REPORT
25th SOCIAL RETURN OF THE RESEARCH
CANCER

VALIDATION OF THE CALCIUM CHANNELS AS THERAPEUTIC TARGETS AGAINST PRIMARY AND RECURRENT GLIOBLASTOMA

Dr Carles Cantí Nicolàs
IRBLl Institut de Recerca Biomèdica de Lleida
Dr Marta Maria Alonso Roldán
Clínica Universitaria de Navarra - Universidad de Navarra

1. Summary

Glioblastoma multiforme (GBM) is an incurable cancer, with a patient median survival below 14 months despite extensive surgery and standard radiotherapy and chemotherapy. In recent years, different plasma membrane calcium channels have been unveiled as key contributors of GBM proliferation and survival, thus becoming potential chemotherapeutic targets. Application of some pharmacological blockers of a particular class -the T-type channels (TTCC or Cav3 family)- reduces cancer cell viability, and clinical trials have been performed to determine the safety of these TTCC blockers in GBM patients. However, we have found that these compounds have limited selectivity for TTCC in cancer cells.

Here we have studied the effects of targeting TTCC and other calcium channels in GBM. First, we have analyzed the expression panel of different voltage gated calcium channels, regulatory subunits and potassium calcium- dependent channels in GBM cell lines to test the effects of their pharmacological or gene targeting. GBM cells exposed to tetralin derivative NNC-55-0396 (NNC), display high levels of ER stress pathways and autophagy, adaptive cellular processes playing pivotal roles in tumor resistance. We have defined the mode of action of NNC, which causes massive cell death of tumoral cells through the mobilization of calcium from ER stores, activating the IRE1□ branch of the unfolded protein response to ER stress and deregulating the autophagic cell process at different levels. On the other hand, in mouse GBM preclinical models we have observed improved survival of animals treated with NNC.

We have also analyzed the effects of depleting GBM cells of this type of channels in a *Drosophila* GBM model. Fly gliomas silenced for the Cav3 gene ortholog Ca-α1T showed reduced glial cell proliferation and decreased activation of proliferation pathways.

2. Results

We found that GBM cells express calcium channels of the Cav1, Cav2, and Cav3 families, as well as their auxiliary subunits, β and α2δ. Additionally, we found evidence that Cav3.1 forms functional tandems with two types of calcium-dependent potassium channels expressed in GBM cells: KCa1.1 and KCa3.1.

We have focused on the attack of Cav3 channels in the GBM. On the pharmacology front, we observed that a NNC-55-0396, a member of the tetralin family with the ability to block Cav3 channels, negatively affect GBM cells viability, and have described their mode of action: it acts off-target (that is, acting in tumor cells on molecular targets that are not the channels they were intended to block). However, we found that this compound induce massive death of GBM cells in culture, dependent on the IRE1α pathway of ER stress and disrupting the autophagic process (on which they play a dual role, inducing and blocking it at the same time). *In vivo* experiments, furthermore, indicate that NNC-55-0396 moderately increases the survival of mice with GBM, and that the tumors exhibit increased macrophage infiltration.

On the other hand, on the gene silencing approach, in the GBM model of *Drosophila melanogaster*, we have showed that silencing Ca-α1T (ortholog of Cav3), Cacophony (ortholog of Cav2) or Slowpoke (ortholog of KCa1.1) reduce the proliferation of GBM cells. In addition, we have found that signaling by Ca-α1T involves the PI3K and ERK pathways and that Ca-α1T expression is increased in the *Drosophila* glioma, thus promoting higher intracellular calcium levels with a possible oncogenic implication.

3. Relevance with future implications

Our results demonstrate that T-type calcium channels (Cav3 family) play a relevant role in tumor physiology and confirm them as targets for gene or pharmacological action against GBM, together with other channels (calcium-dependent potassium channels) with whom they form functional tandems. Additionally, our data demonstrate that the cytotoxicity due to the application of pharmacological blockers of the aforementioned channels is through a non-specific mechanism involving endoplasmic reticulum stress and autophagy, thus dysregulating two key adaptive mechanisms that support tumor resistance.

Although the clinical application of these findings is not immediate, our work contributes to establishing the foundations of new chemotherapies aimed at reducing the resistance of GBM to current treatments. It is important to highlight that the molecular targets of our study also play key physiological roles within the tumor environment, including neurons, microglia and astrocytes. Our results are framed in an

emerging field that aims to control tumor growth through the modulation of ion channels and associated proteins, using pharmacological tools developed in neurological research (particularly in the fields of epilepsy and the physiology of pain).

4. Bibliography

1: Alza L*, Visa A*, Herreros J, Cantí C. T-type channels in cancer cells: Driving in reverse. **Cell Calcium.** 2022 Jul;105:102610. doi: 10.1016/j.ceca.2022.102610. Epub 2022 Jun 6. PMID: 35691056. (* co-authors).

2: Visa A, Alza L, Cantí C, Herreros J. Tetralol derivative NNC-55-0396 induces glioblastoma cell death by activating IRE1α, JNK1 and calcium signaling. **BiomedPharmacother.** 2022 May;149:112881. doi: 10.1016/j.biopha.2022.112881. Epub 2022 Mar 31. PMID: 35367758.

3: Visa A*, Alza L*, Casas-Benito A, Herreros J, Cantí C. Targeting T-type channels in cancer: What is on and what is off? **DrugDiscovToday.** 2022 Mar;27(3):743-758. doi: 10.1016/j.drudis.2021.11.021. Epub 2021 Nov 24. PMID: 34838727. (* co-authors).

4: Bayona C*, Alza L*, Randelovic T, Sallán MC, Visa A, Cantí, Ochoa I, Oliván S* & Herreros J*. Tetralol derivative NNC-55-0396 targets hypoxic cells in the glioblastoma microenvironment: an organ-on-chip approach. **Cell Death Disease**. 2024. Feb 10;15(2):127. doi: 10.1038/s41419-024-06492-1 (* co-authors).

5. Visa A, Marrodan L, Jonathan S, Casals M, Darabi A, Alonso MM, Herreros J, Canti C. "Anti-tumoral compound NNC-55-0396 induces calcium-dependent autophagy that becomes defective by concurrent lysosomal alkalinization in GBM cells". In preparation.

6. Alza L, Montes-Labrador P, Casali A, Casas-Tintó S, Herreros J, Cantí C. "Voltage-gated Ca2+ channels and Ca2+-dependent K+ channels regulate tumor cell proliferation and membrane extension in fly gliomas". In preparation.