cancer. In comparison with normal breast tissue from 10 controls, all cases of carcinoma had an increased frequency of aneusomy of chromosomes 1 and 17. Similar cytogenetic abnormalities were observed in the adjacent uninvolved tissues and the axillary metastatic lymph nodes. the mean number of disomic spots differed significantly between the breast carcinomas and the benign cases. The difference between adjacent tumor-free tissues and benign cases for the fraction of nuclei with two spots was also significant. The number was not statistically different between invasive carcinomas and the metastatic lymph nodes. Taken together, these data suggest that aneuploidy for chromosomes 1 and 17 is an early cytogenetic change in breast cancer tumorigenesis that is stable over progression of the disease.

Percentages of nuclei with 2 spots (mean ± SD)

Chromosome 1			Chromosome 17		
Carcinoma	Adjacent breast tissue	Normal breast tissue	Carcinoma	Adjacent breast tissue	Normal breast tissue
34.7 ± 7.9	51.0 ± 11.6	69.5 ± 3.5	35.8 ± 11.2	48.5 ± 13.2	73.1 ± 5.1
	0.0001			0.0002	
		0.0001			0.0001
	0.0001			0.0001	

Multicentricity of breast cancer: new findings and their clinical and biological implications

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We have studied the spatial relationship within the breast between multicentric foci (MCF) and the primary tumour in 30 modified radical mastectomy specimens using Egan's correlated pathologic-radiological method using 5 mm slices of the whole breast. The relative positions within the breast of the primary tumour and MCF were used to calculate the relative distribution of primary tumour and MCF in the 4 quadrants of the breast and the percent breast volume that would be required to be excised to include all MCF. Nineteen (63%) breasts harboured MCF. The relative distribution of primary tumour and MCF in the 4 breast quadrants was significantly different ($p = 0.034$). MCF were present beyond the index quadrant (25% of breast volume including the tumour) in as many as 79% (15/19) of breasts that harboured MCF; and, in half the cases (15/30) when all breasts were considered. Thus, even if a quadrant were excised, 50% of patients would still have MCF left behind. This is in variance with the suggestion put forward by Holland et al (1985) that MCF are contained within the index quadrant in 90% of cases. Although the number of patients in the present series is small, the probability of our finding being due to play of chance is 1 in 1500.

The biological and clinical significance of this study is, in a way, paradoxical. In large studies of breast conservative therapy > 90% of early breast recurrences have been found to occur in the index quadrant. This is true whether or not radiotherapy is given. In light of the new findings, if MCF were giving rise to these recurrences then half would have occurred in other quadrants and therefore, MCF probably do not give rise to early breast recurrence. We also know that local recurrence can occur in the index quadrant in the presence of clear margins, which suggests that it does not always arise from residual tumour. We propose that local recurrence arises a) from circulating metastatic cancer cells lodging in the highly vascular surgical bed (local relapse does harbinger a poorer prognosis) or b) from local transfection of surrounding breast epithelium by nuclear material released from the original malignant clone resulting in insertional mutagenesis. Thus although the margins of excision are morphologically clear, they may be genetically unstable. This has been corroborated by a recent paper which demonstrated loss of heterozygocity in morphologically normal tissue at the excision margins of breast cancer. In any case, if MCF do not give rise to breast recurrence then why should we treat them with either mastectomy or whole breast radiotherapy? It is as necessary or as unnecessary as treating the contralateral breast! We have begun pilot studies of a clinical trial to test whether radiotherapy to the index quadrant alone can achieve good local control.

Breast gross cystic fluids. I. Protein biochemistry and electrophoretic MAP

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Gross cystic disease (GCD) is a common breast pathology, affecting about 7% of premenopausal women. There is convincing evidence that women with GCD are at a 2–4-fold greater risk of developing breast cancer than normal females. According to the epithelium lining the cyst, two subpopulations have been identified: those lined by apocrine metaplastic epithelium and $Na/K < 3$ (Type I), and those lined by flattened epithelium and $Na/K \geq 3$ (Type II). Needle-aspirated fluid of breast cysts (BCF) has a distinctive composition with unusual profiles of many biochemical constituents. Until now, BCF composition has been the subject of many studies in an attempt to understand the mechanism of cystic initiation-progression and possible linkage of cyst components to carcinogenesis. As cyst fluid proteins are believed to be secretory products of epithelial cell surrounding the cysts, we performed a more detailed protein map, with the identification of the major components of breast secretions and human milk too. BCF was obtained from 102 patients with GCD, and breast fluid nipple secretions (NAF) from 80 patients attending our Senology Centre. Polyacrylamide gel electrophoresis, immunoblot, two-dimensional electrophoretic analyses and protease identification were carried out with Bio-Rad apparatus and protocols. In Type I apocrine cyst, four major protein bands were identified with Coomassie BB (70, 44, 24 and 15 kDa), while silver enhancement revealed at least 25 distinct bands. Whereas in Type II cyst, three major components were identified with Coomassie BB (44, 24 and 15 kDa) and 20 protein bands with silver stain. Two-dimensional electrophoresis allowed us to obtain an improved protein mapping, with the quantification and identification of at least 15 known proteins. Moreover, six classes of proteases were identified in BCF, with a higher expression in Type I cysts in respect to a very limited content in Type II cysts. The protein composition of NAF revealed two types of secretions, with several common constitutive proteins. Type I NAF contained three major distinctive bands at 44, 24 and 18 kDa, while those designated as Type II presented characteristic bands at 120, 80 and 12 kDa. Silver staining revealed 30 protein bands in Type II NAF and 22 polypeptide spots in Type I NAF. NAF contained much more proteases than BCF, with peculiar expression of gelatin-degrading enzymes in Type II NAF. About 90% of women with benign breast disease had secretions with Type I polypeptide pattern, while about 64% of secretions from women with breast carcinoma presented Type II protein pattern. Human milk showed a protein pattern similar to Type II NAF and Type I BCF, except for the specific presence of (bb)-casein. Further studies are needed to evaluate our improved protein mapping as a useful complement to early screening and for corroborating the potential clinical interest of BCF and NAF protein analysis.