ELSEVIER

Cancer Letters 150 (2000) 165–170

CANCER Letters

www.elsevier.com/locate/canlet

Allelic loss at the *BRCA1*, *BRCA2* and *TP53* loci in human sporadic breast carcinoma

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Received 23 August 1999; received in revised form 20 October 1999; accepted 20 October 1999

Abstract

Abnormalities in several genes are known to confer susceptibility to breast cancer. In the present study, we investigated the incidence of allelic imbalance at the *BRCA1*, *BRCA2* and *TP53* loci, in 82 sporadic breast carcinomas using a bank of highly polymorphic microsatellite markers located at the *BRCA1*, *BRCA2* and *TP53* regions. Genetic alterations were observed in 58/82 (71%) cases in at least one microsatellite marker, at one of the three regions. Twenty-seven out of 82 (33%) cases exhibited loss of heterozygosity (LOH) at *BRCA1* locus while in 20/82 (34%) cases LOH was observed for the *BRCA2* region. Allelic deletions were detected in 28/82 (34%) cases for the *TP53* locus. Our results suggest that allelic deletion at the above genetic loci play an important role to the development of sporadic breast tumours.

Keywords: Heterozygosity; Breast cancer; *BRCA1*; *BRCA2*; *TP53*

1. Introduction

Breast cancer is the most common cancer in women in Europe, currently affecting one in 12 [1]. Since family history remains the strongest single predictor of breast cancer risk, attention has focused on the role of highly penetrant, dominantly inherited genes in cancer-prone kindreds. A major breast and ovarian cancer susceptibility gene, *BRCA1*, is located on chromosome 17q21 [2]. The frequent finding of putative loss of function germline mutations in breast and ovarian cancer families and the loss of the wild-type allele in corresponding tumours suggests that *BRCA1* is a tumour-suppressor gene. A second hereditary breast cancer gene, *BRCA2*, was subsequently isolated. It is located on chromosome 13q12–q13, proximal to the retinoblastoma (RB1) gene at 13q14 and is involved in the development of familial breast and ovarian tumours through germline mutations [3–5]. Loss of heterozygosity (LOH) at the *BRCA2* locus was also observed in sporadic breast and ovarian tumours, implying that *BRCA2* acts as a tumour-suppressor gene [6].

TP53 is considered to be a tumour suppressor gene exerting growth-regulatory functions in response to DNA damage by directly inhibiting DNA replication [7] or inducing apoptosis [8]. Mutations in the *TP53* locus are among the most common genetic alterations detected in human tumours while they have also been

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0304-3835/00/$ - see front matter
PII: S0304-3835(99)00386-9

reported in sporadic breast cancer and are generally associated with adverse prognosis [9,10]. Furthermore, analysis of a small series of breast and ovarian tumours arising in carriers of mutant *BRCA1* alleles revealed a high incidence of *p53* mutation [11]. Tumours with abnormal *TP53* lose cell-cycle checkpoints, resulting in neoplastic transformation. However, the evidence linking the tumour-specific targets of genomic alteration to *TP53* allelic imbalance is limited. Loss of heterozygosity analysis using polymorphic microsatellite markers is a common methodology employed for the localization of potent onco-suppressor genes and important regulatory genes in non-malignant syndromes [12]. In the present study, we investigated the incidence of genomic changes at the *BRCA1*, *BRCA2* and *TP53* regions using microsatellite markers flanking the three loci, in 82 sporadic breast carcinomas. We demonstrate that genetic alterations are detectable events in these tumours and are distributed equally at the three genes providing implications for the understanding of both *BRCA*-associated sporadic breast cancer and *TP53* biology.

2. Materials and methods

2.1. Biological materials

The breast tumour specimens corresponding to primary breast tumours were obtained from the Elena Hospital, Athens. Clinical data (histological type, stage, grade, lymph node metastasis, oestrogen and progesterone receptors, age, family history) were available. A matched normal DNA control from blood was analysed.

