Landes Bioscience www.landesbioscience.com

Supplemental Material to:

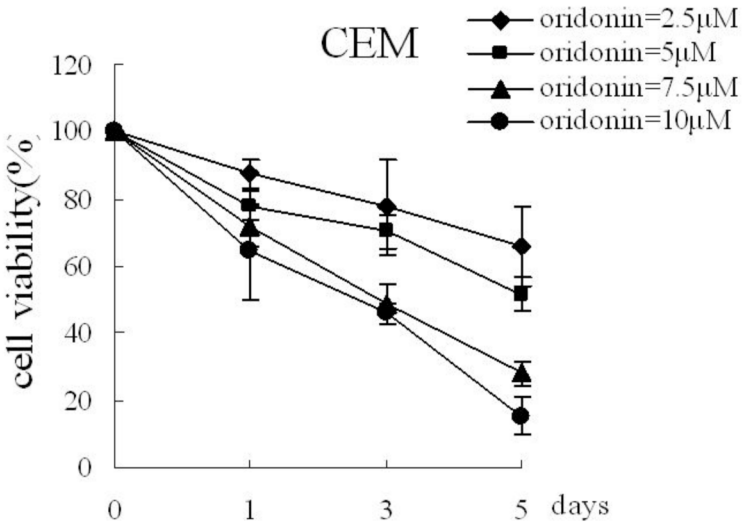
Yong Guo, Qingping shan, Yuping Gong, Juan Lin, Xi Yang and Ruqing Zhou

Oridonin in combination with imatinib exerts synergetic anti-leukemia effect in Ph+ acute lymphoblastic leukemia cells in vitro by inhibiting activation of LYN/mTOR signaling pathway

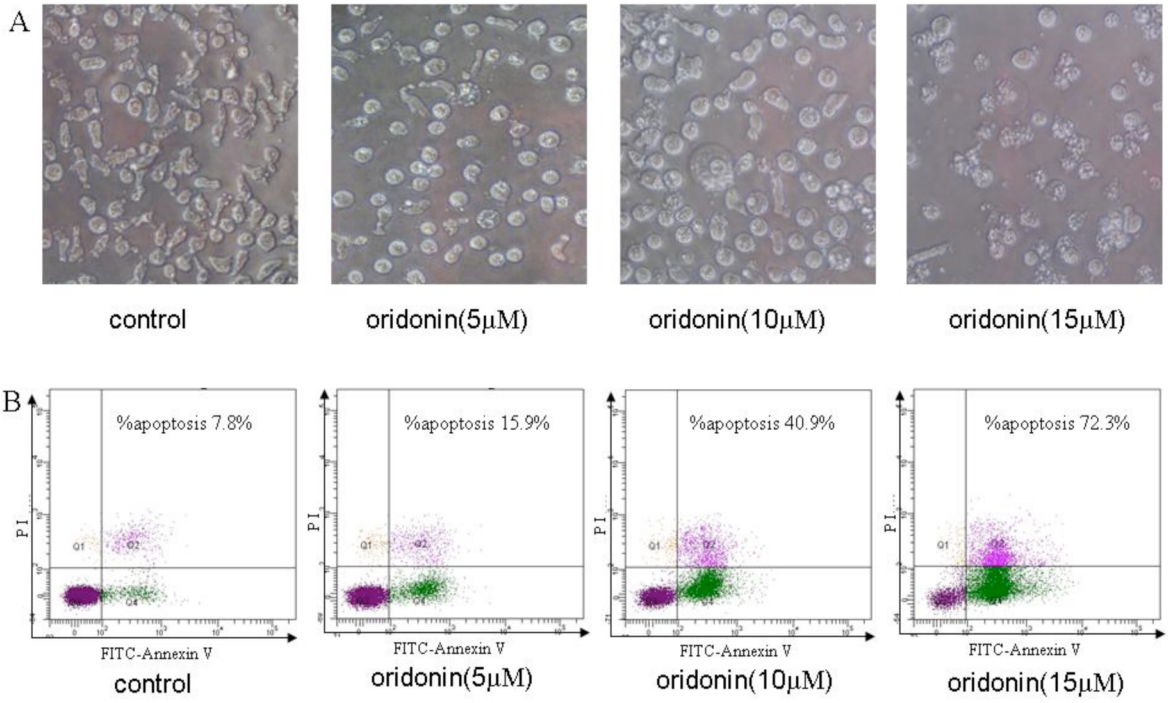
Cancer Biology & Therapy 2012; 13(13)
http://dx.doi.org/10.4161/cbt.21460

