Targeted treatment on PTEN mutated GBM cell lines using metformin and chlorpromazine

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Abstract

Glioblastoma multiforme (GBM) is among the most aggressive and lethal of all human cancers. Characteristically, GBM is genetically heterogenous with different tumors portions exhibiting vastly different genetic profiles. Due to this, GBM patients are plagued with poor prognosis and high recurrence rates. Within the last decade there has been an increase in knowledge of the molecular finger print of GBM but improvement in patient outcome has been slow as personalized treatment regimens have not been linked to significant improvement. However, there is hope with feasible multiagent personalized regimens as well as expanding the amount of treatment options with repurposed agents and immunologic modulators improving patient outcomes. One hypothesized gene of interest in tumor development and progression is PTEN. In this study we investigate two repurpose agents, metformin and chlorpromazine, which are thought to depress downstream oncogenic proteins specific to the PTEN pathway through a cell culture model. For this study, four Xenograft cell lines with differing PTEN status were treated with titrated concentrations of metformin and chlorpromazine. After treatment, results were quantified by SiRNA function using cell-titer glo assay, a marker for cell viability. Results showed no difference cell susceptibility in regards to PTEN status when treated with chlorpromazine. Metformin appear to spare PTEN wild type cell lines while inconsistently targeting PTEN mutated cell lines. We hope that our data will add to the growing knowledge of understanding the molecular mechanism PTEN targeted therapy with metformin within GBM patients.

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Introduction

This investigation attempts to quantify the effects of repurpose agents metformin and chlorpromazine on PTEN mutated vs wild type GBM cell lines. These agents were chosen based on good blood brain barrier penetration as well as prior evidence suggesting PTEN pathway targeting. Thus, we hypothesize that PTEN mutated cells lines will be sensitive to these agents when compared to PTEN wild type cell lines. PTEN tumor suppressor protein is thought to regulated the PI3/AKT pathway, thus PTEN is pro- apoptotic and limits metabolic cell growth. The role of chlorpromazine and metformin in the treatment of GBM has been long since postulated. Chlorpromazine, a first-generation antipsychotic, has been found to be experimentally effective on multiple cancer types including GBM. However, the exact mechanism pertaining to GBM cell lines remains unknown and is inconsistent throughout prior investigations. Metformin, a biguanide and diabetic medication, has been shown to exhibit anti-tumoral effects in GBM. These effects have been postulated to be both dependent and independent from PI3/AKT regulation. Prior experiments have found that metformin has the potential to augment the cytotoxic effects of TMZ and radiotherapy In PTEN mutated cell lines. We believe that additional investigations on other PTEN status could confirm these results and help deceiver the molecular mechanism by which metformin displays its cytotoxic effects on PTEN mutated GBM cell lines.

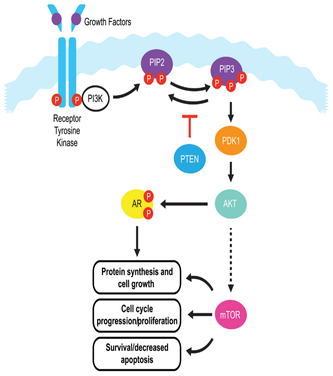

Figure 1.1 outlining IP# AKT pathway and PTEN regulation

Methods

Xenograft cell lines that express unique genetic alteration that is exhibited in four actual patient tumors have been obtained from Mayo Clinic and have been genetically verified by the Translational Genomics Research Institute. These cell types include A172 with wild type PTEN status and three mutated cell lines SF767, U87, and T98G. These cell lines were the plated at a concentration of 5 to 10 thousands in 96 well plates. Cells were then treated with metformin and chlorpromazine with titrated concentrations against control Ly29004 control agent, a known PI3 inhibitor. To analyze cell viability cell titer-glo assay was then performed.

Results

As seen in Figure 1.2, chlorpormazine showed no difference in cell susceptibility to PTEN wild type compared to mutated cell lines. However, metformin did appear to spare PTEN wild type cell lines and appeared to reduce cell susceptibility in some but not all PTEN mutated cell lines (see figure 1.3). Cell response to control agent Ly29004, a known PI3 inhibitor, shows susceptibility to of all PTEN mutant cell types and sparing A172 wild type cell line.

Westernblot analysis for varying concentrations of chlorpromazine shows decreased S6 protein production with the concentration of other upstream cell signaling proteins such as AKT and MTOR remaining constant in wild type PTEN status cell lines. In comparison to the western blot of metformin, not only is decreased s6 protein seen but also akt appears to be mildly decreased (see figure 1.4) .

