic alterations, but potentially dozens.

Base editing is a genome editing method that directly generates precise point mutations in genomic DNA or in cellular RNA without directly generating DNA double-strand breaks (DSBs) or requiring a donor DNA template. DNA base editors (BEs) comprise fusions between a catalytically impaired Cas nuclease and a base-modification enzyme that operates on single-stranded DNA (ssDNA) but not double-stranded DNA (dsDNA). To faithfully model *in vivo* a variety of brain tumour-associated mutations, we have combined CRISPR/Cas9-BE3 base editing with the RCAS-TVA system. We recently generated 2 different BE3-TVA mouse strains by crossing Ntv-a and Gtv-a mice with a tetracycline-responsive BE3 mouse model, Tg.tetO-BE3, kindly provided by the Dow Laboratory at Weill Cornell Medicine in New York. In these new strains, BE3 expression is transiently activated by the transduction of a RCAS-Tet-Off vector that carries the tetracycline transactivator (tTA) protein and is subsequently silenced by treating the mice with doxycycline. A continuous expression of the BE3 editor could potentially lead to undesired base pair deletions over time. Together with the RCAS-Tet-Off, mice are injected with RCAS-gRNA constructs for the desired point mutation. Lastly, these vectors can be combined with either RCAS-PDGFA or RCAS-Sonic Hedgehog (Shh) to model gliomas and medulloblastomas, the most frequent brain tumours in adults and children, respectively. Such a versatile model will now allow us to generate relevant animal models that more closely recapitulate a given patient's tumour. ■

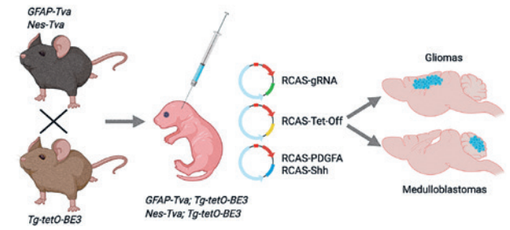

FIGURE BE3-RCAS-TVA system to model brain tumour-associated point mutations.

PUBLICATIONS

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