2.2. DNA extraction, PCR amplification, microsatellite analysis

DNA was extracted as previously described [13] and stored at 4°C until polymerase chain reaction (PCR) amplification. Specimens were investigated for genetic alterations at the three different genetic regions using 11 microsatellite markers for PCR amplification. For the BRCA2 locus polymorphic microsatellite markers used were D13S171, D13S217, D13S289 and D13S290. For analysis of the *BRCA1* genetic locus the microsatellite markers D17S250, THRA1, D17S855 and D17S579 were used. The corresponding markers for the *TP53* locus were D17S515, TP53 and D17S678 (Research Genetics, Inc., USA).

Furthermore, we used markers D10S1768 and D10S1555 located at 10pter-qter, a region with no known tumour-suppressor gene, as a control.

PCR analysis was performed in a 50-μl reaction volume containing 200 ng of genomic DNA, 1 μM of each primer, 250 μM dNTPs, 5 μl of 10 × buffer (670 mM Tris (pH 8.5); 166 mM ammonium sulphate; 67 mM magnesium chloride; 1.7 mg/ml BSA; 100 μM β-mercaptoethanol;1% (w/v) Triton X-100) and 1 unit of Taq DNA polymerase. The reactions were denatured for 5 min at 95°C and the DNA was subsequently amplified for 30 cycles at 95, 55 and 72°C each step. Gels were inspected visually by two independent viewers, comparing the intensity of alleles from normal and tumour DNA. Any absence or significant decrease (>50%) of one tumour allele relative to the other was considered loss of heterozygosity. The analysis in the LOH positive cases was repeated at least twice and the results were highly reproducible.

2.3. Statistical analysis

Package SPSS 6.0 (for Windows) was used for statistical analysis of the results and significance was set at $P < 0.05$.

3. Results

Eighty-two tumour specimens from patients with breast carcinomas were assessed for microsatellite alterations at the loci of *BRCA1*, *BRCA2* and *p53* genes. Representative examples of specimens with LOH are shown in Fig. 1. As presented in Table 1, loss of heterozygosity was observed in 58/82 cases, (71%). LOH was detected in 27/82 specimens (33%) at the *BRCA1* region while both *BRCA2* and *TP53* loci exhibited the same proportion of LOH, 34% (28/82).

Markers D17S250, D17S515, *TP53* and D17S678 were most frequently altered (Table 1) with the highest incidence of LOH for the marker TP53. The incidence of LOH for the marker D13S171 (*BRCA2*) was reversely correlated to that of marker D17S515 (TP53) ($P = 0.07$). Furthermore, LOH for the markers D13S217 and D13S289 (*BRCA2*) was rever-

