Chapter 5

Imaging of glycolytic metabolism in primary glioblastoma cells with RIMChip

5.1. Introduction

Glioblastoma(GBM) is one of the most common brain tumors[1]. It is composed of heterogeneous subpopulations of phenotypically distinctive tumor that express different level of oncogenes such as epidermal growth factor receptor variant III (EGFRvIII)[2]. GBM is also remarkably resistant to targeted therapies, including EGFR inhibitors despite frequent expression of EGFRvIII. Previous works have presented a number of important mechanisms underlying resistance[3,4], but the contribution of cellular heterogeneity to resistance is not well understood.

In spite of cellular heterogeneity, some solid tumors exhibit hallmarks of homogeneous systems. For example, Lander's group has reported that luminal, basal, and stem-cell phenotypes purified from a breast tumor can, over time, regenerate the characteristic cellular heterogeneity (the phenotypic equilibrium) of the original tumor[5]. This behavior, in turn, has consequences for therapeutic resistance[6].

In this chapter summarizing the current work, I will introduce the preliminary results showing the heterogeneous glucose metabolism in cancer cells and the possibility of RIMChip as a screening tool for drug treatments. We begin with a patient-derived, primary glioblastoma cell line (GBM39)[7], which has heterogeneous EGFRvIII expression. By examining the glycolytic activity of cells according to their expression level of EGFRvIII,

we expect to see the relationship between gluocose metabolism and EGFR signaling pathway.

Also, we will explore the possibility of RIMChip platform as a drug screening tool by applying EGFR+ subpopulations of GBM39 cells undergoing different types of drugs.

5.2. Experimental Methods

5.2.1. Preparation of cells

Primary glioblastoma cells, GBM39 cells, were cultured to form neurospheres in DMEM F-12 (Invitrogen) supplemented with 2% of B27 (GIBCO), 1% of Glutamax (GIBCO), 1% of PSQ (GIBCO), 20 ng/mL of EGF (Sigma), 20 ng/mL of FGF (Sigma) and 1μg/mL of Heparin (Sigma). Every 3 days, growth factors were added to the cell culture media, and the growth media was changed every 2 weeks. In the case of ERZ cells, GBM39 cells having the resistance to erlotininb, neurospheres were cultured in the growth media having 5 μM of erlotinib (Chemietek) to maintain the resistance.

In order to make a single-cell suspension, neurospheres were collected by the centrifugation at 400 g for 4 min. These neurospheres were then re-suspended in 3mL of TriplE (Invitrogen) for 5 min at cell incubator, followed by the addition of the growth media to neutralize TriplE. This solution was spined down again, and the single-cell suspension was obtained after the discard of supernatant followed by the resuspension of cell pellet into the growth media.

5.2.2. Cell sorting

In order to study the glycolytic metabolism along with the expression level of EGFRvIII in glioblastoma cells, single-cell suspension of GBM39 cells were prepared. Cells were washed with cold PBS followed by the resuspension to 20 million/mL in FACS buffer, PBS supplemented with 2% of FBS. After the removal of 20uL cell solution for the negative control, EGFRvIII antibody was added to the single-cell suspension to be reacted with cells for 20 min on ice. Cells were washed twice with ice-cold PBS, and re-suspended in FACS buffer containing secondary Anti-Mouse 488 (Jackson Immunolabs) for tagging with chromophore. After the incubation for 20 min on ice, cells were washed with ice-cold PBS twice, followed by the resuspension into Sorting Buffer, PBS supplemented with 0.5% FBS, at the concentration of 20 million/mL. According to their fluorescent signals, cells were sorted by a flow cytometer.

In order to measure the effect of drug treatment to metabolism of the EGFR+ subpopulation of GBM39 cells, the single-cell suspension was applied to the magnetic beads conjugated with EGFR antibody. After the sorting, cells were released and placed in a laminin coated cell culture petri for overnight to eliminate dead cells floating among growth media instead of binding to the laminin coated surface.

5.2.3. In vitro radio assay with RIMChip

The detailed procedure to fabricate RIMChip is introduced in Chapter 3. Cells at a concentration of 3×10^6 were injected into the microchip from inlets by pressing a pipettor for 5 sec to sufficiently cover all microchannels with cells followed by the incubation in a CO_2 incubator at 37 °C for 4 h. After incubation, the microchannels were flushed 2× with

flowing ~ 20 µl PBS using the same pipettor, each time for 30 sec, to flush out any residual glucose. About 10 µl 18F-FDG in PBS was loaded into the microchannels by pressing the pipettor for 15 sec. The whole microchip was then incubated for 30 min in a CO2 incubator at 37 °C. It was the flushed 2× with flowing ~ 20 µl PBS using the same pipettor. The cells in the microchip were then for imaging by the beta-box.

For the imaging of glycolytic metabolism in cells under drug treatment, we introduced CC214, U012610, or the combination of CC214 and 5 µM erlotinib, to EGFR+ GBM39 cells 24 hour prior to the experiment. The remaining procedure was the same as above.

