

Enhanced antiproliferative effects of combined treatment of gamma tocotrienol with erlotinib or gefitinib is mediated through the suppression of Akt and Stat3 mitogenic signaling in +SA mammary tumor cells.

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Clinical use of ErbB receptor tyrosine kinase inhibitors such as, erlotinib and gefitinib has shown limited success in the treatment of breast cancer. The failure to inhibit cancer cell progression by ErbB receptor inhibitors, which target a single member of ErbB receptor family, is believed to be partly due to the coexpression and heterodimer cooperation of multiple ErbB receptors. Studies have shown that gamma tocotrienol, a rare member of the vitamin E family of compounds, is a potent anticancer agent and significantly inhibits EGF-dependent growth of +SA cells by decreasing ErbB3 receptor tyrosine phosphorylation. Recently, it has been shown that combined treatment of subeffective doses of erlotinib or gefitinib with gamma tocotrienol resulted in a significant decrease in mammary tumor cell growth, which was associated with a large suppression in the ErbB3 and ErbB4 receptor activation. Studies were conducted to investigate the intracellular signaling mechanisms mediating the antiproliferative effects of combined gamma tocotrienol and erlotinib or gefitinib treatment in +SA cells. Cells were maintained in serum free defined media with EGF (10 ng/ml) as mitogen. Combined treatment with subeffective doses of erlotinib (0.25 mM) or gefitinib (0.5 mM) with subeffective doses of gamma tocotrienol (0-3 mM) inhibited the growth of +SA cells in a dose-dependent manner over a 4 day culture period as determined by MTT colorimetric assay. Additional studies showed that similar doses of erlotinib, gefitinib, or gamma tocotrienol alone had no effect on the phosphorylated (active) intracellular levels of Stat3, PDK1, Akt or Erk1/2, as compared to controls. In contrast, combined treatment induced a relatively large decrease in the levels of phospho-Stat3, phospho-PDK1, and phospho-Akt but had no effect on the levels of phospho-Erk1/2, phospho-Src and phospho-IRS-1. These combined treatments did not alter the total levels of Erk1/2 but did cause a reduction in the levels of total Stat3 and Akt. Furthermore, the suppression in Akt activation was not associated with an increase in PTEN or PP2A phosphatase activity. These findings show that the enhanced antiproliferative effects of combined gamma tocotrienol and erlotinib or gefitinib treatment is mediated through suppression of Akt and STAT3 mitogenic signaling. Taken together, these results demonstrate that low dose combination therapy targeting multiple ErbB receptors is more effective than high dose monotherapy, and strongly suggest that combined gamma tocotrienol and erlotinib or gefitinib therapy may improve therapeutic responsiveness in the treatment of breast cancers in women.

Short Title: ErbB receptors, vitamin E and cancer.