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Investigation of Methuosis in Glioblastoma Cell Lines in Response to Treatment with Novel Chalcones

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Abstract

The knowledge of cell death mechanisms following cancer therapies is continuing to expand. What was formerly understood as either apoptotic or necrotic cell death has evolved with recent research into non-classical cell death pathways. Our interest in non-classical cell death pathways resulted from our recent investigations of synthetic chalcone derivatives in glioblastoma cell lines. We previously reported on the cytotoxicity of two of our synthetic chalcones, RK6 and RK15, in glioblastoma cell lines (IC_{50} values: 10-20 μM). In our investigations into the mechanisms of action for these chalcones, we found that they induced cell death through a non-caspase mediated mechanism. Others have suggested that treatment of glioblastoma cells with chalcone-like compounds may induce methuosis, a nonapoptotic form of cell death involving the formation of vacuoles from macropinosomes which leads to cell lysis. With this in mind, we sought to determine if cell death caused by RK6 and RK15 was mediated through methuosis.

Methuosis involves activation of the Ras signaling pathway followed by activation of Rac1, leading to vacuole formation and ultimately cell death. Rac1 inhibitors, such as EHT1864, have been shown to inhibit methuosis by preventing vacuole formation. We hypothesized that EHT1864 would reduce the cytotoxic effects of RK6 and RK15 in glioblastoma cell lines. To test our hypothesis, we used the XTT assay to assess cellular viability after treating glioblastoma cell lines, A-172, T98G, and U87, with various concentrations of our synthetic chalcone compounds, RK6 and RK15, with and without EHT1864. In each of our experiments, EHT1864 failed to prevent cell death caused by RK6 and RK15, thereby suggesting our initial hypothesis was incorrect. Interestingly, in several instances, EHT1864 actually enhanced the cytotoxic effects of RK6 and RK15. This data suggests that methuosis is likely not involved as a cell death mechanism for RK6 and RK15. However, the enhancement of RK6 and RK15-induced cytotoxicity by the Rac1 inhibitor, EHT1864, warrants further investigation.

Introduction

Chalcones are plant derived polyphenolic compounds that have been demonstrated to have a large variety of biological effects, such as anti-inflammatory and anti-cancer properties. There are multiple proposed targets for chalcones, however, there is a lack of evidential support for the precise molecular targets for these compounds. The chalcone scaffold consists of an α,β unsaturated ketone system with two aromatic rings. We previously completed structure activity relationship studies to identify RK6, RK7, and RK15 as lead compounds and presented the screening data of these compounds in glioblastoma cell lines. Methuosis is one proposed mechanism by which chalcone-like compounds may trigger cell death of glioblastoma cells. Methuosis involves activation of the Ras signaling pathway, leading to activation of Rac1, and ultimately culminating in a nonapoptotic form of cell death involving formation of vacuoles and cell lysis. EHT1864 prevents methuosis by inhibiting Rac1 and preventing vacuole formation. Here we present the impact of treatment of glioblastoma cells with EHT1864 along with RK6 and RK15.

Effect of RK6 with EHT1864 on Cell Viability

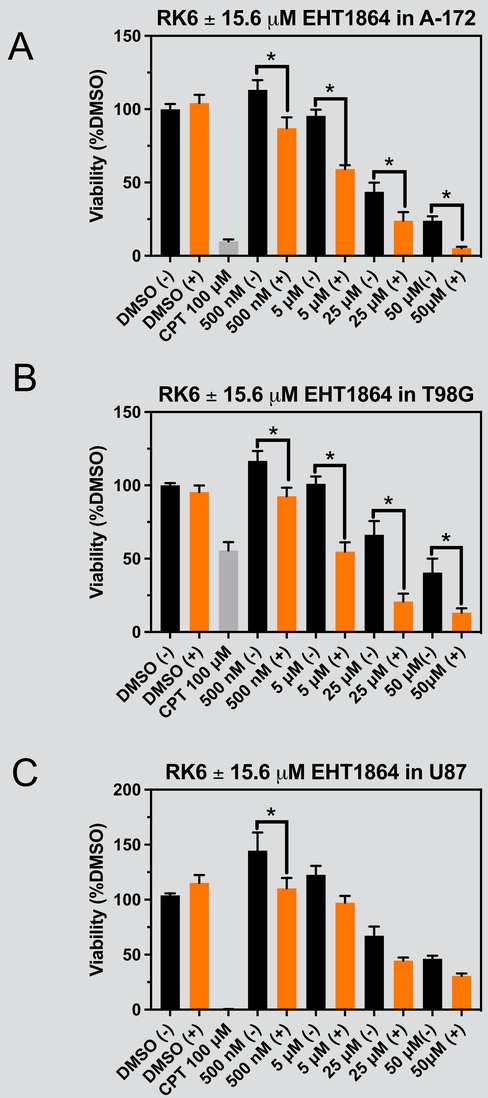

Figure 1: Comparison of RK6 with (+) and without (-) EHT1864, in A-172 (A), T98G (B), and U87 (C) glioblastoma cell lines. The effect of RK6 on cell viability in the presence and absence of Rac1 inhibitor, EHT1864, was investigated using the XTT viability assay. Columns represent an average of at least 3 separate experiments conducted in quadruplicate + SEM . *P<0.05 (One-Way ANOVA with Sidak multiple comparisons tests)

