cystic hypersecretory (*sensu* Rosen) changes were devoid of expression of all the eight mucins sought. It is concluded that several mucins are secreted in benign conditions, unlike current conventional belief, and, that the patterns of mucin expression in them and carcinomas are not random. The biological functions of these mucins in the breast are mostly unknown, and potential prognostic or clinical management implications are as yet unexplored.

510 ORAL

Loss of heterozygosity (LOH) in normal epithelial and myoepithelial cells from tissues adjacent to breast carcinoma

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Purpose: The multistep model for breast carcinogenesis suggests that invasive carcinoma arises via a series of intermediate (Ehyperplastic, and neoplastic stages. Using the method of loss of heterozygosity (LOH), we have previously demonstrated that genetic alterations identified at high frequency in invasive carcinoma are already present in in-situ carcinoma atypical hyperplasia and in non-atypical hyperplasia indicating that at least a proportion of these preinvasive lesions are clonal, neoplastic proliferations. LOH has also recently been demonstrated in apparently normal lobules adjacent to carcinomas. This has implications on the clonal nature of the normal lobule and the significance of LOH in carcinogenesis.

Methods: Using a microdissection technique and established methods to isolate and clone luminal and myoepithelial cells from breast specimens, we have investigated LOH independently in these two breast cell types. 7 microsatellite markers on chromosomes 3p, 11p, 13q, 16q, 17p and 17q were studied. Invasive carcinoma, ductal carcinoma in-situ and normal lobules were microdissected from paraffin embedded tissue in 3 cases. In two of these cases, 8–12 clones each of luminal epithelial and myoepithelial cells (total 40 clones) were also analysed. In one case, 12 clones of fibroblasts were also available.

Results: LOH was found in normal cells in 3/8 cases of breast cancer. In 2 cases, LOH was identified at the locus on chromosome 13q in the carcinoma as well as the adjacent (Enormal lobule or luminal and myoepithelial clones, with all samples exhibiting loss of the same allele. Loss of heterozygosity has not been identified in normal cells cloned from tissues away from the tumour. In 1/8 cases, LOH was identified in a single (Enormal, clone but this LOH was not seen in the adjacent tumour. One of 56 clones from 2 reduction mammoplasty specimens showed LOH at the locus on chromosome 13q.

Conclusion: The data confirm that LOH is present in normal lobules adjacent to carcinoma. The finding of LOH at the same locus independently in luminal and myoepithelial cells provides evidence for the presence of a common stem cell. Hence, genetic alterations predisposing to sporadic cancer probably occur very early in breast development.

511 ORAL

Loss of heterozygosity (LOH) and allelic imbalance (AI) in apocrine metaplasia and apocrine adenosis of the breast

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41 cases of apocrine metaplasia and 17 cases of apocrine change within sclerosing adenosis (apocrine adenosis) were analysed for LOH/AI at 8 loci reported to be involved in invasive and in situ breast cancer using a microdissection technique, polymorphic microsatellite markers and the polymerase chain reaction (PCR). Within apocrine metaplasia, examples of LOH and/or AI were identified in 2/28 (7.1%) of informative cases at 1p (MYCL1), 2/14 (14.3%) at 11q (INT2), 1/15 (6.7%) at 13q (D13S267), 3/22 (13.6%) at 16q (D16S539), 2/23 (8.7%) at 17p (TP53), 3/16 (18.8%) at 17q (D17S250) and 2/11 (18.2%) at 17q (D17S513). The frequency of abnormalities in apocrine adenosis was found to be higher in percentage terms with LOH/AI being detected in 3/12 (25%) informative cases at 1p (MYCL1), 2/7 (28.6%) at 11q (INT2), 1/3 (33.3%) at 13q (D13S267), 2/12 (16.7%) at 16q (D16S539) and 2/10 (20.0%) at 17q (D17S250). Neither LOH nor AI have been identified at 1p (D1S252), 17p (TP53) or 17q (D17S513) in apocrine adenosis. These findings indicate that a small percentage of apocrine metaplasia cases appear clonal and the finding of a higher percentage of abnormalities in apocrine adenosis suggests a possible progression of apocrine lesions to in-situ and invasive breast cancer.

512 ORAL

An audit of grading and typing of invasive breast carcinoma on needlecore biopsy specimens

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Purpose: An audit of the assessment of pathological prognostic factors on breast needlecore biopsies (NCB) was carried out.

Methods: Over a 9 month period 191 malignant NCBs with follow-up excision specimens were received. Histological grade and tumour type were assessed by routine methods.

Results: There was excellent correlation between grade of invasive carcinomas on NCB and excision samples ($p < 0.0001$); 120 of the 173 cases with sufficient tissue for assessment were correctly classified. Scores for tubules, pleomorphism and mitotic counts were also individually highly significant (all $p < 0.0001$). For mitoses NCB tended to underestimate the overall scores (61 out of 67 cases) but the scores for tubules and pleomorphism were more randomly distributed. The accuracy of classification of type of invasive carcinoma was also high ($p < 0.0001$) with 126 of 173 being correctly identified.

