Triplet repeat instability correlates with dinucleotide instability in primary breast cancer

CLAUDIA PIZZI[1], MASSIMO DI MAIO[1], SANTA DANIELE[1], PAOLO MASTRANZO[1], ILARIA SPAGNOLETTI[1], GENNARO LIMITE[2], GUIDO PETTINATO[3], ANTONELLA MONTICELLI[4,5], SERGIO COCOZZA[4] and ALMA CONTEGIACOMO[1]

Dipartimenti: [1]Endocrinologia e Oncologia Molecolare e Clinica, [2]Clinico Medico-Chirurgico di Patologia dell'Apparato Digerente e di Chirurgia Generale e Oncologica, [3]Scienze Biomorfologiche e Funzionali, Sezione di Anatomia Patologica, [4]Biologia e Patologia Cellulare e Molecolare, Facoltà di Medicina, Università di Napoli 'Federico II'; [5]IEOS, Istituto di Endocrinologia ed Oncologia Sperimentale, CNR, Via S. Pansini 5, I-80131 Napoli, Italy

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Abstract. The expansion of triplet repeat microsatellite sequences is the molecular correlate of anticipation in a number of rare Mendelian neurodegenerative disorders. This finding prompted us to study these sequences in primary breast cancer in which there is evidence of genetic anticipation. We used a PCR/silver stain method to determine whether triplet-repeat instability (TRI) was present in DNA from malignant breast tumors, and analyzed microsatellite instability (MSI) in triplets SCA1, SCA2, SCA3, SCA6, HD, DRPLA and X25-GAA. We studied 54 consecutive primary breast cancers previously analyzed for dinucleotide instability (DI) at 9 loci. Microsatellite instability (TRI and/or DI) was found in 28/54 (52%) cases, ranging from 0 to 56% in each patient. Dinucleotide instability occurred at ≥2 loci in 19/54 (35%) cases and TRI in 6/54 (11%). Considering single locus instability, we found DI in 26/54 (48%) tumors and TRI in 13/54 (24%). Triplets DRPLA and X25-GAA were most frequently unstable (14% of cases); SCA2 instability was not detected. Interestingly, most tumors with TRI had DI (11/13, 85%). There was a correlation between TRI and DI in the same tumor (42 vs 7% in DI+ and DI- tumors respectively, p=0.0028). Furthermore, TRI appears more frequently associated with lymph node metastases and more advanced clinical stages and more frequent in patients <50 years old, with positive steroidal hormone receptor status, positive p185 and negative p53. These findings are of interest because they demonstrate a relationship between TRI and the clinicopathological characteristics of cancer aggressiveness. Triplet repeat alterations can interfere with gene expression and proteomic functions, which suggests they can play a role in the neoplastic progression of mammary cells. Furthermore, the association of TRI and DI in the same tumor suggests that alterations in the DNA repair gene could culminate in selective phenotypes and breast cancer progression in a considerable number of patients.

Correspondence to: Dr Alma Contegiacomo, Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università di Napoli 'Federico II', Via S. Pansini 5, I-80131 Napoli, Italy
E-mail: contalma@unina.it

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Introduction

Eukaryotic genomes contain large numbers of repeated sequence elements of one to a few nucleotides ('microsatellites'). Interest in mutation rates in repeat sequences was stimulated by reports of associations between microsatellite instability (MSI) and human diseases and tumors (1,2). Microsatellite instability is characteristic of cells lacking DNA mismatch repair, for instance, tumor cells from patients with hereditary non-polyposis colorectal cancer (3-5) and from a variety of sporadic cancers (1,2). This form of instability is genome-wide and usually consists of the loss or gain of one or a few repeat units from mono-, di-, tri- and tetranucleotide microsatellites. Microsatellite instability is also associated with the hereditary neurological diseases such as Huntington disease, dominant spinocerebellar ataxy (SCA1-SCA2-SCA3 or Machado-Joseph disease-SCA6), dentatorubral-pallidolusyan atrophy (Haw-River syndrome) and Friedreich ataxia (6-13). These diseases are characterized by genes harboring tri-nucleotide repeat (TR), CNG (where N, any nucleotide) and GAA sequences that are at risk of dynamic high-frequency mutations that expand or contract the repeat tract. Trinucleotide repeat instability (TRI) is locus-specific and can arise from gene conversion or by error-prone DNA repair whether the cell is dividing or not, since most cell types have recombination and repair properties (14,15). Indeed trinucleotide repeat polymorphisms are inherently different than dinucleotide polymorphic loci. In TRI, for some repeats, large alleles above a certain threshold (i.e., expanded alleles) were shown to be pathogenic (6-14).

