An NQO1 dependent ROS and RIP1/RIP3 mediated necroptosis induced in glioma cancer cells by MAM

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Background: Glioblastomas (GBM) are the most malignant brain tumors in humans and have a very poor prognosis. New therapeutics are urgently needed. 2-methoxy-6-acetyl-7-methyljuglone (MAM) was reported to induce necroptosis in lung cancer and colon cancer cells, but no report in glioma cancer cells. Thus, it is needed to clarify whether MAM could cause necroptosis in glioma cancer cells and investigated its underlying mechanisms.

Methods: Cell viability was assayed by MTT and ATP assays. Morphological alterations were evaluated by light microscope and fluorescence microscopy with Hoechst 33342/PI double staining, respectively. The apoptosis and necrosis rate was measured by flow cytometry with Annexin V/7AAD double staining. The level of reactive oxygen species (ROS) and calcium level was analyzed by flow cytometry using redox-sensitive dye DCFH2-DA and calcium dye fluo-3AM. The expression level of proteins was analyzed by western blotting and co-immunoprecipitate.

Results: MAM markedly induced cell death in U87 and U251 glioma cancer cells without caspase activation. MAM increased PI uptake, ATP depletion, phosphorylation of RIP1 and RIP3, the formation of RIP1/RIP3, which are markers of necrotic cell death. The cell death induced by MAM was attenuated by the pharmacological or genetic blockage of necroptosis signaling, including RIP1 inhibitor necrostatin-1s (Nec-1s) and siRNA-mediated gene silencing of RIP1 and RIP3, but was unaffected by caspase inhibitor z-vad-fmk or necrosis inhibitor 2-(1H-Indol-3-yl)-3-pentylmaleimide (IM54), further confirmed this process was necroptosis. MAM induced necroptosis through cytosolic calcium (Ca2+) accumulation and sustained c-Jun N-terminal kinase (JNK) activation. Both calcium chelator BAPTA-AM and JNK inhibitor SP600125 could attenuate cell death. We also found that MAM-induced ROS production has an important role in necroptosis, however, independent with RIP1/RIP3 activation. Finally, MAM-induced necroptosis was inhibited by dicoumarol (a NQO1 inhibitor), and NQO1 expression with correlated with sensitivity to MAM. Dicoumarol exposed glioma cancer cells were resistant to MAM-induced RIP1/RIP3 phosphorylation and ROS generation.

Conclusions: Take together, our results demonstrated that MAM induced necroptosis through the NQO1-dependent ROS and RIP1/RIP3 pathway. Our study also provided new insights into the molecular regulation of necroptosis in human glioma cancer cells and a promising approach for GBM treatment.