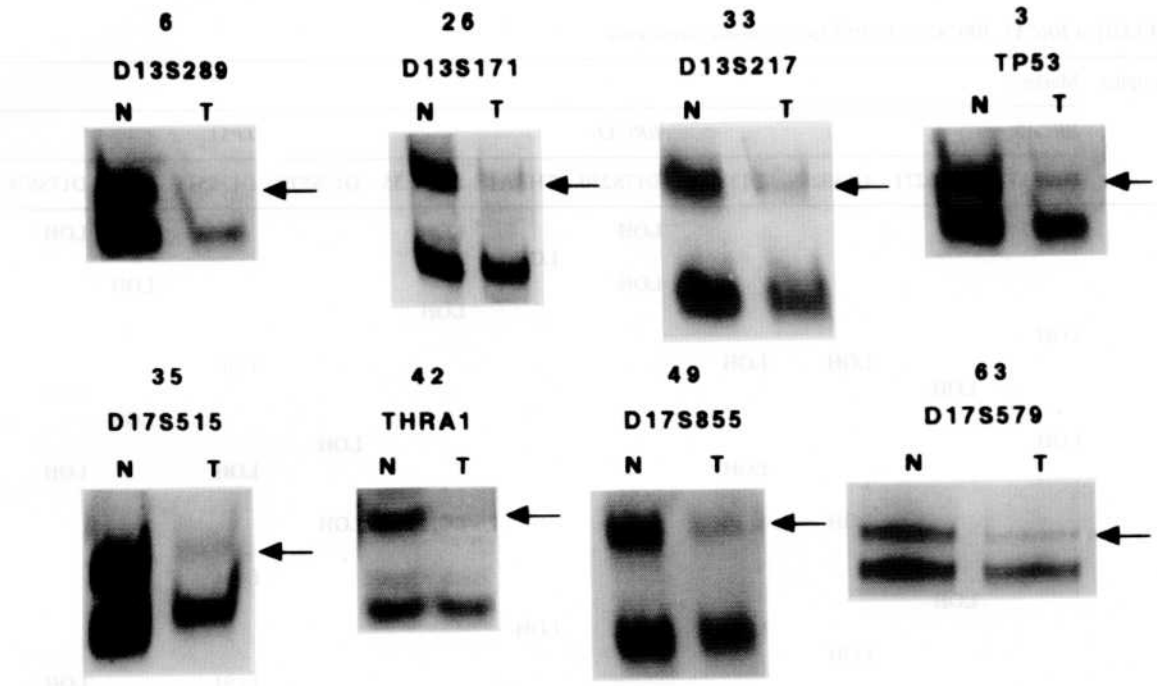

Fig. 1. Representative examples of specimens exhibiting LOH. N, normal DNA; T, tumour DNA. Arrows indicate the position of a deleted allele. The faint bands in the position of the deleted alleles are interpreted as contamination from the adjacent normal tissue.

sely correlated with that of marker D17S855 (*BRCA1*) ($P = 0.08$). Six specimens displayed only partial or interstitial deletions at *BRCA1*, while the same phenomena were observed in five and three samples for the *BRCA2* and *TP53* loci, respectively. These genetic deletions may be due to multiple mitotic recombination events.

We also tested markers D10S1768 and D10S1555 which are located at chromosome 10, known not to contain any tumour-suppressor gene involved in breast cancer. The incidence of LOH for markers D10S1768 and D10S1555 was limited to only 4 of 82 (5%) specimens tested. These data support our suggestion for implication of *TP53*, *BRCA1* and *BRCA2* loci as tumour-specific targets for genomic deletions in breast carcinomas.

The pattern of LOH using the various markers is shown in Table 2. No statistically significant link of allelic deletion was detected between the three loci examined. Only 3/82 carcinomas (4%) exhibited microsatellite instability in at least one marker and no particular specimen exhibited high incidence of microsatellite instability, indicating the absence of the 'mutator phenotype', similar to that reported in hereditary non-polyposis colon cancer [14].

No clinical correlation between genetic alterations and any of the clinical parameters, such as histological

Table 1
Incidence of LOH at *BRCA1*, *BRCA2* and *TP53* loci in breast tumours

Locus	LOH (%)	Marker	No LOH	LOH (%)
BRCA1, 17q21	27/82 (33)	D17S250	10	14
		THRA1	7	10
		D17S855	9	13
		D17S579	8	12
BRCA2, 13q12–q13	28/82 (34)	D13S171	9	13
		D13S217	8	12
		D13S289	8	12
		D13S290	7	10
TP53, 17p13	28/82 (34)	D17S515	10	13
		TP53	11	16
		D17S678	11	15
Total	58/82 (71)			

Table 2
Pattern of LOH at *BRCA1*, *BRCA2* and *TP53* loci in breast carcinomas

No. of samples	Markers										
	BRCA2				*BRCA1*				*TP53*		
	D13S171	D13S271	D13S289	D13S290	D17S250	THRA1	D17S855	D17S579	D17S515	TP53	D17S678
1					LOH		LOH				LOH
3						LOH					
1					LOH					LOH	
2							LOH				
4	LOH										
1			LOH	LOH					LOH		
1		LOH									LOH
3										LOH	
1	LOH							LOH			
1				LOH					LOH		LOH
1					LOH						
1			LOH				LOH	LOH			
1					LOH					LOH	
5									LOH		
5		LOH									
1				LOH		LOH				LOH	
2			LOH		LOH						
2									LOH		LOH
2		LOH			LOH						
1								LOH			
1	LOH						LOH				
2			LOH	LOH							
1							LOH	LOH			
1				LOH						LOH	
2											LOH
1								LOH			LOH
1									LOH		
1					LOH				LOH		
1			LOH							LOH	
1						LOH	LOH	LOH			
2								LOH		LOH	
1	LOH		LOH								LOH
1	LOH									LOH	
1							LOH		LOH		
1					LOH	LOH					
1				LOH							

type, stage, grade, lymph node metastasis, and estrogens and progesterone receptors was observed.

