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Allelic imbalance at chromosome 17p13.3 (YNZ22) in breast cancer is independent of p53 mutation or p53 overexpression and is associated with poor prognosis at medium-term follow-up

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Summary Molecular and immunohistochemical studies of genetic events on chromosome 17p were prospectively compared with conventional clinical and pathological parameters and disease behaviour at a minimum of 72 months follow-up. In a series of 91 patients with primary operable breast cancer, 37 out of 91 (41%) patients had disease relapse and 23 out of 91 (25%) had died during the follow-up period. Allelic imbalance at the YNZ22 locus (17p13.3), demonstrated in 33 out of 63 (52%) informative patients, was significantly associated with disease recurrence ($P < 0.01$, 2 d.f. Cox analysis) and showed a trend towards impaired survival ($P = 0.08$, 2 d.f. Cox analysis) after a mean follow-up of 84 months for survivors. By contrast, p53 mutation (in 10 out of 60, 17% of cancers), p53 allelic imbalance (in 23 out of 56, 41% informative patients), p53 mRNA expression (in 47 out of 87, 54% patients), p53 mRNA overexpression (in 24 out of 87, 28%) or p53 protein expression (detected in 25/76, 32%) were not associated with disease behaviour. There was no significant association between allelic imbalance at YNZ22 and any abnormality of p53 DNA, RNA or protein. Allelic imbalance at 17p13.3 (YNZ22) serves as a marker of poor prognosis in breast cancer. As yet unidentified genes on 17p13.3, distinct from and telomeric to p53, are therefore likely to be of clinical importance in breast cancer.

Keywords: breast cancer; p53; YNZ22; prognosis

Molecular lesions involving the short arm of chromosome 17 are among the commonest aberrations found in human breast cancer. Up to two-thirds of tumours may show allelic imbalance at the YNZ22 locus at 17p13.3 (Mackay et al, 1988; Devilee et al, 1989; Thompson et al, 1990; Chen et al, 1991; Singh et al, 1993; Thorlacius et al, 1993; Cornelis et al, 1994; Harada et al, 1994; Stack et al, 1995) and this finding has been associated with markers of tumour aggression (Thompson et al, 1990; Chen et al, 1991; Merlo et al, 1992; Harada et al, 1994; Ito et al, 1995).

For the p53 gene (Hall et al, 1996) at 17p13.1, mutation (demonstrated at the DNA level) has been associated with poor prognosis on 3–6 years follow-up (Andersen et al, 1993; Thorlacius et al, 1993; Elledge et al, 1994; Silvestrini et al, 1996). Furthermore, the precise location of the mutation may add further information of prognostic value (Bergh et al, 1995; Borressen et al, 1995) and predict response to chemotherapy (Elledge et al, 1995; Aas et al, 1996). p53 protein expression has been identified as a predictor of disease recurrence (Iwaya et al, 1991; Barnes et al, 1993; Friedrichs et al, 1993; Marks et al, 1994), even for patients without nodal involvement at the time of diagnosis (Allred et al, 1993; Barnes et al, 1993; Silvestrini et al, 1993; MacGrogan et al, 1995) and for poor survival (Isola et al, 1992; Allred et al, 1993; Silvestrini et al, 1993; Elledge and Allred, 1994; Borg et al, 1995; MacGrogan et al, 1995; Silvestrini et al, 1996). Although molecular lesions at p53 and at YNZ22 loci occur independently (Coles et al, 1990), it is not clear to what extent allelic imbalance on chromosome 17p telomeric to the p53 locus may reflect direct or indirect involvement of p53 itself. However, there is increasingly strong evidence for potential tumour-suppressor genes telomeric to p53 (Chen et al, 1991; Thorlacius et al, 1993; Cornelis et al, 1994; Harada et al, 1994; Nagai et al, 1995; Stack et al, 1995; Wales et al, 1995; White et al, 1996). To test the hypothesis that allelic imbalance at YNZ22 is an independent predictor of poor prognosis in breast cancer and to examine the associations between YNZ22 allelic imbalance and abnormalities of p53, this study examined YNZ22 allelic imbalance, p53 mutation status, p53 allelic imbalance, p53 mRNA expression, p53 immunostaining and clinical outcome with medium-term follow-up.

