Supporting Information

Baicalin Induces Apoptotic Death of Human Chondrosarcoma Cells through Mitochondrial Dysfunction and Downregulation of the PI3K/Akt/mTOR Pathway

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**Fig. 1S** Baicalin-induced cytotoxicity was highly selective between human chondrosarcoma cells and normal chondrocytes. **A** Human chondrosarcoma cells (SW1353, CH2879) and normal chondrocytes (C28/I2) were treated with Bai for 48 h. **B** Normal chondrocytes (CHON-001) were treated with Bai, Dox, or Cyc for 48 h. Cell viability, which was detected by MTT, is presented as the mean ± SD from three separate experiments. The IC₅₀ of each cell line was determined from the dose-response curves by using GraphPad Prism software. Con: control; Bai: baicalin; Cyc: cyclophosphamide; Dox: doxorubicin.
Fig 2S Baicalin-induced caspase activation in SW1353 cells. SW1353 cells were incubated with baicalin (5 and 10 µM) for 48 h. A The levels of activate form of caspase-3 and caspase-9 were examined by Western blot analysis. B The expression of the bands was semiquantitatively analysed by using image analysis software. The results are representative of three independent experiments; **p < 0.01 compared to the control (Con).
Fig. 3S Baicalin blocked the PI3K/Akt/mTOR signalling pathway in SW1353 cells. A SW1353 cells were treated with baicalin for 6 h, and the whole cell lysates were analysed by immunoblotting. B Bars represent the densitometric intensity of the bands, which is expressed by using image analysis software. The results are representative of three independent experiments; *p < 0.05, **p < 0.01 compared to the control (Con).
**Fig. 4S** Baicalin blocked downstream target (S6K and 4E-BP1) activation of mTOR in SW1353 cells. **A** SW1353 cells were treated with baicalin for 6 h, and the whole cell lysates were analysed by immunoblotting. **B** Bars represent the densitometric intensity of the bands, which is expressed by using image analysis software. The results are representative of three independent experiments; **p < 0.01 compared to the control (Con).
Fig. 5S Baicalin reduced the tumour weight of nude mice bearing SW1353 cell xenografts. The mice were treated with physiological saline, baicalin (50 mg/kg), LY294002 (PI3K phosphorylation inhibitor, 10 mg/kg), and SC79 (Akt activator, 10 mg/kg) for 21 days. The results are expressed as the mean ± SD; n = 5. **P < 0.01 compared to the control; #p < 0.05, ##p < 0.01 compared to the mice treated with baicalin only.
The PI3K/Akt pathway was involved in baicalin-induced cytotoxicity and antitumour activity in chondrosarcoma. A-C SW1353 cells were treated with baicalin (alone or in combination with 5 µM SC79/ LY294002), SC79, and LY294002 with the same concentration gradient (0, 1, 5, 10, 20, 40, 60, 80, or 100 µM) for 48 h. The IC₅₀ values of baicalin are shown. Cell viability, which was detected by MTT, is presented as the mean ± SD from three separate experiments. D-F Nude mice bearing SW1353 cell xenografts were treated with physiological saline for control, baicalin (50 mg/kg), baicalin (50 mg/kg) + LY294002 (10 mg/kg), baicalin (50 mg/kg) + SC79 (10 mg/kg), LY294002 (10 mg/kg), or SC79 (10 mg/kg) for 21 days. The tumour weights are recorded. The results are expressed as the mean ± SD; n = 5. *P < 0.05, **p < 0.01 compared to the control; *p < 0.05, **p < 0.01 compared to the mice treated with baicalin only.