levels of the ER co-activator AIB-1 are thought to be responsible for the agonist-like activity of tamoxifen linked with endocrine resistance. Here we assess differences in estrogen function in endocrine- sensitive and endocrine insensitive breast cancer cells.

Methods: ERa, AIB1, and the ER target gene, cyclin D1 were localised by immunohistochemistry and immunofluorescence. Breast cancer cell lines; tamoxifen sensitive, MCF7 and the tamoxifen resistant, transformed LY2 cells were treated with 17b-estradiol and tamoxifen. Protein and mRNA expression was assessed by Western and Northern blotting, respectively. Proliferation was determined using standard MTT assays.

Results: ERa and AIB1 were found to be expressed predominantly in the nuclei, and cyclin D1 in the cytosol, of tumour epithelial cells. Immunofluorescence demonstrated co-localisation of both AIB1 and cyclin DI with ERa. Estrogen induced cell proliferation and cyclin D1 expression in MCF-7 cells, which was inhibited by tamoxifen, whereas in LY2 cells, treatment with both estrogen and tamoxifen resulted in breast cancer cell growth and target gene expression. Expression of the ER co-activator AIB1 at both the mRNA and protein level was found to be greater in LY2 cells compared with their parent MCF-7 cells.

Conclusion: In endocrine resistant breast cancer, tamoxifen induced ER activity may be due, at least in part to increased expression of the ER-co-activator AIB1.

338 Poster

Galectin-1 expression in human breast cancer tissues

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Introduction: Galectins are a family of lectins, defined by having at least one characteristic carbohydrate recognition domain (CRD) with the affinity for β-galactosides. There are many reports regarding the function of this group of proteins, mostly using the tissue culture media model, and the proposed roles of the galectins are as follows, regulation of immunity and inflammation, regulation of specific developmental processes, and regulation of the development and the progression of cancer. But most studies took the methods of investigating the galectin level in the cell lines, and among some studies using the human breast cancer tissues, only galectin-3 and galectin-9 are investigated. Up to the present, there is no report of galectin-1 expression level in human breast cancer tissues, and moreover, there is also no report of different levels of galectin expression directly related to the stages of human breast cancer. In this study, we examined the level of galectin-1 expression in the human breast cancer tissues and investigated its correlation to the tumor stages, the presence of lymph node metastasis, the tumor size, the tumor invasiveness, and the status of hormone receptors.

Materials and Methods: From the institution's surgical database, we randomly selected 100 breast cancer patients who were operated in the Gyeongsang National University Hospital from January 2000 to November 2003. Breast tissues were immunohistochemically stained with diluted primary antibody against galectin-1 using LASBP Kit. Antigen retrieval was facilitated with microwave method, and the rest of the staining procedure followed the usual ABC method. The staining results were further categorized into 'weak' and 'strong' group, which represents the groups of 0 and 1+, and the groups of 2+ and 3+, respectively. The compared information includes tumor invasiveness, tumor size, presence of lymph node metastasis, stage, hormone receptor status, and tumor recurrence.

Results: *(1) Levels of galectin-1 expression in cancer cells:* Galectin-1 was stained both in cancer cells and in stromal cells. The levels of galectin-1 expression in cancer cells were analyzed to the pathologic and clinical information. The levels of galectin-1 expression did not show any statistically significant differences according to the tumor size, the tumor invasiveness, the presence of lymph node metastasis, the tumor stage, and hormonal status.

(2) Levels of galectin-1 expression in Cancer-related stromal cells: In contrast to the results of galectin-1 staining in the cancer cell, the staining results of the cancer-related stromal cells showed significant changes along the pathologic variables of the breast cancer patients. High levels of galectin-1 expression in cancer-related stromal cells were observed in the tissues of invasive carcinoma compared to the tissues of non-invasive carcinoma ($p = 0.005$), and the levels of galectin-1 expression in cancer-related stromal cells were correlated with the T stages ($p = 0.034$). The levels of galectin-1 expression were also higher in the advanced stages of the breast cancer with a statistical significance ($p = 0.035$). The levels of galectin-1 expression according to the presence of axillary lymph node metastasis did not reach a statistically significant point, but showed some tendency of increased expression in the lymph node metastasis group ($p = 0.128$). The galectin-1 expression did not show any statistical association with tumor recurrence or hormonal receptor status.

