Intracellular hyper-acidification cancer cell death potentiated by the synergistic effect of metformin and simvastatin

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1. Introduction

Conventional chemotherapy, while common, only improves overall survival rate to a limited extent, especially for the more invasive cancer cell lines. Thus, this study targets the Warburg effect, where cancer cells undergo aerobic glycolysis to produce large amounts of lactate, which ionises to form lactic acid, acidifying the intracellular environment [1]. To maintain an intracellular pH homeostasis, the lactic acid will be extruded via monocarboxylate transporter 4 (MCT4). Hence, this study hypothesises that by boosting glycolysis and inhibiting MCT4 using metformin (Met) and simvastatin (Sim) respectively, the two clinically approved drugs synergise to result in lactic acid entrapment, thus inducing intracellular hyper-acidification and cancer cell death. This study also aims to determine the optimum concentration of Sim and Met for synergistic effect.

2. Methodology

This study investigated cervical cancer: HeLa Parental (HeLa P) and HeLa C5, a radio-resistant cell line, and breast cancer: MCF7 and MDA-MB-231, the latter being the invasive cell line. Four assays were employed: crystal violet assay for determining cell viability, Bradford assay for protein quantification, intracellular and extracellular lactate assay for lactate quantification, and ratiometric fluorescence analysis for determining intracellular pH (pHi). CompuSyn, a computer software, was used to determine the synergistic concentration of Sim and Met.

3. Results

By determining cell viability of cancer cells after single-drug treatment of Sim and Met, IC50 (half maximal inhibitory concentration) of Sim and Met for the four cancer cell lines was determined. For HeLa P, HeLa C5, MCF7 and MDA-MB-231, IC50 of Sim (uM) is 16, 12, 24, 0.92 respectively, and IC50 of Met (mM) is 4.5, 5.6, 3.2 and 5.5 respectively.

Based on the IC50 values, we tested different concentrations of Sim and Met on the cancer cells for no-treatment (NT), single drug treatment (Sim, Met) and combination treatment (Combi) which consists of Sim and Met, and determined the cell viability. By using CompuSyn, we determined that the optimum concentration of Sim and Met is 7.5uM and 3mM respectively for HeLa P and HeLa C5, 12.5uM and 15mM for MCF7, and 0.5uM and 5mM for MDA-MB-231.

Using these combinations of drug concentration, we determined extracellular and intracellular lactate and protein of NT, Sim, Met and Combi-treated cancer cells.

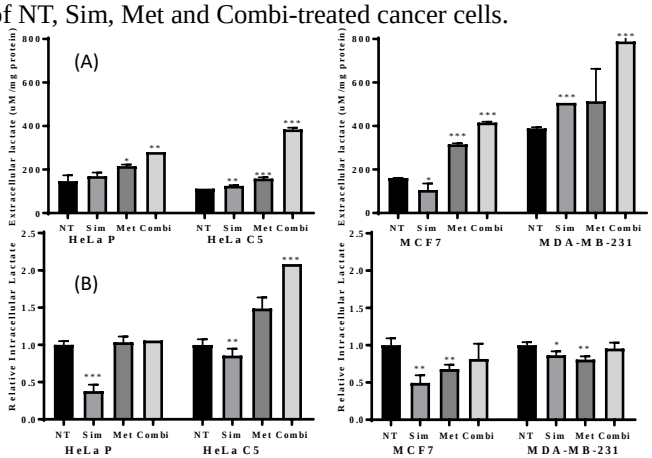

Figure 1(A): Extracellular lactate (uM/mg protein) of NT, Sim, Met, Combi-treated cells. (B): Relative intracellular lactate of NT, Sim, Met, Combi-treated cells.

In 1(A), all cell lines showed profound increase in extracellular lactate levels after Combi treatment due to the overwhelming of intracellular lactate threshold and upregulation of MCT1 expression. In 1(B), although majority of the lactate is pumped out to the extracellular environment, the net increase in intracellular lactate levels leads to pHi decrease (Figure 2).

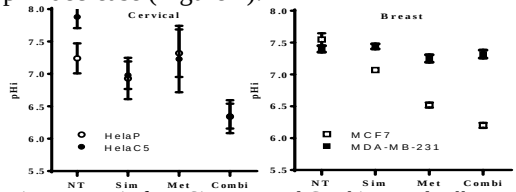

Figure 2: pHi of NT, Sim, Met and Combi-treated cells

4. Conclusion

In conclusion, the synergistic usage of low doses of clinically approved drugs – simvastatin and metformin – to induce intracellular hyper-acidification in cancer cells serves as a promising cancer therapy with faster clinical trials.

5. References

[1] Epstein T, Gatenby RA, Brown JS (2017). https://doi.org/10.1371/journal.pone.0185085