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.

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

R.S. Lakhani[1], R. Chaggar[3], S. Davies[2], C. Odell[3], M. O'Hare[2].
[1]*Departments of Histopathology;* [2]*Breast Cancer Laboratories, LICR/UCL;* [3]*University College London Medical School & The Ludwig Institute for Cancer Research, Branch, UCL, UK*

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.

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

S. Selim, A. Ryan, C. Wells. *St. Bartholomew's Hospital, Department of Histopathology, 3rd Floor, West Wing, Royal Hospitals NH, St. Bartholomew's Hospital, West Smithfi, London EC1A 7BE, UK*

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.

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

H.E. Denley, S.E. Pinder, C.W. Elston, I.O. Ellis. *Nottingham City Hospital Breast Team, UK*

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.

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

S.E. Pinder[1], S. Murray, I.O. Ellis[1], H. Trihia, C.W. Elston[1], R.D. Gelber, A. Goldhirsch, J. Lindtner, H. Cortés-Funes, E. Simoncini, M.J. Byrne, R. Golouh, C.M. Rudenstam, M. Castiglione-Gertsch. [1]*Histopathology Department, BA Gusterson; City Hospital NHS Trust, 1PB NG5 and International Breast Cancer Study Group (IBCSG), UK*

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.

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

G. Mac Grogan, L. Mauriac, M. Durand, F. Bonichon, A. Floquet. *Department of Medicine, 180, rue de Saint-Genes, F-33076 Bordeaux, France*

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.