Breaking the malignant triangle in glioblastoma - ErbB1/nucleolin/Ras

Sari Trangle Schokoroy 1, Yona Goldshmit1,2, Yoel Kloog1, Ronit Pinkas-Kramarski1

1Department of Neurobiology, Tel-Aviv University, Ramat-Aviv, Israel
2Australian Regenerative Medicine Institute, Monash University, Australia

Correspondence: Ronit Pinkas-Kramarski
E-mail: lironit@post.tau.ac.il
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Glioblastoma is one of the most malignant tumors in humans, with poor diagnosis and a low survival rate. These tumors overexpress the ErbB1 receptor and have high levels of cell surface nucleolin, which serves as an indicator for disease grade. We have previously identified a crosstalk between three oncogenes: ErbB1, Ras and nucleolin. This lead us to suggest a combined treatment using FTS, to target Ras protein, and GroA, to target cell surface nucleolin, in order to break this malignant synergy. Here we review our recent findings and suggest a model explaining drugs activities: inhibition of Ras using FTS mainly reduces cell viability and motility and inhibition of nucleolin using GroA reduces the cell proliferation. The combined treatment has a more pronounced effect on glioblastoma growth.

Keywords: Glioblastoma; ErbB1; Ras; nucleolin; FTS; GroA


Signal transduction pathways are at the center of a diverse range of essential biological processes within cells, including proliferation, differentiation, morphogenesis, and cell death [1,2]. Cells sense the environment through several classes of receptors, which bind growth factors and hormones [3,4]. The ErbB subfamily of receptors belongs to the receptor tyrosine kinases family (RTKs), which lies at the head of a complex signal transduction cascade. ErbB1 also known as epidermal growth factor receptor (EGFR) is one of the four ErbB subfamily members. Deregulation of these receptors' expression and their growth factors might lead to excessive cell proliferation, survival, and eventually, to the development of malignancy. The majority of tumors originating in the central nervous system are gliomas. Gliomas are a heterogeneous group of neoplasms that overexpress the EGFR receptor [5,6]. One of the most severe subtypes of gliomas is the glioblastoma. Despite notable recent achievements in oncology, malignant glioblastoma multiform (GBM) has a 14 months prognosis after diagnosis, posing a challenge to researchers [7].

Nucleolin is a multifunctional, ubiquitously expressed phosphoprotein, whose expression often correlates with increased cellular proliferation [7-9]. Nucleolin is localized predominantly in the nucleoli, but it transports between the nucleus and cytoplasm, and it can be also found on the cell surface [8,10,11]. Cytoplasmic and cell surface nucleolin was found to be highest in tumors and other rapidly dividing cells and consequently its expression has become an indicator in studies of cell proliferation and terming the malignancy grade of the glioblastoma [8,12-14]. Expression of extensive levels of nucleolin has been demonstrated in glioblastoma cell lines and primary astrocytoma cells, contrary to astrocytes in normal human brain [15]. It was previously shown that inhibition nucleolin at the cell-surface and blocking its activities, suppresses growth of glioblastoma cell
lines that may also express high levels of activated Ras protein. We have previously identified an oncogenic crosstalk between nucleolin, ErbB1 and Ras proteins, and suggested that combined treatment may have stronger inhibitory effects than treatment with each drug alone.

Recently, we tested the effect of combined treatment of two drugs; the first is GroA (AS1411), a quadruplex-forming G-rich oligonucleotide aptamer that can bind specifically to nucleolin by recognizing its three-dimensional structure. The second is FTS (Salirasib), a small synthetic Ras farnesylesteine imitative molecule, which was demonstrated to specifically disrupt the association of active Ras proteins with the plasma membrane. We tested the effect of combined treatment with the two drugs on the human glioblastoma cancer cell line (U87-MG). Our results revealed that combined treatment of FTS and GroA impairs cell viability effectively and beyond the inhibition observed by each drug alone. We have used the proliferation marker Ki67 and Bromodeoxyuridine (BrdU) incorporation assay in order to test whether the observed reduction in cell viability was due to cell proliferation inhibition. While treatment with each of the drugs alone reduced the levels of Ki67 protein, treatment with GroA had a better effect than treatment with FTS alone, and the combined treatment was the most effective treatment. Similarly, treatment with GroA alone inhibited BrdU incorporation almost completely, even in the presence of the ligand, EGF, therefore, we concluded that the interaction of EGFR and nucleolin mediates cell proliferation. Next, we examined whether part of the cell growth inhibition was due to cell death. For that matter we used the Hoechst dye exclusion assay and active caspase-3 immunostaining assays. In both cases results demonstrated that treatment with FTS mainly induced cell death (Figure 1 and 2). Additionally, we examined the combined treatment effect on cell migration and demonstrated that treatment with each drug alone had an inhibitory effect on the gap closure, with FTS having the more significant effect. Therefore, we suggested that in vitro, each drug affect cell viability differently; GroA mainly influences the proliferation and FTS the cell survival.

We recently published similar results using four cell lines of human colon and prostate cancers, showing the combined effect of FTS and GroA on cell viability and cell migration. These results establish the strong inhibitory effect of co-treatment with FTS and GroA. Furthermore, in U87-MG cells, the combined treatment of FTS and GroA reduced the ErbB1 receptor phosphorylation, as well as the interaction between the receptor and nucleolin both in vitro and in vivo. The combined treatment inhibited the two main pathways activated by the ErbB1 receptor: the Ras /MAPK and the PI3K signaling pathways, both of which can affect various biological effects within the cells. Further In vivo experiments using U87-MG tumor xenografts have shown similar effect to those observed in the in vitro experiments; while FTS induced activated caspase-3, GroA inhibited cell proliferation. When the co-treatment was applied, both active caspase-3 and cell proliferation inhibition were observed. However, the combined treatment of FTS and GroA had no additive or synergistic effect on tumor size, compared to each drug alone.

In conclusion, we have demonstrated that in glioma cells, while GroA has a strong inhibitory effect on cell proliferation, FTS enhances cell death and inhibits cell migration, each drug affects different pathway (Figure 2). Taken together, these data suggest that the combination of FTS and GroA can affect different cellular pathways, leading to a stronger and more effective treatment against glioblastomas.
Conflicting interests

The authors have declared that no competing interests exist.

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