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Introduction

The subject of this research project is potential breast cancer therapy through the use of agents that inhibit the proliferation of malignant cells while allowing the proliferation and function of normal cells. The purpose of this work is to: 1) evaluate the efficacy of a mevalonate analogue, 6-fluoromevalonate (Fmev), in selective inhibition of breast cancer cell growth, and 2) to determine the molecular mechanism by which Fmev can inhibit proliferation of transformed cells. The scope of this research is confined to analysis of the effects of Fmev on proliferation and mitogenic signal transduction in breast epithelial cells, and breast tumor-derived cell lines.

Body

Award DAMD17-97-1-7093 originally to Dr. J. Cuthbert was functionally transferred to my laboratory on or about December 1, 1999. This report covers our progress from December 1, 1999 to August 31, 2000.

Fmev is not a commercially available compound. Therefore, our initial effort was to synthesize a supply of Fmev in collaboration with Dr. C. Falck (UT Southwestern). The synthesis reactions were carried out according to the methods of Quistad (1).

Our next effort was to evaluate the sensitivity of normal diploid breast epithelial cells to the anti-proliferative effects of Fmev. We chose HME50-5E as our model cell line as it mimics the normal growth characteristics of primary cell lines in culture but will not undergo replicative senescence due to a spontaneous immortalization event (2). As shown in Figure 1, the proliferation of HME50-hTERT is affected by Fmev with an approximate IC₅₀ of 20 μ M final concentration. Consistent with Dr. Cuthbert's reported results in leukemic cell lines (3), the total levels of cellular Ras were reduced in HME50-hTERT cells treated with concentrations of Fmev that inhibited proliferation. Levels of active ERK1 and ERK2, MAP kinases regulated by Ras, were also reduced by Fmev. As activation of ERK1/2 directly correlates with cell cycle progression, these results suggest a possible mechanistic explanation for the growth-inhibitory effects of Fmev.

