Sorafenib induces a selective depletion of human glioblastoma cancer stem cells by down-regulation of MCL-1

F. Barbieri[1,2], F. Mattioli[1], C. Fucile[1], E. Carra[3,4], D. Marubbi[3,4], A. Pattarozzi[1], R. Wurth[1], M. Gatti[1], R. E. Favoni[3], A. Daga[4], T. Florio[1,2]

[1]Section of Pharmacology - Dept of Internal Medicine (DIMI); University of Genova; Genova, Italy
[2]Center of Excellence for Biomedical Research (CEBR); University of Genova; Genova, Italy
[3]Dept of Experimental Medicine (DIMES); University of Genova; Genova, Italy
[4]Gene Transfer Lab; IRCSS AOU San Martino-IST; Genova, Italy

Glioblastoma (GBM) is the most common and aggressive brain tumor of adults, characterized by high vascularization and invasiveness. Median survival of patients with GBM after standard-of-care radiation and chemotherapy with temozolomide is about 14 months. GBMs display cellular hierarchies containing tumorigenic stem cells (cancer stem cells, CSCs), a rare subpopulation originated by the acquisition of altered self-renewal activity in either adult neural stem cells, differentiated neurons or glial cells. CSCs display high radio- and chemo-resistance that cause treatment failure, tumor relapse and metastasis formation, through their exclusive ability to self-renew and repopulate the tumor. Thus, the identification of new therapeutic approaches affecting CSC survival and proliferation represents a great challenge. Using protocols developed for neural stem cells, primary cultures highly enriched in GBM CSCs can be obtained in vitro, representing a reliable model to identify drugs selectively targeting this cell population.

The aim of this study was to investigate the effects of the multi-kinase inhibitor sorafenib on the survival and growth of cells isolated from six human GBMs and maintained in CSC-enriching conditions.

CSC cultures were obtained by selection of GBM cells in a stem-permissive medium, supplemented with EGF and bFGF; differentiation was induced by shifting the cells in a medium devoid of growth factors and containing fetal calf serum. Cell viability, proliferation and apoptosis were assessed by MTT assay, BrdU incorporation and annexin V binding, respectively. Confocal immunofluorescence and Western blot were used to assess stem, glial and neuronal marker expression and intracellular signaling pathways. Tumorigenic potential of cultures was assayed by orthotopic xenotranplantation of cells in non-obese diabetic severe combined immunodeficient (NOD-SCID) mice.

Sorafenib reduced the proliferation of GBM cultures, and this effect was dependent, at least in part, on the inhibition of PI3K/Akt and MAPK pathways, both previously reported to be involved in gliomagenesis. Moreover, sorafenib significantly induces apoptosis/cell death via downregulation of the pro-survival factor Mcl-1. To confirm the depletion of the CSCs pool by sorafenib, the expression of differentiation (glial fibrillary acidic protein, GFAP and microtubule-associated protein 2, MAP2) or stem (nestin, Olig2 and Sox2) markers was evaluated in treated and untreated cells. Sorafenib selectively affects GBM CSC survival, since it caused culture enrichment in GFAP and MAP2 positive differentiated cells, and down-regulation of the expression of stem markers required to retain malignancy. Moreover, sorafenib impairs the clonogenic potential of GBM CSCs in vitro, suggesting its preferential effects on cells with self-renewal capacity. The selectivity of these effects on GBM stem cells is further supported by the significantly lower sensitivity of differentiated GBM cultures as compared with their undifferentiated counterpart. To confirm in vitro results, mice were orthotopically transplanted with CSCs survived to 48h exposure to sorafenib or left untreated. Tumor development was observed in 85% of mice injected with untreated CSCs, while only 28% of mice injected with sorafenib-treated cells formed a tumor mass, confirming that the drug inhibits the tumorigenic potential of sensitive GBM stem cultures.

Our data clearly demonstrated that sorafenib effectively induces selective anti-proliferative and pro-apoptotic effects on GBM CSC-enriched cultures, resulting in impairment of clonogenic ability in vitro and tumorigenicity in vivo. Since current GBM therapy enriches the tumor in cancer stem cells, the evidence of a selective action of sorafenib on these cells is therapeutically relevant, at least as proof of principle, since, so far, results from first phase II clinical trials did not demonstrate the same efficacy in vivo.

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