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PATIENTS AND METHODS

Ninety-one female patients mean age 57 years (range 30–78 years) at diagnosis with primary, previously untreated, breast cancer underwent surgery. Node status was determined on axillary node sampling or axillary clearance, with 45 out of 91 (50%) patients node-positive and 46 node-negative on histological examination. The oestrogen receptor content of the tumours was measured using enzyme immunoassay (Abbott Lab, North Chicago, IL, USA) and tumour oestrogen receptor protein of 20 fmol mg^{-1} protein or more was considered oestrogen receptor moderate or rich. Median follow up was 84 months (range 72–95 months) or to death.

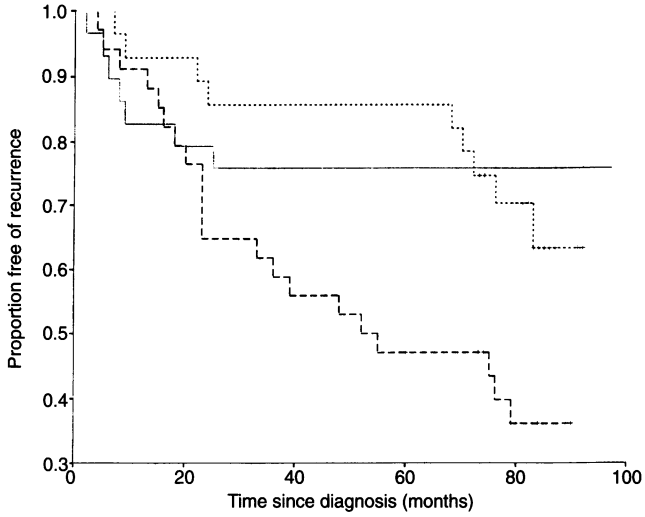

Figure 1 YNZ22 allelic imbalance and disease recurrence in breast cancer. Disease recurrence plotted against time since diagnosis (in months) for 91 patients with allelic imbalance (------), no allelic imbalance (——) and homozygotes (·····). Disease recurrence significantly associated with YNZ22 allelic imbalance ($P = 0.008$)

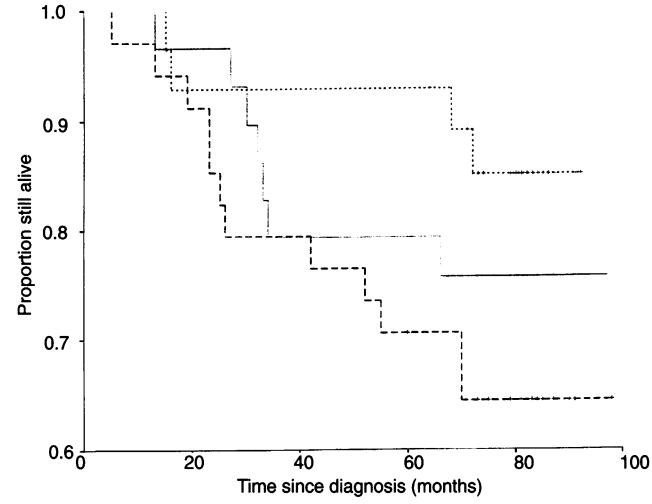

Figure 2 YNZ22 allelic imbalance and survival in breast cancer. Survival plotted against time since diagnosis (in months) for 91 patients with allelic imbalance (------), no allelic imbalance (——) and homozygotes (·····). Poor survival was associated with YNZ22 allelic imbalance ($P = 0.08$)

YNZ22 and p53 studies

Southern blots of paired blood/tumour DNAs were probed with YNZ22, MCT35.1 and pBHp53 for p53 for allelic imbalance in the tumour DNA (Coles et al, 1990). Screening for p53 mutations in exons 5–9 was performed using the hydroxylamine osmium tetroxide (HOT) technique and confirmed by direct sequencing (Prosser et al, 1990). Northern blots of total ribonucleic acid (RNA) were probed for p53 mRNA expression then for alpha-actin mRNA as an internal control (Thompson et al, 1990). For p53 protein expression, the 1801 antibody was used on Bouins-fixed sections and DO1 and DO7 on conventionally fixed tissues (Voijtesek et al, 1992). Results were scored independently by three observers.