The discovery that expansion of triplet repeat microsatellite sequences is the molecular correlate of anticipation

in a number of rare Mendelian neurodegenerative disorders prompted us to study these sequences in primary breast cancer in which genetic anticipation has been considered (16,17). Furthermore, TRI has been reported, at various frequencies, in several sporadic tumors, i.e., colon, prostate, kidney, pancreas and testicle (18-23). Data on TRI in breast carcinomas are not extensive and mainly relate to the androgen receptor exon 1 CAG repeat length (2,17,24-27). On the other side, dinucleotide repeat instability has been well characterized in primary breast cancer and correlated with histotype, tumor size, lymph node-positive phenotype, clinical stage and poorer prognosis, young age and negative p53 expression (24,28-30).

Considering that triplet repeat alterations can interfere with gene expression and proteomic functions, the aim of this study was to analyze primary breast cancer to investigate TRI in relation to dinucleotide instability (DI) and to biological and clinicopathological features in an attempt to describe new breast cancer phenotypes.

Materials and methods

Patients and tissues. Fifty-four consecutive breast-cancer patients (53 females and 1 male) were enrolled in the study. All had undergone partial or total mastectomy, with axillary lymph node clearance, and all gave their consent to the study, which was conducted in accordance with the ethical standards of the Ethics Committee of the University of Naples 'Federico II'. The age of the patients ranged between 31 and 82 years; 18 patients (33%) were ≤50, and 36 patients (67%) >50 years old.

Immediately after surgery, a small portion of the tumor was rapidly frozen in liquid nitrogen and stored at -70˚C for DNA determination. A second specimen was placed in TC 199 tissue-culture medium (Gibco, UK) and processed for the determination of proliferative activity by the thymidine labeling index (TLI) technique (28). The remaining part was fixed in 10% buffered formalin and embedded in paraffin. Sections measuring 5 μ were used for pathological and immunohistochemical studies. Only tumors with at least 50% of tumor cells were included in the study.

Tumors were classified according to histotype, grade of differentiation, tumor size, axillary lymph node status, steroid hormonal status and cell kinetics. These parameters, together with age of the patient and clinical stage of the disease, were analyzed in relation to MSI. Tumors were histologically classified according to the World Health Organization criteria (1981). The degree of differentiation of invasive ductal carcinomas was defined 'high-to-moderate' (G1/G2) or 'low' (G3), according to the Bloom and Richardson system. Steroid hormone receptor status was considered positive when the tumor expressed estrogen and/or progesterone receptor.

Thymidine labeling index. Cell kinetics was assessed by TLI technique as previously described (28). A TLI of 2.8% distinguished between tumors with low and high cell kinetics (<2.8 and ≥2.8%, respectively).

Microsatellite analyses. Paired blood and tumor samples were rapidly frozen in liquid nitrogen and stored at -70˚C. The PCR/silver stain method we used to identify TRI in DNA from malignant breast tumors is reported elsewhere (28,30). We analyzed MSI in 7 triplets: SCA1 (chr. 6), SCA2 (chr. 12), SCA3 (chr. 14), SCA6 (chr. 19), HD (chr. 4), DRPLA (chr. 12) and X25-GAA (chr. 9). The trinucleotide expanded was CAG for all genes except for X25, which was GAA. We studied 54 consecutive primary breast patients for DI at 9 loci. The dinucleotide microsatellite markers analyzed were: D1S104 (1q21-q23), D2S71 (chr. 2), D2S123 (chr. 2), D3S1611 (3p21.3), D5S107 (5q), ACTC (15q11), D17S250 (chr. 17), D17S261 (chr. 17) and D18S34 (18q21). D2S123, D5S107 and ACTC are in the reference panel recommended by the National Cancer Institute's International Guidelines for the evaluation of MSI in colorectal cancer (2,3). MSI at dinucleotide and trinucleotide repeats was detected as previously reported (Fig. 1) (13,28,30).

