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.

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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.

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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.

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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*

amplified cases as compared to polysomic 17 or *HER-2/neu* negative cases.

Conclusion: Based on the current findings, we hypothesize that the biological significance of polysomy 17 is different from that of *HER-2/neu* gene amplification in breast cancer. The observation that polysomic 17 cases showed ER and PR expression rates similar to those encountered in *HER-2/neu* negative breast cancers is in line with this hypothesis.

References

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Expression of insulin-like growth factor-1, aromatase and oestrone sulphatase breast cancer tissue

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Aim: Local oestrogen production by Aromatase and Oestrone Sulphatase enzyme (STS) and Insulin-like Growth Factor-1, IGF-1 play pivotal roles in growth stimulation of breast cancer cells. We investigate the influence of the local production of IGF-1 of breast tumours on the expression of these oestrogen producing enzymes.

The Aromatase(Cyp-19 gene), STS and IGF-1 gene expressions in 71 oestrogen receptor positive breast cancer tissue and their corresponding adjacent normal tissue (ANT) were analysed using real-time quantitative-PCR. Their expression levels were compared to that of beta-actin (housekeeping gene). Data was interpreted using Spearman's Correlation test and paired sample T-test.

Results: The mean IGF-1 mRNA levels were higher in ANT than in tumour tissue but this was not statistically significant ($p = 0.212$). There was no correlation was found between the tumour tissue and ANT in terms of IGF-1 expression in each case($p = 0.844$). There was an inverse relationship between tumour IGF-1 and tumour STS expression ($p = 0.000$). This relationship was also present between IGF-1 expression in ANT and tumour STS expression ($p = 0.014$). ANT IGF-1 expression also had an inverse relationship with tumour Cyp-19 expression but this was only marginally significant ($p = 0.079$). Tumour expressed IGF-1 did not correlate with tumour Cyp-19 ($p = 0.129$).

Conclusion: IGF-1 expression in higher in ANT than in tumour tissue. These results suggests that IGF-1 expression in tumour and ANT may act as downregulators to STS expression and to a lesser degree to Cyp-19 expression.

Thursday, 23 March 2006 16:00–16:45

POSTER SESSION

Pre-operative management

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Additional work-up of new lesions found in breast MRI for staging purposes in 345 patients with breast cancer

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Purpose: Breast MRI is increasingly being used as a staging tool in breast cancer. Our purpose is to evaluate the additional work-up procedures generated by the integration of this technique in everyday practice.

Material and Methods: We staged 345 consecutive patients with fine needle aspiration biopsy (FNAB) or core-biopsy (CB) proven breast cancer or cancer of unknown origin (CUP) syndrome. T1-weighted FLASH 3D pre- and post-contrast images were obtained. Morphologic and semi-quantitative analysis was done in all patients. Additional lesions with potential change in therapeutic approach were evaluated with second-look ultrasound and FNAB or CB when needed. If necessary, new lesions were localized with a radiotracer (Tc-99m). BIRADS 3 lesions were followed-up with breast MR. Our gold standard was the pathologic report in all cases. All the procedures were carried out by dedicated breast radiologists.

Results: Additional lesions in the same or contralateral breast were found in 97 patients (28.1%). These lesions were due in part to multicentricity or multifocality in 67 patients (19.4%), also due to contralateral lesions in 25 patients (7.2%), extensive intraductal component in 7 patients (2%) and CUP syndrome in 5 patients (1.4%). Work-up of all these additional lesions originated 89 ultrasound procedures (in 25.7% of patients), 28 pre-surgical localizations with radiotracer (8.1%), 26 FNAB (7.5%) and 16 core-biopsies with 14G (4.6%). Additional breast MR exams were needed in 47 patients with BIRADS 3 lesions. Mean follow-up time was 13.8 months and no malignant new lesions were seen. For multicentric-multifocal disease, sensitivity was 75% (CI 62–83%) and specificity 99% (CI 96–99%). After all the work-up was done, patients were reevaluated and breast MR changed therapeutic approach correctly in 66 patients (19.1%) and incorrectly in 12 patients (3.4%).

Conclusions: Additional lesions are found in almost a third of patients staged with breast MR and work-up of these lesions is cumbersome but it changes therapeutic approach in 1 out of every 5 patients in our series and is therefore recommended in the staging of women with breast cancer.

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Screen detected breast cancer: is preoperative staging necessary?

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Introduction: Staging investigation to exclude metastatic disease in patients with early stage Breast cancer is routinely carried out. However such a practice has not often been scrutinised for its clinical value and cost effectiveness. Expectedly the earlier the stage of the disease the lesser the chance of finding evidence of metastatic disease with commonly used methods. Consequently the use of routine preoperative investigations may be of little value but adding more financial strain on already stretched resources. This instigated the current study to evaluate the benefit of commonly used tests, full blood count (FBC), liver functions (LFS) and chest x-ray (CXR) as preoperative staging in women with mammographically detected breast cancer diagnosed by pre-operative core biopsy.

Methods: The results of routine preoperative investigations, FBC, LFS and CXR in patients with mammographically detected breast cancer were reviewed. Breast cancer diagnosis was established preoperatively by core biopsy. Patients with palpable tumours and those who had investigations for symptomatic reasons were excluded from the study,

Results: Total of 146 patients with mammographically detected breast cancer were included in the study. Patients aged between 37–90 years. One hundred and sixteen cases had invasive carcinoma and 30 had DCIS only. In those with invasive disease histological tumour size ranged from 1.8 mm to 30 mm. Twenty cases had axillay node involvement. Tumour grade was I, II and III in 36, 49 and 27 cases respectively. Four cases could not be graded because of small tumour size. There was no evidence of metastatic disease detectable on preoperative staging in any of these patients.

Conclusion: This study does not support the routine practice of pre-operative staging in patients with mammographically detected breast cancer. Therefore routine bloods and chest x-ray should not be carried out in this cohort of patients. Omitting these tests not only prelude wasting valuable resources but should also have positive financial implications.

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Prevention of wound infection in breast cancer surgery with a strategy based on administration of antibiotic prophylaxis in patients at high risk of wound infection occurrence

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Background: In a previous study, we showed that neoadjuvant chemotherapy and immediate breast reconstruction were associated with an increased risk of wound infection (WI) in patients undergoing breast cancer surgery. The objective of this study was to evaluate the impact on WI occurrence of a preventive strategy based on administration of