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Preclinical evidence that SSR128129E – A novel small-molecule multi-fibroblast growth factor receptor blocker – Radiosensitises human glioblastoma

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Abstract Resistance of glioblastoma to radiotherapy is mainly due to tumour cell radioresistance, which is partially controlled by growth factors such as fibroblast growth factor (FGF). Because we have previously demonstrated the role of FGF-2 in tumour cell radioresistance, we investigate here whether inhibiting FGF-2 pathways by targeting fibroblast growth factor receptor (FGFR) may represent a new strategy to optimise the efficiency of radiotherapy in glioblastoma.

Treating radioresistant U87 and SF763 glioblastoma cells with the FGFR inhibitor, SSR12819E, radiosensitises these cells while the survival after irradiation of the more radiosensitive U251 and SF767 cells was not affected. SSR128129E administration to U87 cells increases the radiation-induced mitotic cell death. It also decreased cell membrane availability of the FGFR-1 mainly expressed in these cells, increased this receptor's ubiquitylation, inhibited radiation-induced RhoB activation and modulated the level of hypoxia inducible factor, HIF-1α, a master regulator of hypoxia, thus suggesting a role of FGFR in the regulation of hypoxia pathways. Moreover, treating orthotopically U87 xenografted mice with

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SSR128129E before two subsequent local 2.5 Gy irradiations significantly increased the animals neurological sign free survival (NSFS) compared to the other groups of treatment. These results strongly suggest that targeting FGFR with the FGFR blocker SSR128129E might represent an interesting strategy to improve the efficiency of radiotherapy in glioblastoma.
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1. Introduction

Standard treatment of patients with glioblastoma (GBM) associating radiotherapy and temozolomide has only increased the median survival by 3 months compared to radiotherapy alone [1]. Almost all the patients will die of a relapse in the radiation fields as a result of low tumour sensitivity to radiation. This tumour radioresistance is not only due to the modulation of different biological signal transduction pathways but also to a cross-talk between the tumour cells and the microenvironment. Our team, as well as others [2,3], have shown that fibroblast growth factor (FGF)-2 regulates cell response to ionising radiation. Expressing FGF-2 in tumour cells induces a radioresistant phenotype [4] through the small GTPase RhoB [5,6]. αvβ3 integrins also control GBM U87 cells radioresistance via RhoB as well as HIF-1α expression [7,8]. Moreover, fibroblast growth factor receptor (FGFR)-1 and αvβ3 integrin expression in tumour cells are independent factors of poor prognosis of overall survival but also, for FGFR-1 expression, of time to progression in glioblastoma [9] while a combined profile of FGF-2/β3 integrin expression is a signature of the worst local control in patients exclusively treated with chemo-radiotherapy for locally advanced lung cancer [10]. These results led us to hypothesise that inhibiting the biological pathways of FGF-2 might overcome the radioresistance of glioblastoma by controlling tumour cell radiosensitivity and microenvironment.

FGFs execute their biological actions by binding and activating cell surface FGF receptors (FGFRs). The four types of FGF receptors (FGFR-1, FGFR-2, FGFR-3 and FGFR-4) are composed of an extracellular ligand-binding domain, a single transmembrane domain and a cytoplasmic domain containing the catalytic protein tyrosine kinase core. FGF-2 binds to FGFR-1, 2 and 4 (for review [11]). We very recently described an extracellularly acting small molecule, allosteric inhibitor of FGF receptor signalling at nanomolar concentrations, SSR128129E [12,13]. In that report, we investigated for the first time whether this new class of FGFR inhibitor might reduce glioblastoma radioresistance. Our results demonstrated that SSR128129E significantly increased the radiosensitivity of human glioblastoma cells *in vitro* and *in vivo* and modulates *in vitro* HIF-1α expression under hypoxia.

2. Material and methods

2.1. Cell culture

Glioblastoma U87, U251, SF763 and SF767 cell lines (obtained from ATCC, Manassas, VA, United States of America (USA)) were maintained as monolayers at 37 °C in DMEM medium 10% FCS. Hypoxic conditions were obtained as previously described [8].

2.2. Flow cytometry

Exponentially growing cells were incubated 1 h at 4 °C with 5 μg/ml of the specific antibodies (anti-FGFR-1 (Ab823;Abcam); anti-EGFR (Ab-4 (cloneF4); Neomarkers); or matched control isotype IgG1 (purified immunoglobulin isotype, Dako (Trappes, France)) at similar concentrations, followed by secondary fluorescein-labelled IgG1 (anti-mouse IgG1 coupled to FITC, Dako (Trappes, France), then analysed in a FACScan flow cytometer (Becton Dickinson, Franklin Lanes, NJ) using Cell Quest acquisition and analysis software. To evaluate membrane antigen expression, we determined the mean fluorescence intensity (MFI) and/or the mean specific fluorescence index (SFI). The SFI was calculated with the following formula: (MFI with the specific antibody-MFI with the isotype control)/MFI with the isotype control. Data on 30^4 cells were collected for analysis and all experiments were performed at least three times.

2.3. RhoB activation assay

The Rho binding domain of Rhotekin (GST-Rhotekin RBD pulldown assay) was performed as previously described [7]. A detailed protocol is given in Supplementary methods.

2.4. Radiation survival determination

Clonogenic and limited dilution assays were performed as already described [4,6]. A detailed protocol is given in Supplementary methods.

2.5. Measurement of cell death

Mitotic cell death was determined by quantifying the percentage of giant multinucleated cells [6,7]. (See Supplemental material and methods).

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