Conclusion: NCB of the breast is recognised as a reliable test for the diagnosis of invasive breast carcinoma and can also accurately predict the grade and type. This may be clinically relevant in some situations.

513 ORAL

The prediction of response to chemotherapy in invasive breast carcinoma by determination of histological grade

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Purpose: We have examined the role of "Nottingham" histological grade of invasive breast cancer in predicting response to chemotherapy.

Method: Grade (HG) was examined in a group of 465 patients from the IBCSG randomised clinical trial of adjuvant chemotherapy (peri-operative or prolonged) (formerly Ludwig Trial V).

Results: HG predicted overall survival (OS) in both lymph node (LN) negative and LN positive breast cancer ($p = 0.045$ and $p < 0.001$ respectively). Hazard ratios of 1.651 ($p < 0.001$) and 1.437 ($p = 0.045$) respectively were seen for an increase of 1 grade in LN+ and LN− disease. In LN+ patients an increase by 1 grade gave a significant OS disadvantage regardless of whether prolonged or peri-operative chemotherapy was given. However, in LN− disease this survival disadvantage was seen only in patients receiving peri-operative chemotherapy. No observed difference in survival of LN− patients was seen according to whether peri-operative treatment was received or not, when grouped by HG. However LN+ patients with grade 3 tumours obtained a significant OS and DFS benefit from prolonged compared to peri-operative chemotherapy ($p = 0.016$ and $p = 0.013$ respectively); those with grade 1 or 2 tumours had comparable survivals for both treatment arms.

Conclusion: Histological grade predicts OS and can, in particular, identify a group of grade 3, LN+ patients who may benefit from chemotherapy.

514 ORAL

Predictive factors of response to neo-adjuvant chemotherapy by immunohistochemistry (IHC)

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Neo-adjuvant chemotherapy is currently used for locally advanced and operable breast tumors not assessable for immediate conserving surgery due to their large size. The number of mastectomies is dramatically decreased and survival is identical to that obtained by mastectomy and medical adjuvant treatment. Conserving treatment rates are in correlation with several factors: type and intensity of neo-adjuvant chemotherapies, tumor size and characteristics. An immunohisto-chemichal study was performed on tumor samples from 128 patients enrolled in a randomized trial comparing mastectomy to neo-adjuvant chemotherapy (Ann Oncol 1991; 2: 347–54). Specific antibodies to p53, c-erbB-2 (Her-2/neu), Mib1 (antiKi-67), pS2, GSTpai, estrogen receptors (ER) and progesterone receptors (PR) were used to correlate these factors to tumor shrinkage during neo-adjuvant chemotherapy.

Univariate statistical analysis showed that negative ER detection by IHC (and also by dextran charcoal coated method) was highly correlated with chemosensitivity ($p = 0.001$). A high percentage of Mib-1 tumor cells (higher than 40%), as well as initial tumor size less than 4 cm were also correlated with tumor responsiveness to chemotherapy ($p = 0.009$ and $p = 0.03$).

By multivariate analysis, IHC-ER, Mib-1 and initial tumor size were independent predictive factors of response to neo-adjuvant chemotherapy, the last parameter being the most important.

515 ORAL

The expression of thymidine phosphorylase in fibrocystic disease, fibroadenoma, papilloma and carcinoma of breast

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Purpose and Methods: We have shown that human thymidine phosphorylase (TP) is identical with the platelet-derived endothelial cell growth factor and has angiogenic activity. In this study, we examined the expression of TP in fibrocystic disease, fibroadenomas, papillomas and mammary carcinomas, using biochemical and immunohistochemical methods. Moreover, in order to evaluate the significance of TP expression in mammary carcinomas, we studied its relationship with the vascular density and various clinicopathological factors in patients with a mammary carcinoma.

Results and Conclusion: TP expression was much common in mammary carcinomas, was slight in fibrocystic disease and fibroadenomas and was intermediate in papillomas. The number of microvessels in mammary carcinomas was generally correlated with the number of TP-positive cells. TP expression and the vessel density were significantly high in fumors negative for ER or positive for c-erbB2 and in tumors positive for TP or c-erb B2, respectively, TP expression and vessel density were not modified by any status of nodal metastasis, PR expression, menopause, age, and p53 expression.

516 POSTER

Power doppler vascularity in malignant breast tumours: Correlation with pathological blood vessel counts, microvessel density and other histopathological prognostic factors

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Purpose: Neoangiogenesis is a recognised characteristic of malignant breast tumours. We evaluated if power doppler (PD) detected vascularity and vessel morphology correlated with histological vascularity and known histopathological prognostic factors.

