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Poster Abstracts

Biomarkers

P1
Circulating adiponectin levels as a predictive factor of breast cancer in a double-blind 2×2 phase II trial of low-dose tamoxifen and fenretinide for breast cancer prevention

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Background: The assessment of breast cancer risk is a key step for an effective preventive treatment. Besides the established risk assessment models, independent predictive factors like circulating biomarkers may also be important to better select high risk subjects and to determine efficacy of chemopreventive treatment.
Adiponectin is a peptide hormone secreted from the adipose tissue that has been inversely related to breast cancer risk. In the present study, we measured plasma adiponectin levels in premenopausal women participating in a phase II trial of low-dose tamoxifen and fenretinide for breast cancer prevention.
Methods: Premenopausal women (n = 235) were randomly assigned in a double-blind 2×2 trial to receive tamoxifen 5 mg/d, fenretinide, a vitamin A derivative, 200 mg/d, both agents, or placebo for 2 years. A total of 181 premenopausal women with ductal intraepithelial neoplasia (DIN) and 54 unaffected women at higher risk according to the Gail model were enrolled.
Mammographic percent density was centrally measured using the computer-assisted method described by Boyd. Plasma adiponectin was measured by use of a commercial enzyme-linked immunosorbent assay kit.
Preliminary results: According to disease status (DIN vs Gail) at baseline, median plasma adiponectin levels were lower in women with a DIN (10 ug/ml; interquartile [IQ] range: 7.1–14) compared to unaffected women with a Gail risk (12 ug/ml; IQ range: 8.7–14.7) (p = 0.05).
Importantly, after a median follow-up of 5.5 years, plasma adiponectin levels were lower in women who had a breast cancer event (7.7 ug/mL; IQ range: 6.46–12.78) compared to women without event (11.02 ug/mL; IQ range: 7.8–14.4) (p = 0.015). This difference is also maintained after adjusting for BMI and treatment allocation (p = 0.011).
Considering the distribution of adiponectin levels at baseline, we observed a 9% reduction in the hazard ratio of breast cancer events by each unit increase of adiponectin levels (95% CI: 0.83–0.96; p = 0.02, cox model adjusted for BMI, treatment allocation, mammographic density and disease status at baseline).
Conclusion: We found that subjects with lower plasma adiponectin levels had a significantly higher risk of breast cancer event. The association appeared independent of known risk factors such as BMI and mammographic breast density. Further studies to better understand and confirm the role of adiponectin as independent predictive risk factor are warranted.

P1a
Promoter methylation of the BRCA1 gene in young breast cancer patients with no significant family history

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Background: Premenopausal breast cancer represents < 25% of all breast cancers, and only 5–10% of all breast cancer can be attributed to mutations in BRCA1/2 genes. Therefore, 90–95% of premenopausal patients may have a sporadic form of breast cancer. Although these women do not have germline mutations in the BRCA genes, it is possible that epigenetic modifications which lead to BRCA gene silencing may play a role and be a risk factor in these sporadic cases. Previous research has identified epigenetic changes in the BRCA1 gene in as many as 10–20% of sporadic cases. More recently, other investigators used peripheral blood cells (PBC) to evaluate the presence of somatic methylation of the BRCA1 promoter and suggested that promoter methylation in normal PBC can be identified and correlated with development of triple negative breast cancer. However, currently the presence and rate of BRCA1 promoter methylation is not well described in women with early onset breast cancer and high risk women. Therefore, in this current study our aim was to examine the rate of BRCA1 promoter methylation in breast cancer patients diagnosed at or under 40 who had no detectable BRCA1 mutations and in an effort to correlate BRCA1 promoter methylation with premenopausal breast cancer development.
Methods: 52 U.T. MD Anderson patients were identified from a prospective study database. 35 patients had a history of breast cancer or ducal carcinoma in situ (DCIS) who tested negative for a deleterious mutation in the BRCA1gene were enrolled in the study. As a control, 6 BRCA1 positive patients with breast cancer and 11 BRCA negative unaffected high risk patients were also examined. Median age was 35 (range 24–40). Twenty-eight (75.7%) of the patients had a positive family history of breast cancer, however only 12 (34.3%) had a first degree relative with breast cancer, other patients were tested for BRCA mutations based on their young age. Three (8.1%) had stage 0, 3 (8.1%) had stage I, 22 (59.5%) had stage II, 6 (16.2%) stage III, and 3 (8.1%) stage IV breast cancer. The estrogen receptor (ER) was positive in 27 (72.9%) of the samples, progesterone receptor (PR) in 20 (54.1%)

and Her-2/neu was positive (based on IHC scores of 2–3 + and above, or FISH +) in 10 (27%) of the patients. Seven (18.9%) were ER, PR, and Her-2/neu (triple) negative. DNA was extracted from PBC then subjected to bisulfite conversion (Invitrogen, MethylCode Bisulfite Conversion kit) and the promoter region was examined using methylation specific PCR techniques (–157 to +57, +1 serving as transcription start site).