Conclusion: Higher levels of Galectin-1 level were observed in the patients with advanced stages, and in patients with positive axillary lymph node metastasis. Authors propose possible roles of Galectin-1 in the tumor growth and metastasis of human breast cancer, and this study can be a starting point of research in the lectin-targeted treatment of breast cancer, using Galectin-1.

339 Poster

Associations and interactions between the co-regulatory protein SRC-1 and Ets-2 in breast cancer

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In breast cancer associations between p160 co-activator proteins and the development of resistance to endocrine treatment have been shown. We hypothesized that nuclear co-regulatory proteins may interact with non-steroid receptors. We investigated the effect of silencing the co-activator, SRC-1, on tumour cell growth *in vitro*. We also examined the MAPK activated transcription factors, Ets, as possible interaction proteins of the co-activator SRC-1 in human breast cancer. The effect of SRC-1 silencing on the Ets target genes was also investigated. SiRNA technology was used to inhibit estrogen induced cell growth of breast cancer cells *in vitro*. Protein-protein interactions between SRC-1 and Ets-2 were assessed using co-immunoprecipitation. It was found that Ets-2 interacted with SRC-1 under basal conditions and that the addition of growth factors further increased the level of interaction. Recruitment of SRC-1 to the Ets response element was demonstrated in primary breast tumour cell cultures and in the SKBR3 cell line using electromobilty shift assay. It was shown that growth factors induced interaction between Ets and their DNA response element and stimulated recruitment of co-activators to the transcription factor-DNA complex. Silencing of SRC-1 was found to down-regulate expression of the Ets target gene, c-myc.

Expression and co-expression of Ets and the co-regulatory protein SRC-1 was investigated using immunohistochemistry and immunofluorescence in a cohort of breast tumour patients ($n = 132$). Ets-2 was found to be associated with reduced disease-free survival ($p < 0.0001$), as was expression of SRC-1 ($p < 0.0001$). Co-expression of Ets-2 and SRC-1 significantly reduced the period of disease-free survival ($p < 0.0001$).

These data describing associations and interactions between non-steroid transcription factors and co-regulatory proteins may provide the basis for a new model of co-activator mediated endocrine resistance in breast cancer.

340 Poster

Implication of polysomy 17 in HER-2/neu overexpressing breast cancers

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Introduction: In breast cancer, the implication of polysomy 17 in the evaluation of the *HER-2/neu* status remains poorly understood. We studied *HER-2/neu* gene and chromosome 17 copy numbers in HER-2/neu overexpressing breast cancers, thereby evaluating the distribution of estrogen (ER) and progesterone (PR) receptor expression.

Methods: A series of 80 formalin-fixed paraffin-embedded breast carcinomas, showing HER-2/neu overexpression on immunohistochemistry (IHC 2+ and 3+ scores), were subjected to FISH analysis. Using a dual-probe system (Vysis), the *HER-2/neu* gene and the centromeric region of chromosome 17 were enumerated simultaneously. A mean *HER-2/neu*-to-chromosome 17 ratio > 2 was considered amplified for *HER-2/neu* and a chromosome 17 copy number > 3 was considered indicative of polysomy 17. All cases were further examined by IHC for the expression of ER and PR using the rabbit SP1 and SP2 monoclonal antibody respectively (NeoMarkers). The Allred-score was used to evaluate ER and PR staining.

Results: All 44 cases scoring 3+ on IHC showed *HER-2/neu* gene amplification. In the majority of cases (55%), this was accompanied by polysomy 17. Of 36 IHC 2+ cases, only 6 (17%) showed *HER-2/neu* gene amplification whereas 17 had a normal *HER-2/neu*-to-chromosome 17 ratio. However in a high proportion of IHC 2+ cases (47%), polysomy 17 without *HER-2/neu* gene amplification was found. These findings are in line with our previously published data [1], including four IHC 2+ cases which all showed polysomy 17 without *HER-2/neu* gene amplification nor increased *HER-2/neu* mRNA levels. Polysomic 17 cases showed 88% ER and 53% PR positivity, expression rates that are similar to those observed in *HER-2/neu* negative breast cancers. In contrast, only 49% of *HER-2/neu* amplified cases were ER positive and 47% were PR positive. These results illustrate that ER expression is considerably less frequent in *HER-2/neu*