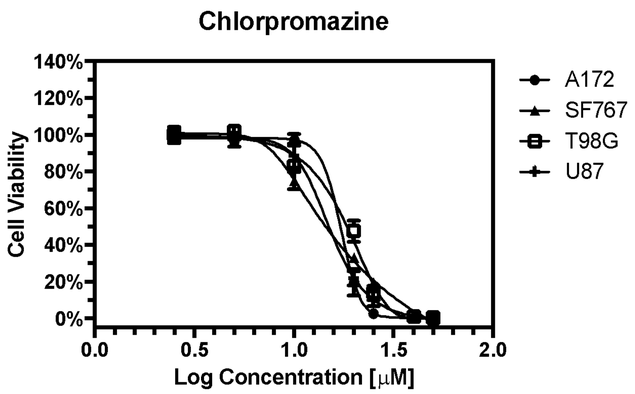

Figure 1.2 Quantitative analysis of cell viability using cell titer-glo assay for chlorpormazine

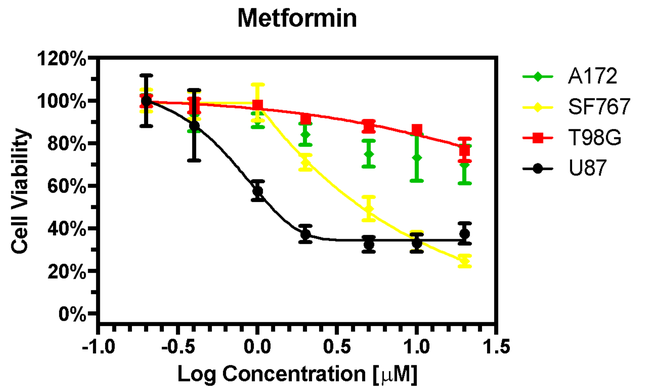

Figure 1.3 Quantitative analysis of cell viability using cell titer-glo assay for control metformin

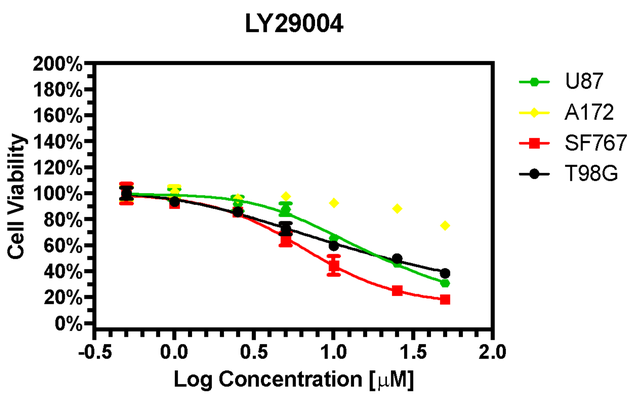

Figure 1.3 Quantitative analysis of cell viability using cell titer-glo assay for control agent Ly29004

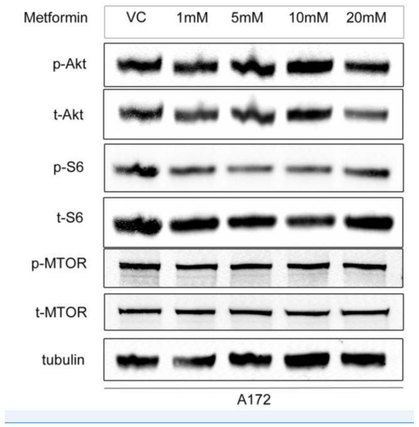

Figure 1.4 Western blot for metformin

Discussion and Conclusions

During this experiment, we hoped to investigate the potential actionable targets in PTEN mutated cells with repurpose drugs metformin and chlorpormazine. Our results show no significant difference in response rate of different cell types when treated with chlorpormazine and only partial differences to metformin in mutated PTEN status cell lines. Thus, there is limited evidence to suggest that targeted blockade is dependent to PTEN status with the chlorpormazine. Likely, the toxicity with chlorpormazine occurs for reasons that have already been postulated. Metformin treatment showed some targeted response to PTEN mutated versus PTEN wild type cell lines suggesting a possible specific response to PTEN status. The potential incomplete response may be secondary to heterogenous nature of mutations within the pathways regulated by tumor suppressor protein PTEN. However, our data is comparable with another cell culture study showing that different PTEN status Glioblastoma cell lines treated with metformin exhibit a dosage response with augmented treatment with TMZ and radiotherapy in vitro.

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