tumor model. Inhibition of both aromatase and sulfatase activities should offer a new therapeutic approach to the treatment of hormone-dependent breast cancer.

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Evaluation of in vitro toxicity and efficacy of ferutinin, a natural promising chemopreventive compound

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The biological activity of the natural phytoestrogen ferutinin have not been extensively examined as yet, in spite of the interest about plant-derived products as possible chemopreventives. In this study, the efficacy of ferutinin on several in vitro experimental endpoints correlated with tumour onset and progression has been compared to that of the well characterized soy isoflavone genistein. Effects of ferutinin and genistein have been examined on cell proliferation and growth inhibition, anchorage-independent growth and Matrigel invasion, cell growth in estrogen depleted media, programmed cell death. Like genistein, ferutinin acts as an estrogen agonist in the E-screen assay and exerts a biphasic effect on cell growth and proliferation in ER-positive MCF-7 cells, with an induction of proliferation at lower concentration (1 μM) and an antiproliferative dose-response effect at higher concentrations (10–100 μM). In MDA-MB-231 ER-negative cells, the dose-related inhibition of cell growth induced by ferutinin or genistein was evident, even if no biphasic effect was shown. In the agar clonogenic assay, ferutinin did not induce any significant increase in colony growth of MCF-7 cells at the assayed doses, while it showed a strong dose-related antiproliferative activity at high concentrations (10–100 μM). The biphasic effect of genistein on anchorage-independent growth was evident. The effect of the phytoestrogens on the malignant phenotype was evaluated in the in vitro Matrigel invasion assay. Ferutinin (1−100 μM) induced a dose-dependent inhibition of the invasive ability of MDA-MB-231 cells. The effect of ferutinin on cell death was also evaluated. The morphology of dying cells suggested the induction of a different mechanism of cell death induced by ferutinin, possibly alternative to apoptosis. The monodansylcadaverine (MDC) assay for autophagic cell death revealed some MDC-positive structures, which could be classified as autophagic vacuoles, in cells treated with high ferutinin concentrations (80 and 40 μM). The results show that ferutinin is more effective than genistein in the assayed in vitro endpoints in human breast cancer cells, suggesting a possible use of this natural compound as a chemopreventive or chemoterapeutic drug, even if the mechanisms of action and the benefit/risk ratio need to be further evaluated.

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Estrogenic effect of ellagic acid in the estrogen sensitive breast cancer cells

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Background: Ellagic acid is a dietary polyphenol present in abundance in strawberries and pomegranate. The antiproliferative activity of ellagic acid is documented and has been extensively studied in colorectal cancer, prostate cancer, and endometrial cancer cells. Ellagic acid exerts its effects via activation of various signaling pathways. Some study showed that the antiproliferative effect of ellagic acid is through mitochondrial pathway in colorectal cancer cell line. But another study reported that ellagic acid has antiproliferative effect by estrogen receptor α. We hypothesized that ellagic acid could be used as a new anticancer drug or new selective estrogen receptor modulator to manage the breast cancer. In the present in vitro study, we have compared the effect of ellagic acid on the proliferation of estrogen receptor negative or positive human breast cancer cells. In addition, the effect of ellagic acid on estradiol-induced stimulation of receptor-positive breast cancer cells was studied. Next, we evaluate the expression of pS2 and c-fos which is the down stream gene of ER.
Material and Methods: The receptor-positive breast cancer cell line MCF-7 and the receptor-negative cell line MDA-MB231 were used. The ellagic acid were tested in the concentration range of 10 um to 100 um. In MCF-7, 17α-estradiol and the mixture of ellagic acid and 17α-estradiol were also evaluated. Cell proliferation was measured after 0 hours, 24 hours, 4 8hours and 72 hours using the MTT assay. Apoptosis was confirmed by flowcytometry. The western blotting for pS2 and c-fos was done.
Results: Ellagic acid was able to significantly inhibit the cell proliferation of MDA-MB-231. But it caused cell proliferation in MCF-7. Flowcytometry showed that ellagic acid caused apoptosis in MDA-MB-231. It provokes cell-cycle arrest in S phase in MDA-MB-231. The western blotting for pS2 and c-fos was done to evaluate the estrogenic effect of ellagic acid on estrogen-receptor positive cell line. The expressions of pS2 and c-fos were higher in MCF-7 which was treated by ellagic acid.
Conclusions: The present data indicate that ellagic acid can inhibit the proliferation of receptor-negative human breast cancer cells. But it also stimulates the proliferation of receptor-positive breast cancer cells. Ellagic acid has different pathway in ER positive and ER negative cell lines. It might be used as anticancer drug in ER negative breast cancer, but it would be forbidden in ER positive breast cancer.

