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## Eupatilin, A Pharmacologically Active Flavone Derived From Artemisia Plants, Inhibits Growth of H-Ras Transformed Human Breast Epithelial Cells Through Cell Cycle Arrest

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Purpose of study: Extracts of *Artemisia asiatica* Nakai (Asteraceae) have been shown to have anti-inflammatory and anti-oxidative activities. Eupatilin (5,7-dihydroxy-3,4,6-tri-methoxy-flavone), one of the pharmacologically active ingredients derived from *Artemisia asiatica*, has been shown to induce apoptosis in promyelocytic leukemia (HL-60) cells.(H.-J. Seo and Y.-J. Surh, *Mutat. Res.*, 496, 191-198, 2001) In this study, we examined the cytostatic effects of eupatilin in H-ras transformed human breast epithelial (MCF10A-ras) cells.

Methods: Effects of eupatilin on growth of MCF10A-ras cells were determined by using MTT reduction and [³H]thymidine incorporation assays. To determine whether the inhibitory effects of eupatilin on growth of MCF10A-ras are mediated through cell cycle blockade, DNA contents were analyzed by a flow cytometry. Furthermore, effects of eupatilin on expression of cyclin-dependent kinases (CDKs), cyclins, CDK inhibitors were assessed by Western blot analysis and RT-PCR.

**Results:** Eupatilin inhibited the growth of MCF10A-ras cells in a concentration-dependent and time-related manner. The anti-proliferative effect of eupatilin was associated with cell cycle arrest. Flow cytometry analysis showed that eupatilin

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(100µM) blocked the cell cycle progression in the G2/M phase. Eupatilin inhibited the expression of Cdk2 and Cdc2, which are responsible for mediating cell cycle progression, and increased the expression level of CDK inhibitors such as p27<sup>kip1</sup> and p53. However, levels of p21<sup>waf/Cip1</sup> were decreased at both protein and mRNA levels. It has been known that expression of p21<sup>waf/Cip1</sup> is regulated by ERK1/2. Eupatilin also inhibited the activation of ERK1/2 in MCF10A-ras cells. Therefore, the inhibitory effect of eupatilin on p21<sup>waf/Cip1</sup> is likely to be mediated via the Raf/MEK/ERK signaling pathway.

**Conclusion:** Eupatilin inhibited the growth of MCF10A-ras cells by blocking the G2/M phase of cell cycle progression.