Methods: We prospectively examined 174 patients presenting with palpable or mammographic solid breast masses. Parameters measured included: number of vessels seen on colour doppler (vessel C) and PD (vessel P), vessel distribution (peripheral, central or penetrating) and morphology (tortuous, branching). 145 lesions had all available H&E sections examined and the number of vessels (separately and grouped) measuring 1 mm or more in diameter at both the centre and periphery of the lesion recorded. CD31 immunostaining was also carried out to determine the highest microvessel density at the periphery of the lesion in the "hot spot"region.

Results: 183 masses were examined and diagnosis confirmed with needle biopsy. There were 117 cancers which formed the basis of this study. 109/117 had surgery and 99 of these were histologically examined for vascularity. Doppler factors significantly associated with histological grade were vessel P ($P < 0.001$), branching vessels ($P = 0.0019$), vessel C ($P = 0.0063$), central or penetrating vessels ($P = 0.0339$) and tortuous vessels ($P = 0.0377$). Regression analysis showed vessel P to be the best predictor of high histological grade. The number of large vessels identified on PD correlated with the number of large vessels found on histology (periphery and centre: $p < 0.0001$ and $p = 0.0075$ respectively). High histological peripheral large vessel counts were associated with higher tumour grade ($p = 0.0012$) and lymph node stage ($p = 0.0073$), whilst the histological central vessel counts corresponded with stage ($p < 0.0001$) but not grade. Increased numbers of large vessels in pathological sections (both peripherally and centrally) were associated with greater size of breast carcinoma ($p = 0.0325$ and $p = 0.0024$ respectively). The mean CD31 counts showed no relationship with histological grade, stage or size.

Conclusion: The study confirmed good correlation between the number of vessels seen on PD and on histological sections. The demonstrated vascularity is associated with known pathological prognostic factors. PD may therefore provide a method of non-invasive prediction of the aggressiveness of malignant breast tumours.

517 POSTER

A national pathology audit of breast cancer cases

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Purpose: In 1997, comprehensive recommendations for pathology reporting of breast cancer were published in Australia. The NHMRC National Breast Cancer Centre undertook an audit of national pathology reporting to give a describe a baseline descriptionmeasure of the completeness and coverage of pathology reporting of breast cancer in Australia before the introduction of the recommendations.

Methods: Pathology reports of breast cancer cases in April-May 1995 from all states and territories in Australia were audited on a standard data collection form. Three pathology registrars recorded completeness of tumour size, histological type, grade, margins of excision, vessel invasion, DCIS and non-neoplastic changes in adjacent breast tissue.

Results: Reports of invasive cancers for 1,542 women were most complete for tumour type (100%) and size (94%, 95%CI 92–95) and less for histological grade (84%, 95%CI 82–86) and presence or absence of DCIS (79%, 95%CI 77–81). Information, except for tumour type and size, was variable across the States and Territories. Reporting of DCIS was comparatively poor.

Conclusion: The capacity for improvement compared with past, more limited, surveys and the variable percentages of complete information in this national audit suggested that continuing efforts were needed to ensure uniform, high quality reporting of breast cancer specimens.

518 POSTER

Comparative studies on cytological findings between early recurrent cases with negative nodes and recurrence-free cases with positive nodes in breast cancer

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Purpose: Axillary lymph node metastasis is the most important prognostic factor in breast cancer. However, early recurrence has been observed in patients without the node metastasis, and node-positive patients have survived without recurrence. Thus, the prognosis after operation is various and sometimes independent of nodal status. In the present study, we examined a significant factor by comparing cytological findings and the immunohistochemical expression of primary cancer cells between the both cases.

Material and Methods: From 735 cases surgically treated in our hospital between 1990 to 1996, 11 node negative and early recurrent cases within two years (group 1), 16 node negative and non recurrent cases for more than 5 years (group 2), 18 node positive and recurrent cases (group 3), and 20 node positive and non recurrent cases for more than 5 years (group 4) are used. Cytological atypia of the primary tumor was analyzed using microscopic morphometry (Olympus micrometer VM-30). In addition, expression of Ki-67, c-erb B2, and p53 were evaluated immunohistochemically.

Results: Significant parameters of cytological findings between group 1 and group 4 were noticed in diameter of nuclear long-length (mean $p = 0.005$, SD $p < 0.001$), diameter of nucleolar long-length ($p < 0.001$), nuclear area ($p = 0.009$), incidence of nucleolus $>2.5\ \mu m$ in diameter ($p = 0.01$), and mitotic index ($p = 0.007$). A cytological grading system (0–11 points) by means of scoring these cytological items and necrosis in each case was significantly different between the two groups (the cut point; 7, $p < 0.001$). Regarding immunohistochemical analysis, expressions of Ki-67, c-erb B2 revealed higher positivity in early recurrent cases without the node metastasis (group 1).

Conclusion: It is proposed that our scoring system of the cytological dysplasia and immunohistochemical expresions of Ki-67 and c-erb B2 may be a useful parameter of early recurrence in breast cancer.