

**Azulene derivatives act on mitochondria and induce apoptosis of cancer cells.**

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Cancer cells obtain anaerobic glycolysis and metabolic abnormalities due to reduced mitochondrial function. We have found that guaiazulene affects the metabolism of the nematode *C. elegans* and cultured mammalian cells. We investigated whether azulene derivatives synthesized from guaiazulene as a precursor have more effective interference with metabolism.

Adding azulene derivatives to cultured mammalian cells, cell growth was suppressed in immortalized cells and cancer cells, but not normal fibroblast. This result suggests that azulene derivatives affect cells with increased metabolic activity. Next, we investigated whether azulene derivative-treated cells did not proliferate due to cell cycle arrest or apoptosis. Whereas the treatment of azulene derivatives did not significantly change the cell cycle in flow cytometry, cleaved PARP was increased in HeLa cells in a time-dependent manner. These results indicate that the azulene derivative induces apoptosis instead of cell cycle arrest. Moreover, we observed the mitochondrial status using MitoProbe™ JC-1. HeLa cells treated with azulene derivative were observed to undergo mitochondrial depolarization. Our findings indicate that azulene derivatives reduce mitochondrial function to induce apoptosis. We are analyzing where azulene derivatives act in the metabolic pathway.