http://www.landesbioscience.com/journals/cbt/article/21460/

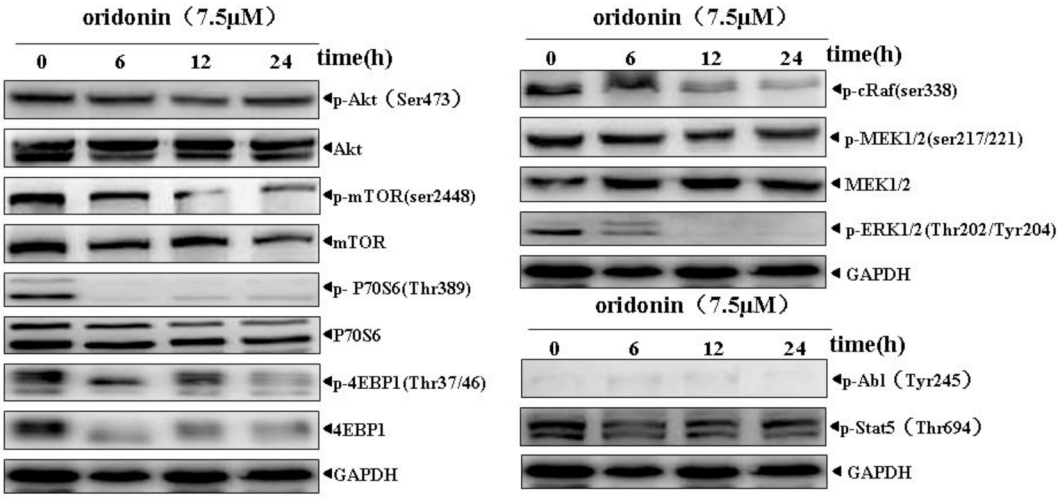
Supplementary Figure 1. Oridonin inhibited the proliferation of CEM cells in a time- and dose-dependent manner. CEM cells were incubated with 0, 2.5, 5, 7.5, 10μM oridonin for 1 day, 3 days, and 5 days. At the end of incubation, the cell survival rates were determined by MTT methods. Cell viability was expressed as the percentage of cell survival compared to the control. Data were from three independent experiments.



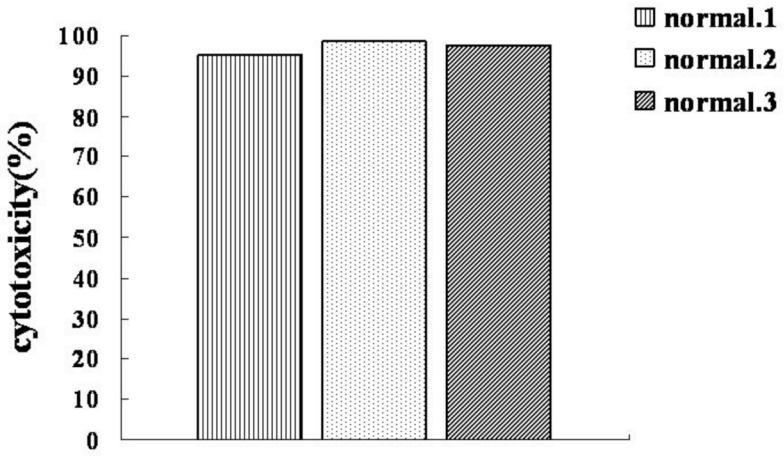
Supplementary Figure 2. Oridonin induced apoptosis in CEM cells. A, The CEM cells were treated with indicated concentration oridonin for 24 h. A light microscope was used to examine the changes in cellular morphology. **B,** The CEM cells were treated with indicated oridonin for 24 h and apoptosis percentage was examined using the AnnexinV-FITC /PI apoptosis detection kit.



Supplementary Figure 3. Oridonin inhibited the constitutive activation of mTOR and RAF/ERK signaling on in CEM cells. The CEM cells were incubated with 7.5μM for 6, 12, 24 h. They were then harvested, and total proteins were extracted. Equal amounts of protein from each sample were separated on SDS‐PAGE and immunoblotted with indicated antibodies. GAPDH was used as a loading control. A, Oridonin inhibited the constitutive activation of mTOR signaling but had no inhibitory effects on AKT kinase. B, Oridonin inhibited RAF/ERK signaling activation. C, CEM cells were nearly negative for Abl kinase activity. Oridonin had no inhibitory effect on STAT5 signaling in CEM cells.



Supplementary Figure 4. Oridonin had minimal cytotoxic effect on normal human peripheral blood mononuclear cells. The normal human peripheral blood mononuclear cells isolated from three blood donor were incubated with 7.5μM oridonin for 72h. MTT methods were used to determine the cell survival rates. Cell viability is expressed as the percentage of cell survival compared to the control.



Supplementary Figure 5. PBMCs from healthy donors were negative for ABL/AKT/mTOR , RAF/MEK/ERK, and STAT5 signaling activation. Total proteins were extracted from PBMCs from three healthy donors. Equal amounts of protein from each sample were separated on SDS‐PAGE and immunoblotted with indicated antibodies. GAPDH was used as a loading control. SUP-B15 cells was used as positive control.