Immunohistochemical analysis. Immunohistochemistry was performed using an avidin-biotin system for mouse primary antibodies (ABC Elite Vectastain, Vector Laboratories, AL) and monoclonal antibody (MAb) DO7 directed against the p53 protein, at a dilution of 1:700 (Novocastra, UK); MAb-1 directed against p185, at a dilution of 1:100 (Triton, USA); 1D5 directed against estrogen receptors, at a dilution of 1:250 (Dako, DK) and 1A6 directed against progesterone receptors at a dilution of 1:250 (EPOQ, EU). Before incubation with primary antibodies, the slides were pre-treated in a microwave oven for three 5-min passages with 0.01 M citrate buffer, pH 6.0. The p53 expressed in tumors was quantified and assigned to one of three categories depending on the percent of cells stained: ≤10%, negative; 11-25%, (+); 25-50%, (++); >50%, (+++). Two histopathologists scored slides independently and a matching hematoxylin and eosin stained slide was used to determine which part of the section was tumor tissue (30).

Statistical methods. Fisher's exact test was used for statistical analysis. P-values were computed after combining cases with an MSI phenotype defined by the presence of instability at one or more loci versus cases with no evidence of MSI. Statistical significance was considered at $p \leq 0.05$.

Results

We investigated the status of microsatellites detected by random size shifts in microsatellite alleles in tumor DNA as compared with blood DNA in 54 primary breast carcinomas. Cases were collected from a consecutive series previously analyzed for DI (30). Table I shows the patients' clinicopathological characteristics. Dinucleotide instability was defined when 1 of the 9 loci analyzed was altered; TRI was defined an expansion or contraction in the length of one of the triplet repeats studied in tumor DNA with respect to blood DNA. As recommended by the National Cancer Institute guidelines for colorectal cancer (2,3), the tumors were also assigned to three MSI classes: tumors with no instability (microsatellite stable); tumors with instability of at least one of the markers; tumors with instability of two or more of the markers. However, the results did not change when only tumors with more than two altered markers were considered 'positive'.

Table I. Clinicopathological characteristics of the 54 cases analyzed.

