

SHORT REPORT

Cytogenetic differences in breast cancer samples between German and Japanese patients

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Background: Japanese and German breast cancer cases differ substantially in the frequency of *egfr* amplification.

Aims: To unravel further the cytogenetic differences between Japanese and German breast cancer cases.

Methods: Forty one Japanese breast cancer cases were evaluated by means of comparative genomic hybridisation (CGH). The results were compared with the CGH results from 161 German breast cancer cases.

Results: The mean number of genetic alterations/case was significantly higher in German premenopausal patients with breast cancer than in their Japanese counterparts. Japanese breast cancer cases revealed a higher number of chromosome 17p losses. Losses of 8p were associated with oestrogen receptor (ER) negativity in Japanese patients with breast cancer, whereas in the German patients gains of 3q and 6q were associated with the lack of ER expression.

Conclusions: The interethnic differences of invasive breast cancer are reflected by cytogenetic aberrations, which are also associated with the differential expression of the ER.

Substantial epidemiological and clinical differences have been reported between Japanese and white patients with breast cancer.¹ Most cytogenetic studies published so far, using comparative genomic hybridisation (CGH) as a screening technique for the detection of unbalanced cytogenetic alterations within a given tumour, have concentrated on white women.^{2–4} In contrast, little is known about the cytogenetic aberrations in breast cancer in patients of Asian origin.⁵

Significant differences are known to exist between white and Asian populations for many polymorphic DNA sequences. A study published by our group previously identified a polymorphic sequence in intron 1 of the epidermal growth factor receptor (EGFR) gene (*egfr*) that had a significantly longer CA repeat stretch in Japanese than in German women. In vitro and in vivo experiments showed that the longer CA repeat stretch in Japanese patients was associated with a higher frequency of *egfr* gene amplification⁶ and EGFR expression. Therefore, it is possible that other polymorphic sites exist that have distinct chromosomal aberrations in the different ethnic subgroups.

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MATERIAL AND METHODS

Forty one cases of Japanese breast cancer were analysed by CGH and these results were compared with those from 161 invasive breast tumours from German women, originating

from the files of the Gerhard-Domagk Institute of Pathology. None of the patients had preoperative treatment. Table 1 provides further details of the investigated tumour samples. The Japanese breast cancer cases originated from the files of the Saitama Cancer Centre Hospital, Japan. The method of CGH analysis, the criteria for the evaluation of genetic alterations, and the immunohistochemical evaluation of the steroid receptor content were performed as described previously.⁷

Statistical tests

Statistics were performed using Fisher's exact test and the Mann-Whitney U test. p Values were two tailed and not adjusted for multiple comparisons; p values < 0.05 were considered significant.

RESULTS

In accordance with the literature, 28 of 41 (68%) of the Japanese and 51 of 161 (32%) of the German patients with breast cancer were premenopausal. The mean age was 51.9 (SD, 13.1) and 59.9 (SD, 15.0) years in Japanese and German patients with breast cancer, respectively (p < 0.01). Table 1 provides a detailed characterisation of the patients. Table 2 provides an overview of the most common quantitative cytogenetic aberrations.

A detailed characterisation of the German patients with breast cancer has been published previously.^{7–9} On average, the German breast cancer cases revealed 8.5 alterations/case (9.6 and 8.1 in premenopausal and postmenopausal patients, respectively), whereas the Japanese patients with breast cancer had a significantly lower average number of alterations/case (7.2; 6.3 and 8.7 in premenopausal and postmenopausal patients, respectively; p < 0.05). ER positive cases had a lower average number of alterations/case compared with ER negative tumours in both subgroups (7.5 v 10.5 (p < 0.01) in German and 6.2 v 8.7 (p = 0.11) in Japanese patients with breast cancer). ER negative tumours showed a similar frequency of aberrations irrespective of menopausal status in German patients, whereas postmenopausal Japanese patients with ER negative tumours showed a clear increase in cytogenetic alterations. The Japanese ER positive subgroup showed a higher rate of 16q losses, whereas ER negative cases showed an increased rate of 8p losses (p < 0.05 for both parameters). German ER positive tumours also had a significantly increased rate of 16q losses (p < 0.05) and a decreased rate of 3q (p < 0.01) and 6q gains (p < 0.05).

The recurrent changes seen most frequently in German and Japanese breast cancer cases were gains of 1q, 3q, 6q, 8q, 17q, and 20q in addition to losses of 6q, 8p, 11q, 13q, and 16q. 17p losses were predominantly seen in Japanese patients with breast cancer (p < 0.001).

Abbreviations: CGH, comparative genomic hybridisation; EGFR, epidermal growth factor receptor; ER, oestrogen receptor

Table 1 Overview of the distribution of clinicopathological parameters in both patient cohorts

Characteristic	Japanese	German
	n = 41	n = 161
Menopausal status		
Premenopausal	28 (68%)	51 (32%)
Postmenopausal	13 (32%)	110 (68%)
Stage		
I	14 (35%)	51 (32%)
IIa	11 (27%)	40 (25%)
IIb	5 (11%)	24 (15%)
IIIa	4 (10%)	18 (11%)
IIIb	4 (10%)	20 (12%)
IV	3 (7%)	8 (5%)
Grade		
I	8 (19%)	18 (11%)
II	18 (44%)	77 (48%)
III	15 (37%)	66 (41%)
Tumour type		
Ductal invasive	36 (87%)	121 (75%)
Lobular invasive	5 (13%)	25 (16%)
Miscellaneous subtypes		15 (9%)
Oestrogen receptor status		
Negative	18 (43%)	65 (40%)
Positive	23 (57%)	96 (60%)

The rate and distribution of high level gains, indicative of gene amplification, was similar in both ethnic subgroups (36% in German patients compared with 44% in the Japanese ones).