5.3. Results and Discussion

5.3.1. Imaging of glycolytic metabolism in glioblastoma cells according to their expression level of EGFRvIII

In order to study the glycolytic metabolism in the subpopulation of glioblastoma cells, we first did RIMChip experiment to image [^{18}F]FDG uptake in EGFRvIII+ and EGFRvIII- GBM39 cells based on the hypothesis that EGFRvIII+ cells, compared with EGFRvIII- cells, would show higher uptake of [^{18}F]FDG due to their uncontrolled active signaling. ERZ cells, GBM39 cells evolved to have erlotinib resistance during the treatment of the drug, were also studied to investigate the effect of erlotininb resistance as shown in **Fig. 5.1.**

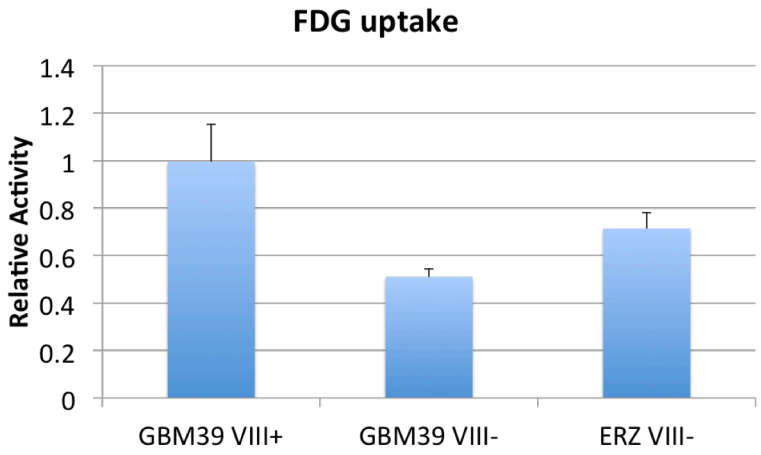

Fig. 5.1. The relation between [^{18}F]FDG uptake and the expression level of EGFRvIII in GBM39 cells.

As I expected, EGFRvIII+ subpopulation showed two times higher uptake of [^{18}F]FDG than EGFRvIII- counterpart. Interestingly, ERZ cells which rarely express EGFRvIII- on their cellular membrane consumed 20% more [^{18}F]FDG than EGFRvIII- subpopulation, which might be related with the evolution of bypass signaling pathway leading to the resistance to the inhibition of EGFR caused by erlotininb treatment.

5.3.2. Imaging of glycolytic metabolism in glioblastoma cells under drug treatment

As mentioned in Chapter 3, RIMChip can work as a rapid screening tool for drug treatments. Here we confirm the capability of RIMChip again with EGFR+ subpopulation of GBM cells undergoing different types of treatment: mTOR inhibition by CC214[8], MEK inhibition by U0126[9] and the dual inhibition of mTOR and MEK by the combinatorial treatment with CC214 and U0126.

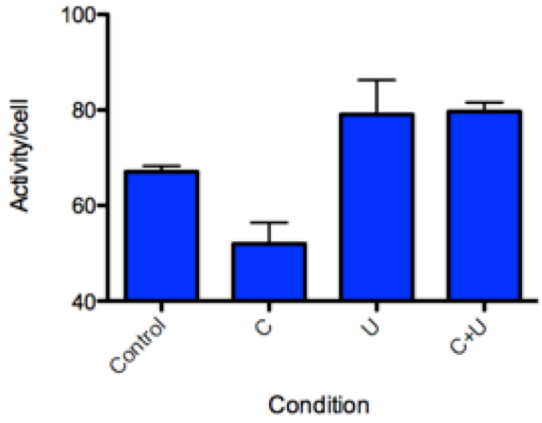

Fig. 5.2. The relation between [^{18}F]FDG uptake and the drug treatment to EGFR+ subpopulations of GBM39 cells C represents CC214, U represents U0126 and C+U represents the combinatorial treatment with CC214 and U0126.

As shown in **Fig. 5.2.,** [^{18}F]FDG uptake is decreased with the inhibition of mTOR by CC214, which can be explained by the diminished activation of glucose transporter at the downstream of mTOR signaling pathway[10]. The increased [^{18}F]FDG uptake was observed with the treatment of U0126, MEK inhibitor, which may be related with the interrupted activity of mitochondria as described in literature[11].

5.4. Conclusions and Future Directions

By using RIMChip platform, we observed the heterogeneity of energy metabolism in tumor cells with the imaging of glycolytic metabolism in the subpopulations of primary glioblastoma cells sorted by the expression level of oncoprotein, EGFRvIII. Also, we confirm the capability of RIMChip as a drug screening tool with the preliminary results of primary glioblastoma cells under treatment of different kinds of drugs. Combined with

further study of drug kinetics, with this platform, it would be possible to screen drugs rapidly with low requirement of biopsy samples.

5.5. References

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