Results: We observed that 5 out of 52 (9.6%) patients with breast cancer who were negative for germline BRCA1 mutations displayed BRCA1 promoter methylation. No promoter methylation was seen in patients with breast cancer who had a deleterious BRCA1 mutation, nor in high risk women who had a deleterious BRCA1 mutation.

Conclusions: These preliminary results suggest that about 10% of sporadic breast cancers have a somatic promoter methylation in the BRCA1 gene. This finding might be important for the development of somatic BRCA1 promoter methylation assay as an assessment of breast cancer risk in women with no deleterious BRCA1 mutations. It would be interesting and important to show increased somatic promoter methylation in the BRCA1 gene in high risk women who do not have a deleterious BRCA1 germline mutation. Such analysis is currently going and will be presented.

P2

"AminoIndex" for cancer detection (1): evaluation as a novel screening tool for colorectal cancer

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Introduction: Amino acids balance is changed in patients of various diseases due to metabolic transition while it is maintained in healthy human. It is also known that the metabolism of cancer cells is totally altered. Therefore, the detection of metabolic transition using amino acid profile is expected to be a promising screening marker of various cancers. We previously demonstrated that significant changes of plasma amino acid profile was observed and classifier composed of plasma amino acid concentrations as explanatory variables "AminoIndex" was high discrimination ability for colorectal cancer patients (Okamoto 2009). In this study, further possibilities of "AminoIndex" for colorectal cancer were investigated.

Subjects and Methods: Plasma samples were collected from Japanese colorectal cancer patients before any medical treatment (N = 220). Those of controls were also collected from subjects who were undergone comprehensive medical examination (N = 4,348). Plasma amino acid concentrations were measured by LC-MS. 80 patients and 400 gender- and age-matched control subjects were chosen as study data set to predict "AminoIndex". And the rest were used as test data set to valid the predicted "AminoIndex".

Results: "AminoIndex" for colorectal cancer was inferred by multivariate logistic regression analysis and classifier composed with six amino acids (Glu, Gly, ABA, His, Trp, and Arg) was predicted as the best model. The ROC curve for each predictive score was calculated, and this gave an AUC of ROC of 0.812 using the study data. Then, validation of predicted "Aminolndex" was performed using test data set and resulted equivalent discriminating performance (AUC of ROC of 0.768).

Further analysis showed that predicted "AminoIndex" for colorectal cancer had potential as a screening marker as follows:

1. The index could discriminate colorectal cancer patients in any stages equally.
2. The index showed higher discrimination performance especially in stages 0, I, and II patients while decrease of sensitivity was observed in existing tumor markers.
3. The distribution of predicted index was independent of those of tumor markers. Therefore, higher detection efficiency would be expected by combinatorial use of "AminoIndex" and tumor markers.

Conclusion and Perspectives: In this study, we demonstrated that further possibilities of "AminoIndex" for colorectal cancer based on plasma amino acid profile. For further evaluation, cohort studies are ongoing.

P3

The expression of WWOX in pancreatic adenocarcinoma

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Introduction: WWOX is a 46 kDa WW domain-containing oxidoreductase protein that has been shown in many different tumour types to have a tumour suppressor role. Few studies have investigated the expression of WWOX in small numbers of pancreatic carcinomas. The aim of this study was to assess the expression of WWOX in the normal pancreas as well as in pancreatic carcinomas and to investigate the correlation of WWOX expression in pancreatic carcinomas with tumour type, grade and stage.

Materials and Methods: WWOX expression was studied in 89 pancreatic adenocarcinomas using immunohistochemistry on formalin fixed paraffin embedded tissue. Of these cases, 76 were classical adenocarcinomas and 13 were invasive carcinomas ex intraductal papillary mucinous tumour (IPMT). The expression of WWOX was assessed in tumours and in adjacent normal pancreatic tissue. WWOX expression was assessed for staining intensity and subcellular distribution. The expression profile was correlated with tumour type, grade and stage.

Results: In the normal pancreas, WWOX was moderately/strongly expressed in the majority of the acini (98%), ductal (97%) and islet cells (91%). Localization of WWOX was only cytoplasmic in acinar and islet cells, but cytoplasmic and apical in most ductal cells (72%). The majority (62%) of classical pancreatic adenocarcinoma cases showed absent or low expression of WWOX. The ex IPMTs demonstrated absent or low expression in 46% of cases. No statistically significant correlation was found between WWOX expression and tumour type, grade or stage.

Conclusion: The results suggest that WWOX may have a physiological role in the normal pancreas, being expressed in all tissue compartments of the endocrine and exocrine pancreas. In addition, our results confirm that WWOX expression is decreased in the majority of pancreatic adenocarcinomas. Reduced WWOX expression shows no statistically significant correlation with tumour type, grade or stage suggesting that the loss of WWOX occurs as an early event in pancreatic tumorigenesis and may play a role in the initiation and progression of pancreatic carcinomas.