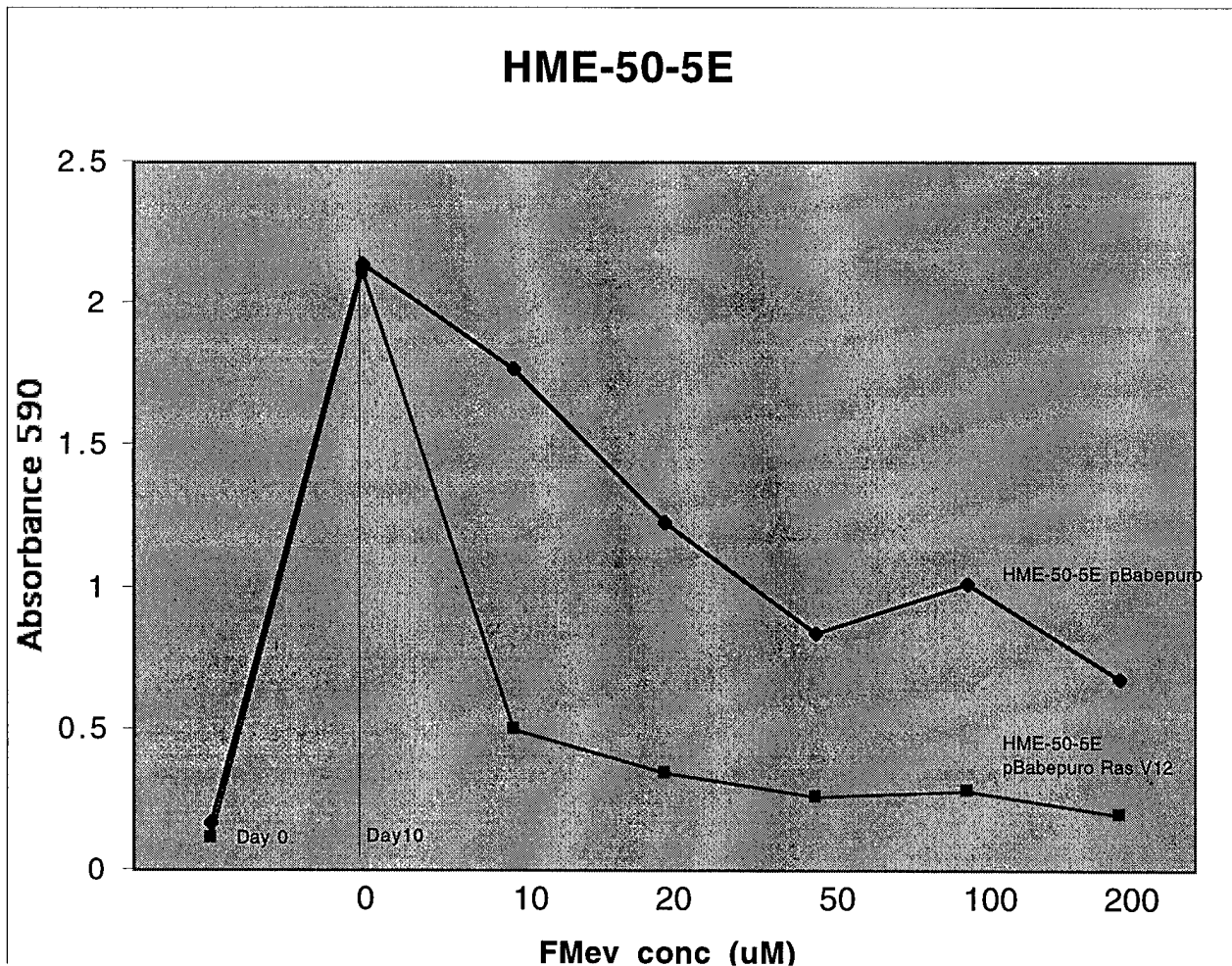


Figure 1. HME50-5E and HME50-5E-ras12V cells were plated at a density of 10,000 cells/well in 24 well plates with the indicated concentrations of Fmev. Following 10 days of culture, cells were fixed and stained with Giemsa. After extensive washing, the stain was extracted with acetic acid, and A590 was measured. The A590 of the starting density is shown for comparison (day 0).

Dr. Cuthbert's earlier work suggests that oncogenic Ras expressing cells may have increased sensitivity to Fmev (4). To test this directly, we established a line of HME-50-5E that stably express H-ras12V from a retroviral LTR promoter. These cells have elevated levels of active ERK1/2 under serum-deprived growth conditions as compared to the parental cells. As shown in Figure 1, ras12V expressing cells are markedly more sensitive to the growth inhibitory effects of Fmev. In contrast to parental cells, the levels of active ERK in ras12V expressing are not detectably affected by Fmev. In addition, we did not detect any reduction in levels of Ras expression. These observations suggest that the growth inhibitory effects of Fmev on cells sensitized by ras12V are not due to inhibition of the mitogenic signaling of Ras to ERK. Other Ras-dependent pathways may be involved. The sensitization of cells to Fmev by Ras12V may be downstream of ERK regulation, or through other Ras effector pathways. This will be explored using partial loss of function Ras mutants, and activated variants of Raf and MEK.

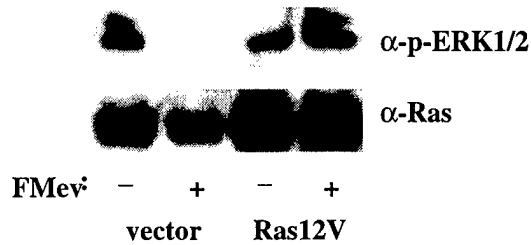


Figure 2. HME50-5E were infected with empty pBabePuro (vector) or with pBabePuro-ras12V (Ras12V). Whole cell lysates were prepared from cells treated or not with 100 uM Fmev for 48 hours. Lysates were separated by SDS-PAGE and immunoblotted with the indicated antibodies.

Key Research Accomplishments

1. Synthesis of Fmev
2. Characterization of the sensitivity of human mammary epithelial cells to growth-inhibitory effects of Fmev.
3. Characterization of the sensitivity of oncogenic Ras expressing human mammary epithelial cells to growth-inhibitory effects of Fmev.

Reportable Outcomes: none

Conclusions

We have established that, at appropriate concentrations, Fmev can preferentially inhibit the growth of human mammary epithelial cells expressing oncogenic Ras versus those that do not. These results give an initial indication that Fmev may have utility as an antagonist to breast cancer cell growth. Future studies will determine if the inhibitory effects of Fmev are through inhibition of cell cycle progression or induction of apoptosis. In addition, we have established that the inhibitory effects of Fmev on HME-ras12V cells is not due to decreased expression of Ras or lower levels of ERK activation. Future studies will focus on determining if the level of oncogenic Ras-dependent sensitization is downstream of ERK regulation, or through other Ras regulated effector pathways.

References

1. Quistad, G.B, Cerf, D. C., Schooley, D. A., and Staal, G. B. (1981). Fluoromevalonate-an inhibitor of insect juvenile hormone biosynthesis. *Pr Nauk Inst Chem Org Fiz Politech Wroclaw* **22**: 163-168.
2. Shay, J. W., Tomlinson, G., Piatyszek, M. A., and Gollahon, L. S. (1995). Spontaneous in vitro immortalization of breast epithelial cells from a patient with Li-Fraumeni syndrome. *Mol. Cell. Biol.* **15**: 425-432.
3. Cuthbert, J. A., and Lipsky, P. E. (1995). Suppression of the proliferation of Ras-transformed cells by fluoromevalonate, an inhibitor of mevalonate metabolism. *Cancer Res.* **55**: 1732-1740.
4. Cuthbert, J. A., and Lipsky, P. E. (1997). Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. *Cancer Res* **57**: 3498-3505.