Determining the role of nicotinamide metabolism in chemosensitivity of glioblastoma multiforme

Mr Richard Perryman*, Mr Kevin O’Neill*, Dr Hector Keun†, Dr Nelofer Syed*

*John Fulcher Neuro-Oncology Laboratory, Imperial College London, UK, †Division of Cancer, Imperial College London, UK

Background

- Glioblastoma multiforme (GBM) is a highly aggressive grade IV glial cancer with a median survival time of only 18 months.
- GBM is treated with temozolomide (TMZ), but tumour recurrence is common with acquired resistance to TMZ.
- Novel drug combinations are required to improve patient outcomes.
- Targeting nicotinamide metabolism may be one way to achieve this.
- Rapidly proliferating cells need to maintain high levels of intracellular NAD to fuel metabolic reactions, maintain redox balance, promote proliferation, and enable the repair of DNA damage.
- Given that TMZ is an alkylating agent known to cause DNA damage, inhibition of NAD synthesis may be one method to improve its efficacy.
- TCGA data shows that expression of enzymes involved in nicotinamide metabolism are predictive of survival in patients with GBM.

Material and methods

LN229 and SNB19 GBM cell lines were used in all experiments. Chemicals purchased from Sigma-Aldrich include: TMZ, FK866, APCP, NMN, NAD. Cell viability was measured using SRB assay. Cell death was measured using trypan blue staining. Apoptosis was measured using the Muse Annexin V staining kit. NAD/NADH quantification was carried out using the Promega Glo™Assay kit. NAD/NADH analysis was carried out on TCGA data (GBM subset) using R (ver. 3.3.0). All graphs generated using GraphPad Prism (ver. 7.0).

Results

- GBM cells are highly sensitive to the NAMPT inhibitor FK866.
- FK866 is affected by the expression of two other enzymes involved in nicotinamide salvage: NAPRT and NTSE.
- Sensitivity to FK866 is highly correlated with NAPRT expression ($R^2 = 0.84$, $p = 0.01$).
- Cells expressing NTSE can convert extracellular NMN or NAD in to nicotinamide riboside, which can be used to fuel NAD synthesis.
- Inhibition or knockdown of NTSE sensitises GBM cells to FK886 and TMZ.
- NTSE null cells exhibit decreased proliferation, increased activation of apoptosis and increased cell death in the presence of TMZ and FK866.
- FK866 rapidly depletes NAD and NADH levels in GBM cells.

Discussion and conclusions

- FK866 has been shown to be safe in phase I/II clinical trials.
- FK866 is highly toxic to GBM cells at very low doses.
- The combined use of FK866 and TMZ is a strategy that could be explored to combat GBM recurrence and improve patient survival, stratified on NTSE and NAPRT status.

References