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Tamoxifen induces degradation of the o6-methylguanine DNA methyltransferase protein via the ubiquitin-dependent proteosome pathway in human cancer cells

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Background: Tamoxifen is a synthetic nonsteroidal anti-estrogen triphenylethylene compound. It is part of a class of anti-cancer drugs known as selective estrogen receptor modulators (SERMs), and could block tumor growth by mimicking estrogen and filling up estrogen receptors which prevents the cancerous growth. However, the current view is that the action of tamoxifen is not only mediated by its anti-estrogenic properties. Previous study have demonstrated that a combination chemo/hormonal therapy regimen for the treatment of patient with neoplastic diseases, for example, the combination of the tamoxifen with the CNU-type alkylating agents, leads to synergistic cytotoxic effects. However, the mechanism of action of combined effect had not been elucidated.
Material and Methods: MGMT activity assay, Western blot analysis, Northern blot analysis, and Immunoprecipitation were used in this study.
Results: Here, we demonstrated that treatment of human colorectal HT-29 carcinoma cells with tamoxifen decreased the expression level of MGMT protein in a dose- and time-dependent manner. This inhibition, independent with estrogen receptor status, was also shown in other common cancer types tested. No difference between MGMT mRNA levels before or after tamoxifen treatment was found. However, MGMT protein half-life was markedly decreased in the presence of tamoxifen compared with that of control. Moreover, the MGMT protein was found to increase its ubiquitinated species after tamoxifen treatment. This tamoxifen-induced MGMT degradation could be reversed by proteosome inhibitors lactacystin and MG-132.
Conclusion: We conclude that tamoxifen induced reduction of MGMT protein levels by accelerating protein degradation via the ubiquitin-dependent proteosome pathway. This result provides the evidence for the combination benefit in chemo/hormonal therapy.

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Alteration of arachidonic acid metabolism in human breast cancer

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Background: The plasma membranes of most cells contain both polyunsaturated and monounsaturated lipids, which are susceptible to oxidative damage by free-radical processes or electrophilic addition reactions, e.g. reaction of hydroxide ion (OH^-) with a double bond. Oxidative stress elevate levels of free radicals that can directly target arachidonic acid, an important mediator of inflammation, bound to phospholipids. This generates a complex mixture of oxidized products, known as isoeicosanoids, that can be cleaved off. In mammary gland tissues lipid peroxidation promotes the production of linoleic acid-derived arachidonic acid, a fatty acid that is most susceptible to lipid oxidation and formation of malondialdehyde (MDA). In this research we have studied alteration of arachidonic acid metabolism, both in model systems and in normal tissue adjacent to breast cancer and not in order to understand features of phospholipids fragmentation pattern in breast cancer.
Materials and Methods: Membrane phospholipids fractions from normal human tissue adjacent to breast cancer and fibroadenoma were extracted with chloroform/methanol (2:1), then sonicated on ice. The lipids chloroform extract was used for thiobarbituric acid assay (TBA) to induce a fragmentation pattern of lipids and evidenced the formation of aldehydic products as malondialdehyde (MDA). Moreover the lipids chloroform extract obtained after TBA assay was analyzed by ES-MS and MALDI-TOF spectroscopic techniques.