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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 [^{3}H]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

(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 $p27^{kip1}$ and p53. However, levels of $p21^{waf/Cip1}$ were decreased at both protein and mRNA levels. It has been known that expression of $p21^{waf/Cip1}$ is regulated by ERK1/2. Eupatilin also inhibited the activation of ERK1/2 in MCF10A-*ras* cells. Therefore, the inhibitory effect of eupatilin on $p21^{waf/Cip1}$ 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.