Statistical methods

Univariate Cox analysis was used to compare YNZ22 allele loss, p53 allele loss, p53 mutation, p53 mRNA expression, p53 protein expression, node status, tumour size, oestrogen receptor status (as continuous variable), menopausal status with disease recurrence and survival. The chi-squared test was used to compare YNZ22 allele loss with p53 allele loss, p53 mRNA expression and oestrogen receptor protein content in the tumours.

RESULTS

Among the 91 patients examined for YNZ22 allelic imbalance, 63 out of 91 (69%) of patients were informative and in 33 out of 63 (52%) of tumours there was allelic imbalance (defined as a ratio of greater than 2:1 in band intensity on laser densitometry). At the p53 locus, allelic imbalance was demonstrated in 23 of 56 informative patients (41%). DNA from 60 of the 91 patients was successfully examined for p53 mutation and mutations confirmed in ten patients (17%). Among the 63 patients informative at the YNZ22 locus, p53 mutation was present in ten cancers (16%), seven of which showed allele imbalance at YNZ22.

p53 mRNA expression was detected in 47 out of 87 (54%) breast cancers successfully examined and p53 mRNA overexpression (> 4 times expression in normal breast tissue) detected in 24 out of 87 (28%) cancers. p53 protein was detected immunohistochemically in 13 out of 44 cancers (30%) using the 1801 antibody. In the 32 cancers examined with DO1 and DO7, the results were identical with the two antibodies and p53 protein was detected in 12 out of 32 cancers. Strong staining was uniform over cancer cells in eight cases but patchy in five, including one cancer with positive p53 protein staining in the absence of any p53 mutation or detectable p53 mRNA expression.

YNZ22 allelic imbalance was associated with oestrogen receptor-poor (< 20 fmol mg^{-1} protein) tumours (chi-squared 3.8, $P = 0.05$, 1 df), but not with p53 allele loss (39 patients informative at both YNZ22 and p53 loci) nor with p53 expression at either RNA or protein level.

After a minimum of 72 months follow-up 37 out of 91 (41%) patients had developed recurrent disease and 23 out of 91 (25%) of these patients had died.

Using univariate Cox analysis, YNZ22 allelic imbalance was significantly associated with disease recurrence ($P = 0.008$; Figure 1), as were axillary node metastasis detected at diagnosis ($P < 0.001$) and tumour size ($P < 0.001$). YNZ22 allelic imbalance was also associated with reduced survival ($P = 0.08$; Figure 2) and axillary node metastasis ($P < 0.001$), tumour size ($P = 0.008$) and low oestrogen receptor ($P = 0.001$) were each significantly associated with poor survival. No other parameter measured (p53 mutation, p53 allelic imbalance, p53 mRNA expression or overexpression, p53 protein expression, age at diagnosis or menopausal status) was significantly associated with disease recurrence or survival.

DISCUSSION

This prospective study has demonstrated that allelic imbalance at the YNZ22 locus is significantly associated with disease recurrence and associated with reduced survival after medium-term follow-up.

YNZ22 allelic imbalance reported in this study (52%) lies within the range (37–65%) previously published by other groups (Chen et al, 1991; Singh et al, 1993; Thorlacius et al, 1993; Cornelis et al, 1994; Harada et al, 1994; Ito et al, 1995; Stack et al, 1995). The proportion of cancers with p53 mutation (20%), p53 allele loss (41%), p53 mRNA expression (54%) and overexpression (28%) or p53 protein expression (32%) are similar to the reported series (Cattoretti et al, 1988; Davidoff, 1991; Iwaya, 1991; Kovach et al, 1991; Osborne et al, 1991; Runnebaum et al, 1991; Varley et al, 1991; Vojtesek et al, 1992; Andersen et al, 1993; Barnes, 1993; Friedrichs, 1993; Martinazzi, 1993; Thorlacius et al, 1993; Tsuda et al, 1993; Elledge and Allred, 1994; Marks et al, 1994; Bergh et al, 1995; Borressen et al, 1995; Stenmark-Askmalm et al, 1995).