Other than the above described correlations between cytogenetic alterations and clinicopathological parameters, no distinct cytogenetic alterations were found in Japanese patients with breast cancer.

DISCUSSION

Recently, significant differences have been shown in relation to the length of a polymorphic sequence within the intron 1 of *egfr*, the frequency of *egfr* mutations, and the expression of EGFR between Japanese and German patients with breast cancer.⁵ Interestingly, similar differences have also been described for activating mutations of *egfr* in Japanese and white patients with lung cancer.¹⁰

The idea of different genotypes leading to a similar phenotype led us to the comparative cytogenetic analysis of Japanese and German patients with breast cancer. In addition to the differences in *egfr* amplification previously described in this series, a significantly higher incidence of 17p losses was found in Japanese breast cancer cases compared with German breast cancer cases. The frequency of 17p losses was even higher than that seen in German ductal invasive G3 carcinomas, which generally display the highest frequency of 17p losses.^{3-4, 11} This feature was not affected by menopausal or ER status, and these results are still open to interpretation. The role of p53 (with its chromosomal locus at 17p13.1) in this scenario remains unclear, because p53 mutations are associated with worse prognosis and a lower degree of histopathological differentiation, features less common in Japanese breast cancer.¹² Alternatively, another putative tumour suppressor gene within 17p13 might contribute to breast carcinogenesis predominantly in Japanese patients with breast cancer^{13, 14}; however, alterations within this suspected tumour suppressor gene appear to be associated with highly proliferative breast cancers with a poor prognosis,¹⁵ which again is contradictory to the tumour biological features seen in Japanese patients with breast cancer in general and also in our series.

A higher average number of cytogenetic alterations/case correlates with an increased recurrence rate in node negative

Table 2 Summary of cytogenetic alterations in the Japanese and German breast cancer subgroups

	Germany	Japan	p Value
<i>Mean number of cytogenetic alterations/case</i>			
All	8.5	7.2	
Premenopausal	9.6	6.3	p<0.05
Postmenopausal	8.1	8.7	
ER+	7.5	6.2	
ER-	10.5	8.7	
	p<0.01	p=0.11	
<i>Frequency of the most recurrent cytogenetic alterations</i>			
1q gains	70%	78%	NS
3q gains	23%	11%	NS
6q gains	11%	14%	NS
8q gains	49%	46%	NS
17q gains	18%	26%	NS
20q gains	20%	17%	NS
6q losses	21%	19%	NS
8p losses	34%	26%	NS
13q losses	26%	19%	NS
16q losses	53%	39%	NS
17p losses	22%	63%	p<0.001
<i>Frequency of the most common chromosomal high level gains</i>			
8q	18%	21%	NS
11q	10%	7%	NS
17q	18%	12%	NS
20q	6%	5%	NS

breast cancer, and therefore with overall prognosis.¹⁶ In accordance with this, there was a slightly increased average number of genetic alterations in all German breast cancer cases and a decreased number of genetic alterations in German postmenopausal breast cancer cases. Interestingly, there was a significantly higher average number of genetic alterations/case in the German premenopausal patients with breast cancer than in their Japanese counterparts.

“It is interesting that, irrespective of menopausal status, our German patients with oestrogen receptor (ER) negative cancer had a significantly higher average number of cytogenetic alterations/case compared with ER positive patients”

The regulation of ER expression between Japanese and German patients with breast cancer is similar in some respects but different in others. Different levels of ER expression in benign breast tissue between Japanese and white populations have been reported as an underlying cause of differences in breast cancer incidence between these two populations. The exact reasons for this observation are unclear, although the influence of food habits has been discussed.¹⁷ A higher rate of ER negativity has been found in Japanese postmenopausal patients with breast cancer compared with matched white patients, and this correlated with a worse prognosis in the Japanese patients.¹⁸ It is interesting that, irrespective of menopausal status, our German patients with ER negative cancer had a significantly higher average number of cytogenetic alterations/patient (p < 0.01) compared with ER positive patients. This was also true for our Japanese patients with breast cancer, with the exception of premenopausal patients, in whom no quantitative cytogenetic impact of ER expression could be measured.

However, similar cytogenetic alterations associated with the regulation of ER expression appear to be present in German and Japanese patients with breast cancer. This is especially true for chromosomal 16q losses in ER positive carcinomas in both ethnic groups, as described previously.^{11, 19} Our series did not reveal the biological importance of 8p

Take home messages

- The average number of genetic alterations/case was significantly higher in German premenopausal patients with breast cancer than in their Japanese counterparts
- There were a higher number of chromosome 17p losses in Japanese patients
- Losses of 8p were associated with oestrogen receptor (ER) negativity in Japanese patients with breast cancer, whereas in the German patients gains of 3q and 6q were associated with the lack of ER expression
- Thus, the interethnic differences of invasive breast cancer are reflected by cytogenetic aberrations, which are also associated with differential expression of ER

losses associated with ER negativity in the German breast cancer cases. Losses at this site were found in all but one CGH study on white patients with breast cancer.^{19,20} The number of Japanese tumours investigated in our series might be too small to draw definite conclusions concerning 3q and 6q gains in Japanese patients with breast cancer because these changes are relatively rare events in breast cancer. Gains of 3q and 6q have been shown to be associated with an increased level of telomerase activity, cytogenetic instability, and tumour proliferation, suggesting an interplay between these parameters.²¹

In summary, our results show that there are cytogenetic differences between Japanese and German breast cancer cases. Further studies are needed to define the extent to which these differences are causative or merely a reflection of other underlying genetic disturbances that are not detectable by means of CGH.

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