4. Discussion

Normal cells have the ability to maintain integrity of the genome by regulating the cell replication cycle, in response to DNA damage. This regulation takes place during transition from G1 to S and G2 to M phases of the cell cycle with the *TP53* gene participating by controlling the first transition. On the contrary, tumour cells with abnormal *TP53* lose these essential functions at the cell-cycle checkpoints and consequently accumulate unrepaired damage, leading to extensive genetic alterations. Previous studies in

breast cancer genomic aberrations, such as gene amplification, allelic loss and chromosome alterations have been linked to *TP53* abnormality [15].

The objective of this study was to investigate the involvement of the *BRCA1*, *BRCA2* and *TP53* regions in a set of sporadic breast tumours and to detect a potential linkage between the tumour-specific targets of genomic alteration to *TP53* allelic imbalance. We found 58 samples with genetic alterations in at least one marker. A high incidence of LOH (34%) was found for the *TP53* locus, confirming that *TP53* gene constitutes a possible target for allelic deletions [16,17]. A similar proportion of LOH was detected for both *BRCA1* and *BRCA2* regions, suggesting that the inactivation of these tumour-suppressor genes plays a critical role in multistage carcinogenesis [18–21].

Since *TP53* and *BRCA1* genes are located at the same chromosome, we cannot exclude the possibility of allelic deletions due to monosomy. Monosomy 17 has been described in other types of human cancers such as acute myeloid leukaemia, myelodysplastic syndrome and carcinomas in pleomorphic adenomas [22,23].

Previous studies revealed a high frequency of genomic alterations in breast tumours from both *BRCA1* and *BRCA2* mutation carriers [24]. Among different genomic alterations, some are indirect events representing genomic instability generally acquired during tumorigenesis. In contrast, those at critical loci are of particular tumorigenic importance, which may imply that cells carrying deletions at these three tumour suppressor loci are more likely to acquire a growth advantage. The latter is supported by the finding that *BRCA1* has a growth inhibitory function [25] and that decreased *BRCA1* expression levels may arrest the cell cycle through activation of p53 checkpoint in human sporadic breast tumours [26]. The fact that no statistically significant correlation of *TP53*, *BRCA1* and *BRCA2* deletion was observed may reflect the importance of all these three genetic loci in regulating the cell. Our results suggest that deletions in each one of these loci are sufficient to give breast cells an oncogenic potential; however, the role of other modifying genes must also be taken into consideration.

Microsatellite instability was not detected in high incidence. The phenomenon was observed only in 4% for the specimens examined, indicating the absence of a true 'mutator phenotype' [27]. Furthermore, the lack of detection of microsatellite instability in *TP53*-LOH-associated tumours suggests that *TP53* allelic imbalance is not the result of a mutator phenotype.

References

[1] D.M. Black, The genetics of breast cancer, Eur. J. Cancer 30A (1994) 1957–1961.

[2] Y. Miki, J. Swensen, K. Shattuck-Eidens, P.A. Futreal, K. Harshman, K. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, W. Ding, R. Bell, J. Rosenthal, C. Hussey , T. Tran, M. McClure, C. Frye, T. Hattier, R. Phelps, A. Haugen-Strano, H. Katcher, K. Yakumo, Z. Gholami, D. Shaffer, S. Stone, S. Bayer, C. Wray, R. Bogden, P. Dayananth, J. Ward, P. Tonin, S. Narod, P.K. Bristow, F.H. Norris, L. Helvering, P. Morrison, P. Rosteck, M. Lai, J.C. Barrett, C. Lewis, S. Nehausen, L. Cannon-Albright, D. Goldgar, R. Wiseman, A. Kamb, M.H. Skolnick, A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*, Science 266 (1994) 66–71.

[3] R. Wooster, G. Bignell, J. Lancaster, S. Swift, S. Seal, J. Mangion, N. Collins, S. Gregory, C. Gumbs, G. Micklem, Identification of the breast cancer susceptibility gene *BRCA2*, Nature 378 (1995) 789–792.

