UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.4

Proponent: Dr. Michela Ceriani

Project Title: Unraveling the secret of ralgps2 tdark: a new potential target in glioblastoma

Scientific background and 'open issues'

Glioblastoma (GB) is the most aggressive form of glioma and the common primary tumor of the central nervous system accounting for approximately 50% of gliomas with a median lifespan of 15 months from diagnosis. GB is classified as a rare cancer (annual incidence 1/33.330 individuals) and is particularly challenging among brain tumors due to its heterogeneous microenvironment representing a strategic tool for treatment escape and recurrence after surgical resection. Currently, no resolutive treatment exists for recurrent GB. In this scenario, communication between cells plays a key role in GB drug resistance: more specifically, GB cells form tunneling nanotubes (TNTs) which are probably involved in tumor progression and recurrence. TNTs are actin-based highly-dynamic membrane protrusions that enable cells to directly communicate with each other over long distances and play a central role in cancer progression and malignancy. One of the proteins involved in TNT formation is RalGPS2, a Ras-independent Guanine Nucleotide Exchange Factor (GEF) for RalA GTPase, whose knock-down in G523NS patient-derived GB cell line affects cell proliferation. RalGPS2 contains a PxxP motif and a PH domain and is classified as a Tdark gene (https://pharos.nih.gov/targets/RALGPS2) due to the minimal knowledge of its biological function, obtained by our research group, and limited tools for its analysis. RalGPS2 is involved in several other cellular processes including cytoskeletal remodeling and actin reorganization, survival, and cell differentiation. Besides, the RalGPS2 PH-PxxP region acts as a dominant negative (DN) for RalA activation, inhibiting actin polymerization and TNT formation similarly to RalGPS2 silencing. Furthermore, another study demonstrated that the overexpression of LGI1 (a tumor suppressor gene involved in abrogating cell migration and invasion) in the T98G glioma cell line down-regulated RalGPS2 gene expression resulting in a reduction of cell motility. The main hypothesis of the proposal is that RalGPS2 in GB can be involved in TNT formation among cancer cells and many different cell subtypes within the TME, and in other cellular processes involved in tumor cell proliferation and progression. The proposal described here is the next step in paving the clinical translation of the proposed approach that ultimately may have a significant impact on the treatment of patients with GB.

Objectives

We will investigate cell-cell communications and metabolism, two crucial factors that have a significant impact on the dynamic adaptation of neoplastic and non-neoplastic cells within

the tumor microenvironment, facilitating proliferation, infiltration, and therapy resistance. Furthermore, we will pay particular attention to the study of the effects of RalGPS2 knockdown considering the results reported in a recent paper demonstrating that downregulation of RalA and RalB reduces the viability of GB cells growing as tumorspheres, suggesting a possible role of these GTPases and their GEFs (RalGPS2) in the survival of GB stem-like cells.

Methodologies

- 2D-3D mammalian cell colture; transient and stable transfection; migration, invasion and co-colture assay.
- Neurosphere growth.
- Microscopy (confocal, fluorescence…)
- develop a smart carrier system for mRNA delivery in GB

Collaboration / Co-tutoring opportunities

External collaborators:
-Prof. Serena Pellegatta, Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta | IRCCS Besta · Division of Molecular Neuro-Oncology

Internal collaborators:
-Dr. Elena sacco
-Prof. Renata Tisi
-Prof. Marzia Lecchi
-Prof. Francesca Re

Project's Sustainability & Mobility

RalGPS2 has been cloned in 2002 by Michela Ceriani. Actually there are only 7 articles on PubMed on RalGPS2 and Michela Ceriani is an author of 5 of them. Therefore, she is the leading experts in the study of this Tdark. Furthermore, she has studied tunneling nanotubes on different cells model (breast, bladder, kidney), included Glioblastoma cells, approaching to different methodologies to characterize their induction, functionality and classification.

Pertinent research article published by the proposer/s

- Taiarol L, Formicola B, Fagioli S, Sierri G, D'Aloia A, Kravicz M, Renda A, Viale F, Dal Magro R, Ceriani M, Re F. The 3.0 Cell Communication: New Insights in the Usefulness of Tunneling Nanotubes for Glioblastoma Treatment. Cancers (Basel). 2021 Aug 8;13(16):4001. Doi:10.3390/cancers13164001. PMID: 34439156; PMCID: PMC8392307.
- D'Aloia A, Berruti G, Costa B, Schiller C, Ambrosini R, Pastori V, Martegani E, Ceriani M. RalGPS2 is involved in tunneling nanotubes formation in 5637 bladder cancer cells. Exp Cell Res. 2018 Jan 15;362(2):349-361. doi: 10.1016/j.yexcr.2017.11.036. Epub 2017 Dec 6. PMID: 29208460.
- Formicola B‡, D'Aloia A ‡, Dal Magro R, Stucchi S, Ceriani M‡, Re F‡*. Differential Exchange of Multifunctional Liposomes Between Glioblastoma Cells and Healthy Astrocytes via Tunneling Nanotubes. Front Bioeng Biotechnol. 2019 Dec 12;7:403.

doi: 10.3389/fbioe.2019.00403. PMID: 31921808; PMCID: PMC6920177. ‡ These Authors contributed equally to this work.

Putative foreign institutions for achieving the required ordinary mobility

- Giampietro Dotti laboratory, University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center