Aida Karina Zaineddin
Dr. sc. hum.

The effect of phytoestrogens on postmenopausal breast cancer risk and possible anticarcinogenic mechanisms

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktormutter: Frau Prof. Dr. sc. hum. Jenny Chang-Claude

Breast cancer is the most common type of cancer in women worldwide. Breast cancer is a hormone related disease; some of the established risk factors include early age at menarche, late age at menopause, and use of exogenous hormones. The observation that breast cancer rates are much higher in Western countries when compared to Asian countries led to the hypothesis that diet or genetic variants may play a role in breast carcinogenesis.
Phytoestrogens are plant-derived substances present in soy products. In Western populations phytoestrogens intake is predominantly derived from the intake of foods rich in lignans, which are present in fruits, vegetables and grains. Due to the structural similarity of phytoestrogens with the mammalian estrogens, phytoestrogens may bind to the estrogen receptor of the cell via hormone-dependent mechanisms, and thereby competing with the endogenous estrogens. Phytoestrogens can also exert hormone independent anti-carcinogenic actions, such as increasing apoptosis and inhibition of angiogenesis. After consumption, plant lignans are metabolized by the gut micro-flora into the mammalian lignans, enterolactone and enterodiol.
So far epidemiological studies investigating the association between phytoestrogens and breast cancer risk showed inconsistent results. Modification by genetic variants has been examined by a limited number of studies, and no definite conclusion can be drawn.

In the present thesis we assessed both dietary phytoestrogens and enterolactone concentrations in serum samples. Effect modification by estrogen, progesterone and HER2 receptor status was investigated for both phytoestrogen groups. Selected genetic polymorphisms were assessed, and analysis of GxE interaction between serum enterolactone and genotypes of selected polymorphisms. Moreover plausibility of anti-carcinogenic mechanisms was investigated by assessing estrogens and SHBG levels in unaffected controls.
Data (2884 cases and 5509 controls) from a population-based case-control study that was carried out in two study centers (Rein-Neckar Karlsruhe region, and Hamburg) was used to assess the association of dietary phytoestrogen intake (plant lignans and mammalian lignans), and specific phytoestrogen rich foods (soy milk, soy beans, tofu, soy spread, seeds) on breast cancer risk. Participants completed a self-administered food frequency questionnaire assessing the dietary habits in the year prior to diagnosis; moreover all participants were interviewed using a standardized questionnaire, to obtain information on possible breast cancer risk factors and sociodemographic factors.
Our results showed no significant association between dietary phytoestrogens and postmenopausal breast cancer risk, and this was not differential by receptor status of the tumor. Significantly reduced postmenopausal breast cancer risk was observed with high consumption of soy beans (OR 0.83, 95%CI 0.70-0.97, P_{trend}= 0.02) and high consumption of sunflower/pumpkin seeds (OR 0.86, 95%CI 0.77-0.97, P_{trend}= 0.02). When stratifying the analyses by receptor status of the tumor, we observed a significantly reduced postmenopausal

breast cancer risk in ER- tumors with high consumption of soy beans (OR 0.66, 95%CI 0.46-0.94, P_{trend}= 0.02). Also a significantly reduced postmenopausal breast cancer risk in ER+ tumors with high consumption of sunflower/pumpkin seeds (OR 0.88, 95%CI 0.707-0.99, P_{trend}= 0.02) was found. But no significant heterogeneity was observed between ER- and ER+. These results could support the biological theory that phytoestrogens have both hormone-dependent and hormone independent anti-carcinogenic properties.
Serum enterolactone and genistein were measured in 1250 cases and 2164 controls from the Rhein-Neckar Karlsruhe region with available serum samples. Significantly reduced post-menopausal breast cancer risk was observed with high enterolactone levels (OR 0.65, 95%CI 0.52-0.83, P_{trend}<0.0001). The association was significantly stronger for ER- tumors, but not differential by PR and HER2 status of the tumor.
Results of both dietary phytoestrogens and phytoestrogen biomarker measurements were not modified by high or low fiber intake, nor by recent or past use of HRT.
A meta-analysis was performed to summarize studies assessing the association between dietary enterolignans and breast cancer risk. No association was observed with premenopausal breast cancer risk pooled RE of 1.01 (95% CI 0.87-1.15), but significantly reduced postmenopausal breast cancer risk was observed pooled RE of 0.85 (95% CI 0.71-0.99). Significantly reduced postmenopausal breast cancer risk was observed with high levels of enterolactone (Pooled OR 0.66, 95%CI 0.55-0.77). When stratifying studies on postmenopausal breast cancer risk by study design, both cohort studies (Pooled OR 0.70, 95% CI 0.52-0.88) and case-control studies (OR 0.64, 95%CI 0.49-0.79) showed a significant reduced pooled estimate for breast cancer.
To examine whether phytoestrogens influence hormone levels, estrone, estradiol and SHBG levels were measured in the controls of the Rhein-Neckar Karlsruhe region (n=1962). When comparing high vs. low enterolactone levels significantly lower levels of estrone and estradiol, as well as significantly increased SHBG levels were observed, after accounting for multiple factors that affect hormone levels. This indicates that enterolactone acts via altering endogenous hormone levels, providing support for the suggested anti-carcinogenic properties of phytoestrogens could be via hormone-dependent mechanisms. No effect modification was observed for genetic variants in the estrogen pathway genes *CYP17*, *CYP19*, *ESR1*, with respect to the association between enterolactone levels and postmenopausal breast cancer risk. Also sex hormone levels (estrone, estradiol) and SHBG did not significantly differ among the genotypes of selected polymorphisms.

In summary, the present findings significantly contribute to the hypothesis that phytoestrogens especially enterolactone can play an important role in reducing the risk of post-menopausal breast cancer in Western populations. The anti-carcinogenic properties of phytoestrogens can be due to hormone-dependent or hormone-independent mechanisms. Our results strengthen the assumption that serum enterolactone plays a role in etiology of postmenopausal breast cancer, yet further studies are needed to confirm our results, and to elucidate the biological mechanisms by which phytoestrogens reduce breast cancer risk.