[4] S.V. Tavtigian, J. Simard, J. Rommens, F. Couch, D. Shattuck-Eidens, S. Neuhausen, S. Merajver, S. Thorlacius, K. Offit, D. Stoppa-Lyonnet, C. Belanger, R. Bell, S. Berry, R. Bogden, Q. Chen, T. Davis, M. Dumont, C. Frye, T. Hattier, S. Jammulapati, T. Janecki, P. Jiang, R. Kehrer, J.F. Leblanc, J.T. Mitchell, J. McArthur-Morrison, K. Nguyen, Y. Peng, C. Samson, M. Schroeder, S.C. Snyder, L. Steele, M. Stringfellow, C. Stroup, B. Swedlund, J. Swensen, D. Teng, A. Thomas, T. Tran, T. Tran, M. Tranchant, J. Weaver-Feldhaus, A.K.C. Wong, H. Shizuya, J.E. Eyfjord, L. Cannon-Albright, F. Labrie, M.H. Skolnick, B. Weber, A. Kamb, D.E. Goldgar, The complete *BRCA2* gene and mutations in chromosome 13q-linked kindreds, Nat. Genet. 12 (1996) 333–337.

[5] J. Lancaster, R. Wooster, J. Mangion, C.M. Phelan, C. Cochran, C. Gumbs, S. Seal, R. Barfoot, N. Collins, G. Bignell, S. Patel, R. Hamoudi, C. Larsson, R.W. Wiseman, A. Berchuck, J.D. Iglehart, J.R. Marks, A. Ashworth, M.R. Stratton, P.A. Futreal, *BRCA2* mutations in primary breast and ovarian cancers, Nat. Genet. 13 (1996) 238–240.

[6] M.W. Beckmann, F. Picard, H.X. An, C.R. van Roeyen, S.I. Dominik, D.S. Mosny, H.G. Schnurch, H.G. Bender, D. Niederacher, Clinical impact of detection of loss of heterozygosity of *BRCA1* and *BRCA2* markers in sporadic breast cancer, Br. J. Cancer 73 (1996) 1220–1226.

[7] L.S. Cox, T. Hupp, C.A. Midgley, D.P. Lane, A direct effect of activated human p53 on nuclear DNA replication, EMBO J. 14 (1995) 2099–2105.

[8] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.

[9] J. Bergh, T. Norberg, S. Sjogren, A. Lindgren, L. Holmberg, Complete sequencing of the p53 gene provides prognostic

information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy, Nat. Med. 1 (1995) 1029–1034.

[10] S. Saitoh, J. Cunningham, E.M. De Vries, R.M. McGovern, J.J. Schroeder, A. Hartmann, H. Blaszyk, L.E. Wold, D. Schaid, S.S. Sommer, J.S. Kovach, p53 gene mutations in breast cancers in midwestern US women: null as well as missense-type mutations are associated with poor prognosis, Oncogene 9 (1994) 2869–2875.

[11] T. Crook, S. Crossland, M.R. Crompton, P. Osin, B.A. Gusterson, p53 mutations in *BRCA1*-associated familial breast cancer, Lancet 350 (1997) 638–639.

[12] G. Zachos, E. Koumantaki, A. Vareltzidis, D.A. Spandidos, Loss of heterozygosity of particular chromosome regions in human sporadic lesions, Br. J. Dermatol. 139 (1998) 974–977.

[13] G. Sourvinos, H. Kiaris, A. Tsikkinis, S. Vassilaros, D.A. Spandidos, Microsatellite instability and loss of heterozygosity in primary breast tumours, Tumor Biol. 18 (1996) 157–166.

[14] L.A. Aaltonen, P. Peltomaki, F.S. Leach, P. Sistonen, L. Pylkanen, J.-P. Mecklin, H. Jarvinen, S.M. Powell, J. Jen, S.R. Hamilton, G.M. Petersen, K.W. Kinzler, B. Vogelstein, A. De la Chapelle, Clues to the pathogenesis of familial colorectal cancer, Science 260 (1993) 812–816.