Effect of RK15 with EHT1864 on Cell Viability

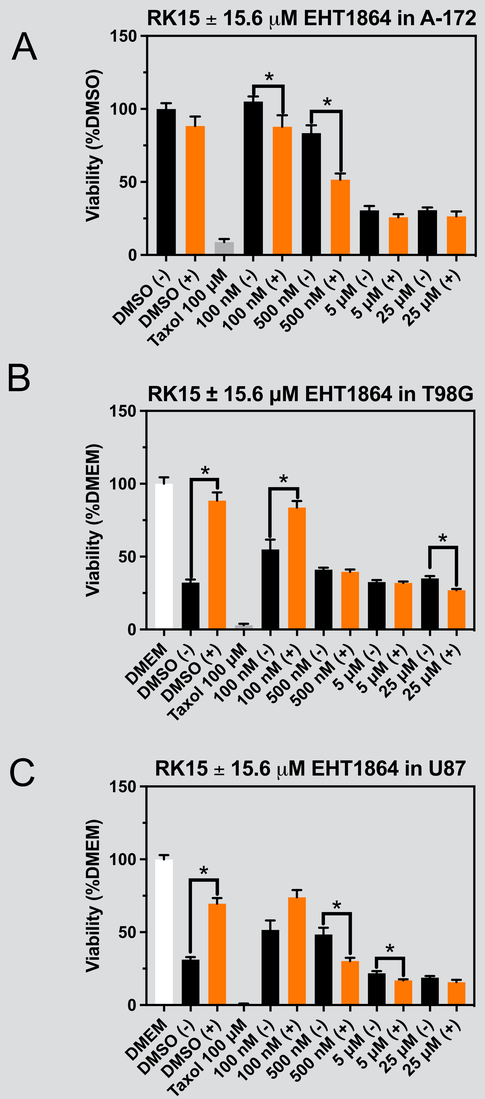

Figure 2: Comparison of RK15 with (+) and without (-) EHT1864, in A-172 (A), T98G (B), and U87 (C) glioblastoma cell lines. The effect of RK15 on cell viability in the presence and absence of Rac1 inhibitor, EHT1864, was investigated using the XTT viability assay. Columns represent an average of at least 3 separate experiments conducted in quadruplicate + SEM . *P<0.05 (One-Way ANOVA with Sidak multiple comparisons tests)

Methods

XTT Assay: Cells were plated in clear flat-bottom 96-well plates at a density of 5,000 to 15,000 cells/well and allowed to equilibrate overnight. The cells were pre-treated with the corresponding Rac1 inhibitor (EHT1864) for one hour followed by a co-treatment with RK6 or RK15 at various concentrations for 48 hours. Cell viability was determined using the XTT Cell Viability Assay (Life Technologies) following a modified version of the manufacturer's protocol. Absorbance was read on Cytation 3 plate reader (BioTek).

Data Analysis: The data was analyzed using GraphPad Prism 9.0 software. The data was normalized to the mean of the DMSO (-) control or DMEM control and a one-way ANOVA test with Sidak multiple comparisons test was conducted to compare the column averages of viability data with and without EHT1864.

IC_{50} Values of Novel Chalcone Compounds

Glioblastoma Cell Lines	RK6 (μM)	RK15 (μM)
A-172	20	0.47
T98G	21	1
U87	30	3

Table 1: Previously reported IC_{50} values for RK6 and RK15 in glioblastoma cell lines A-172, T98G, and U87. RK6 and RK15 are effective at inducing cytotoxicity at low micromolar concentrations, which was previously reported.

Conclusions

- EHT1864 failed to prevent cell death induced by the chalcones, RK6 and RK15, in most instances.
- The effect of enhanced cytotoxicity in the presence of EHT1864 was most prevalent in the A-172 cell line and least prominent in the U87 cell lines.
- In the future, different cell signaling pathways involving Rac1 will be explored for their possible involvement with the observed chalcone-mediated cytotoxicity.

References

- Maltese WA, Overmyer JH. Methuosis: Nonapoptotic Cell Death Associated with Vacuolization of Macropinosome and Endosome Compartments. The American Journal of Pathology. 2014 Jun;184(6):1630-42.