Human Glioma Stem Cells as a personalized model to identify novel biomarkers and therapies

I. Manini[1], D. Mangoni[1], A. Zanello[1], T. Ius[2,3], S. Piazza[4], R. Verardo[4], Y. Ciani[4], C. di Loreto[1], C.A. Beltrami[1], A.P. Beltrami[1], D. Cesselli[1]

[1]Dept. of Medical and Biological Sciences; University of Udine; Udine, 33100; Italy
[2]Dept. of Neurosurgery; Azienda Ospedaliero-Universitaria of Udine; Udine, 33100; Italy
[3]Dept. of Robotics, Brain and Cognitive Sciences, Instituto Italiano di Tecnologia, Genoa, 16163; Italy
[4]CIB, LNCIB, Area Science Park, Trieste; Italy

Background: Low-grade gliomas (LGG) are characterized by a heterogeneous clinical behavior that is partly foreseeable by available prognostic and predictive markers, rendering difficult the decision-making process.
Methods and results: we isolated glioma-associated stem cells (GASC) from LGG (n=40) and HGG (n=73). GASC were characterized by stem cell features, aberrant anchorage-independent growth, tumor-supporting ability mediated by exosomes but were devoid of genetic alterations, thus representing a population of glioma supporting stem cells.
To assess the ability of this patient-based in vitro model to identify novel biomarkers, we started from a case study composed of GASC obtained from HGG (n=13) and LGG (n=12) and utilizing a ROC analysis, we identified 9 GASC surface markers whose expression level could be utilized to correctly classify the two groups. The prognostic value of the score based on the expression of these 9 parameters was assayed on 40 subsequent LGG-patients (median follow-up 36 months, range 13-76). At the multivariate Cox analysis, the GASC-based score was the only independent predictor of overall survival (HR 8.84, CI 95% 2.15-36.28) and malignant progression free-survival (HR 3.74, CI 95% 1.60-8.75), out-performing the state-of-the-art histological, clinical and molecular LGG prognostic factors, including Ki67 expression, IDH1 and 2 mutation, 1p-19q co-deletion and MGMT-promoter methylation.
To establish whether GASC could be used to identify novel therapeutic approaches, we isolated glioma-associated stem cells (GASC) from LGG characterized by good prognosis (overall survival >48 months and without MRI evidence of malignant progression, LGG-good; n=3) and from LGG characterized by a rapid transformation into an anaplastic form (LGG-bad; n=3). Next generation sequencing (NGS) of transcripts and miRNAs of GASC were performed and differential gene expression analysis was performed using a read-count-based statistics employing edgeR. When the transcriptional program of LGG-bad vs LGG-good was evaluated by using Upstream regulators analysis (included in Ingenuity Pathway Analysis), we have identified: 1. IL1, IL6, NFkb as strong up-stream regulator suggesting, in the LGG-bad, the presence of a senescence-associated secretory phenotype that, as shown in the glioblastoma, could favor a pro-tumourigenic milieu; 2. Molecules (drugs, chemical inhibitors and miRNAs) potentially able to revert the observed transcriptional program. Interesting, many of this molecules are known for their anti-inflammatory role (aspirin, cyclosporine) or for their ability to revert senescence (resveratrol). The list includes also dietary substances such as genistein, cucurmin and caffeine. For most of them, including statins, in vitro and in vivo study support an anti tumor effect. Because many of these drugs are characterized by low toxicity and are already used in chronic settings, their putative role as adjuvant treatment can be extremely interesting.
Regarding miRNAs, we have identified miRNA significantly up-regulated in LGG-bad (e.g. miR1-304-3p, miR-873-3p, miR-873-5p, miR-551a and miR-876-3p) and in LGG-good (e.g. miR-1-3p, miR-133a-3p, miR-133a-5p, miR-551b-3p), respectively.
Conclusions: GASC can represent a patient-based approach able to provide a groundbreaking method to predict prognosis and to direct current therapies or to exploit novel strategies aimed at targeting the tumor stroma.