Bufalin

Bufalin, a cardiotonic steroid found in the venom of the Chinese toad Bufo gargarizan, has been shown to inhibit the growth of human cancers, such as colon and bladder.

A study aimed to further clarify the in vitro and in vivo antiglioma effects of bufalin and the molecular mechanism underlying the regulation of drug sensitivity. The anticancer effects of bufalin were determined by colony formation assays, apoptosis assays, and cellular redox state tests of glioma cells. Confocal microscopy was performed to determine the expression changes of the DNA damage biomarker γ-H2AX and the nuclear translocation of p53 in glioma cells. Western blotting and RT-PCR were used to detect the protein and gene expression levels, respectively. Here, we report that bufalin induced glioblastoma cell apoptosis and oxidative stress and triggered DNA damage. The critical roles of the sodium pump α1 subunit (ATP1A1) in mediating the XPO1-targeted anticancer effect of bufalin in human glioma were further confirmed. Mechanistic studies confirmed the important roles of Src and p53 signaling in mediating bufalin-induced apoptosis. Importantly, bufalin also inhibited the growth of glioma xenografts. In conclusion, our study indicated that therapies targeting the ATP1A1 and p53 signaling-mediated mitochondrial apoptotic pathways regulated by bufalin might be potential treatments for human glioma, and these findings will provide molecular bases for developing bufalin into a drug candidate for the treatment of malignant glioma [1)].

Liu et al. investigated the effect of miR 203 expression and bufalin treatment on glioma cell proliferation and stem cell-like phenotypes.

They used cell viability assay, colony formation assay, cell apoptosis assay and neurosphere formation assay to dectect the treatment effect of bufalin on U251 and U87 cells. Cells were transfected with the miR-203 mimic without bufalin treatment or cells were transfected with anti-miR-203 under bufalin treatment, the above expreiments were repeated. RT-PCR was employed to quantify miR-203 expression. Western blot was performed to detect the stem cell-like (CSC) markers, OCT4 and SOX2. Luciferase activity assay was used to determine whether the SPARC is the target of miR-203.

Bufalin treatment inhibited cell proliferation, colony formation, and CSC phenotypes and increased cell apoptosis and expression of miR-203. Furthermore, overexpression of miR-203 led to similar outcomes as bufalin treatment with respect to the cell viability, colony formation, cell apoptosis and the phenotypes of glioma cells. While anti-miR-203 attenuated the inhibitory effects of bufalin as promoting cell proliferation, colony formation and CSC phenotyes and inhibiting cell apoptosis. In addition, we identified SPARC as a novel target gene of miR-203.

These findings suggest that miR-203 plays an important role in bufalin's ability to inhibit the growth of glioma cells and the development of stem cell-like phenotypes [2)].

Zhang et al. investigated the response of U251 and U87MG glioblastoma (GBM) cell lines to bufalin in vitro and found that bufalin impaired several biological processes. First, in both U251 and U87 MG, bufalin reduced cell proliferation and induced a G2/M cell cycle arrest (~10% vs~30%, untreated vs

treated cells, respectively). Second, bufalin disrupted the mitochondrial membrane potential, leading to reduced oxygen consumption and ATP production. Third, homologous recombination (HR) efficiency was reduced by~40% in both cell lines in the presence of bufalin. At the molecular level, bufalin led to decreased RAD51 protein, a central player in HR, and increased γ-H2AX, a marker for the presence of DNA double strand breaks. Finally, bufalin was additive with radiation in the treatment of GBM cells in vitro. Cell death increased significantly under combination treatment compared to radiation treatment alone. Our findings indicated that bufalin led to reduced mitochondrial and DNA repair function and therefore, might be a promising therapeutic drug to increase the sensitivity of GBM cells to radiotherapy [3].

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