Although YNZ22 allelic imbalance may occur in the same tumour as p53 mutation (Chen et al, 1991; Cornelis et al, 1994; seven out of ten patients in this study) it is clearly no longer tenable to suggest that YNZ22 allelic imbalance occurs only in the presence of p53 mutation (Singh et al, 1993) and the two may in fact be quite dissociated (Chen et al, 1996).

The associations between YNZ22 allelic imbalance, disease recurrence and death due to breast cancer with medium-term follow-up provides confirmation of the hypothesis that YNZ22 allelic imbalance was likely to be associated with poor prognosis (Thompson et al, 1990; Nagai et al, 1995). Thus, the associations between YNZ22 allelic imbalance and a high proliferation index (Chen et al, 1991; Merlo et al, 1992), DNA aneuploidy (Chen et al, 1991), with absence of progesterone receptor expression (Ito et al, 1995), low oestrogen receptor expression (Thompson et al, 1990) and the presence of axillary lymph node metastasis at the time of diagnosis (Takhita et al, 1992; Harada et al, 1994) have now been supported in this study by prospective patient follow-up beyond 6 years. In addition to the evidence presented here of a gene or genes from 17p13.3 associated with disease recurrence and poor prognosis in breast cancer, this gene(s) is also implicated in neoplastic proliferation even at the early stages of breast carcinogenesis, including atypical ductal hyperplasia (Lakhani et al, 1995) and ductal carcinoma in situ (Radford et al, 1993; Harada et al, 1994; Munn et al, 1996). Whether the gene marked by YNZ22 behaves as a tumour-suppressor gene involved in the control of tumour cell proliferation (Merlo et al, 1992) or has a role in promoting metastasis to lymph nodes (Harada et al, 1994), or in some way directly or indirectly regulates p53 gene expression (Coles et al, 1990; Chen et al, 1991) remains to be seen. It is possible that the influence of a gene at 17p13.3 may be to stabilize p53 protein and hence account for the disparity between p53 protein expression and the detection of p53 mutations at the DNA level (Thompson et al, 1992; Cornelis et al, 1994). However, the function of the putative gene(s) at 17p13.3 remains speculative.

In the same cohort of patients that has provided evidence that YNZ22 may mark a region of clinical importance, as in two previous studies (Poller et al, 1992; Bland et al, 1995), we have failed to confirm that p53 abnormalities including p53 mutation were closely associated with disease behaviour. This may be due to the comparatively small numbers in the present study or because the association between p53 and prognosis in breast cancer may be comparatively weak (Elledge and Allred, 1994; Bland et al, 1995) or reflect the relationship between p53 and several prognostic factors that indicate an aggressive, rapidly proliferating tumour with an unstable genome (Stenmark-Askmalm et al, 1995). Given that YNZ allele imbalance is more common than p53 mutation in breast cancer, the independence of YNZ22 allele imbalance from any p53 changes suggests that the greater discriminatory power of the YNZ22 locus as a marker for disease behaviour is not simply due to chance. Emerging mapping data for 17p13.3 (Stack et al, 1995; White et al, 1996) have suggested two regions: YNZ22 and a more telomeric region (defined by markers D17S926, D17S695, D17S849) may be of interest in human breast cancer. Alongside the HIC-1 (hypermethylated in cancer), ABR and CRK genes (Heisterkamp et al, 1989; Morris et al, 1995; Wales et al, 1995) in this region, 17p13.3 may carry a gene or genes of both scientific interest and clinical importance in breast cancer.

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