[15] J.E. Eyfjord, S. Thorlacius, M. Steinarsdottir, R. Valgardsdottir, H.M. Ogmundsdottir, K. Anamthawat-Jonsson, p53 abnormalities and genomic instability in primary human breast carcinomas, Cancer Res. 55 (1995) 646–651.

[16] G. Eiriksdottir, R.B. Barkardottir, B.A. Agnarsson, G. Johannesdottir, K. Olafsdottir, V. Egilsson, S. Ingvarsson, High incidence of loss of heterozygosity at chromosome 17p13 in breast tumours from *BRCA2* mutation carriers, Oncogene 16 (1998) 21–26.

[17] D.S. Liscia, R. Morizio, T. Venesio, C. Palenzona, M. Donadio, R. Callahan, Prognostic significance of loss of heterozygosity at loci on chromosome 17p13.3-ter in sporadic breast cancer is evidence for a putative tumour suppressor gene, Br. J. Cancer 80 (1999) 821–826.

[18] J.M. Silva, R. Gonzalez, M. Provencio, G. Dominguez, J.M. Garcia, I. Gallego, J. Palacio, P. Espana, F. Bonilla, Loss of heterozygosity in *BRCA1* and *BRCA2* markers and high-grade malignancy in breast cancer, Breast Cancer Res. Treat. 53 (1999) 9–17.

[19] F. Kerangueven, F. Allione, T. Noguchi, J. Adelaide, H. Sobol, J. Jacquemier, D. Birnhaum, Patterns of loss of heterozygosity at loci from chromosome arm 13q suggests a possible involvement of *BRCA2* in sporadic breast tumours, Genes Chromosomes Cancer 13 (1995) 291–294.

[20] J. Van den Berg, O. Johannsson, S. Hakansson, H. Olsson, A. Borg, Allelic loss at chromosome 13q12-13 is associated with poor prognosis in familial and sporadic breast cancer, Br. J. Cancer 74 (1996) 1615–1619.

[21] U. Hamann, C. Herbold, S. Costa, E.F. Solomayer, M. Kaufmann, G. Bastert, H.U. Ulmer, H. Frenzel, D. Komitowski, Allelic imbalance on chromosome 13q: evidence for the involvement of *BRCA2* and RB1 in sporadic breast cancer, Cancer Res. 56 (1996) 1988–1990.

[22] V. Soenen, C. Preudhomme, C. Roumier, A. Daudignon, J.L. Lai, P. Fenaux, 17p Deletion in acute myeloid leukemia and myelodysplastic syndrome. Analysis of breakpoints and deleted segments by fluorescence in situ, Blood 91 (1998) 1008–1015.

[23] X. Li, T. Tsuji, S. Wen, Y. Mimura, K. Sasaki, F. Shinozaki, Detection of numeric abnormalities of chromosome 17 and p53 deletions by fluorescence in situ hybridization in pleomorphic adenomas and carcinomas in pleomorphic adenoma. Correlation with p53 expression, Cancer 79 (1997) 2314–2319.

[24] M. Tirkkonen, O. Johannsson, B.A. Agnarsson, H. Olsson, S. Ingvarsson, R. Karhu, M. Tanner, J. Isola, R.B. Barkardottir, A. Borg, O.P. Kallioniemi, Distinct somatic genetic changes associated with tumor progression in carriers of *BRCA1* and *BRCA2* germ-line mutations, Cancer Res. 57 (1997) 1222–1227.

[25] J.T. Holt, M.E. Thompson, C. Szabo, C. Robinson-Benion, C.L. Arteaga, M.C. King, R.A. Jensen, Growth retardation and tumour inhibition by *BRCA1*, Nat. Genet. 12 (1996) 298–302.

[26] G. Sourvinos, D.A. Spandidos, Decreased *BRCA1* expression levels may arrest the cell cycle through activation of p53 checkpoint in human sporadic breast tumours, Biochem. Biophys. Res. Commun. 245 (1998) 75–80.

[27] C.J. Yee, N. Roodi, C.S. Verrier, F.F. Parl, Microsatellite instability and loss of heterozygosity in breast cancer, Cancer Res. 54 (1994) 1641–1644.