No.	Age	Histology	T	N	G	Clinical stage	TLI	Hormonal status	p185	p53	TRI	DI	MSI
1	77	CLI	T1	N1	-	III	0.4	+	-	-	+	+	+
2	52	CDI	T1	N0	G3	I	0.4	+	-	-	-	-	-
3	65	CDI	T4	N1	G3	III	ND	+	+	-	-	+	+
4	76	CLI	T2	N1	-	III	1.1	+	-	-	+	+	+
5	57	CDI	T1	N0	G3	I	0.2	+	+++	-	-	-	-
6	61	CDI	T2	N1	G3	III	1	-	-	++	-	-	-
7	72	CDI	T2	N1	G3	III	4.9	-	-	-	-	+	+
8	56	CDI	T2	N0	G3	I	0.2	+	-	-	-	-	-
9	70	CDI	T1	N0	G3	I	1.9	-	-	+++	-	-	-
10	54	CDI	T2	N1	G3	III	1	+	-	-	-	+	+
11	53	CDI	T2	N1	G3	III	2.7	+	-	-	-	-	-
12	31	CDI	T2	N0	G3	I	ND	+	-	++	-	-	-
13	72	CDI	T1	N0	G3	I	0.2	+	-	-	-	-	-
14	57	CDI	T1	N0	G3	I	0.2	+	+++	+	-	-	-
15	61	CDI	T2	N1	G3	III	5.1	+	-	+	-	-	-
16	58	CDI	T2	N0	G2	I	0.4	+	-	-	+	+	+
17	49	CDI	T2	N1	G3	III	1.2	+	-	+++	+	+	+
18	48	CLI	T2	N1	-	III	1.1	+	-	-	-	+	+
19	51	CDI	T1	N1	G2	II	ND	+	-	-	-	-	-
20	41	CDI	T4	N1	G3	III	ND	-	-	-	-	+	+
21	48	CLI	T3	N1	-	III	ND	+	-	++	-	-	-
22	66	CDI	T3	N1	G3	III	ND	-	-	-	-	+	+
23	66	CDI	T4	N1	G3	III	ND	-	+++	-	+	+	+
24	76	CLI	T2	N0	-	II	ND	+	-	-	-	+	+
25	61	CDI	T1	N0	G3	I	ND	-	-	-	-	-	-
26	74	CDI	T2	N0	G3	II	ND	+	+++	+++	-	-	-
27	72	CLI	T1	N1	-	II	ND	+	-	-	+	+	+
28	82	CDI	T2	N1	G3	II	ND	+	++	++	+	-	+
29	61	CDI	T1	N1	G3	II	ND	+	-	-	+	-	+
30	49	CDI	T2	N1	G3	II	ND	ND	ND	ND	-	+	+
31	60	CDI	T2	N1	G3	II	ND	ND	ND	ND	-	+	+
32	59	CDI	T1	N1	G3	II	ND	ND	ND	ND	-	-	-
33	59	CDI	T1	N0	G2	I	ND	ND	ND	ND	-	-	-
34	78	CDI	T2	Nx	G1	ND	2.5	+	-	+	-	-	-
35	47	CDI	T2	N1	G3	III	3.5	+	-	-	+	+	+
36	59	CLI	T2	N2	-	III	2.3	-	-	-	-	-	-
37	37	CDI	T1	N2	G3	III	9.2	+	-	-	-	-	-
38	65	CDI	T3	N0	G3	II	8.4	+	-	+++	-	+	+
39	37	CDI	T1	N1	G3	III	2.4	+	-	-	+	+	+
40	52	CDI	T2	N0	G3	I	0.4	-	-	++	-	-	-
41	49	CDI	T1	N0	G3	I	2.8	+	-	+++	-	+	+
42	43	CDI	T1	N0	G2	I	7.0	+	-	-	+	+	+
43	39	CDI	T1	N0	G3	I	6.3	+	-	-	-	-	-
44	38	CDI	T2	N2	G3	III	0.3	-	-	-	-	+	+
45	42	CLI	T2	N1	-	III	0.9	+	-	-	-	-	-
46	70	CDI	T2	N0	G3	I	3.2	+	-	+++	-	+	+
47	35	CLI	Tx	N0	-	ND	8.0	+	-	-	+	+	+
48	39	CLI	T2	N1	-	III	ND	+	-	-	-	+	+
49	48	CLI	T2	N0	-	I	0.1	+	-	-	-	-	-
50	68	CDI	T1	N0	G2	I	2.7	+	-	-	-	-	-
51	74	CLI	T2	N0	-	I	2.8	+	-	++	-	-	-
52	67	CDI	T2	Nx	G3	ND	4.9	+	+++	++	+	+	+
53	52	CDI	T1	N0	G3	I	2.8	-	-	-	-	-	-
54	39	CDI	T3	N1	G3	III	4.4	+	+++	-	-	+	+

+, 11-25% positive cells; ++, 26-50% positive cells; +++, >50% positive cells. ND, not determinable.

Table II. Triplet and dinucleotide loci status in the 54 carcinomas studied.

No.	SCA1	SCA2	SCA3	SCA6	HD	DRPLA	X25	TRI n	TRI (%)	D1S104	D2S71	D2S123	D3S1611	D5S107	ACTC	D17S250	D17S261	D18S34	DI n	DI (%)	MSI n	MSI (%)
1	-	-	-	+	-	-	-	1	14	-	-	-	-	+	-	-	+	+	3	33	4	25
2	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
3	-	-	-	-	-	-	-	0	0	-	-	-	-	+	-	-	-	+	2	22	2	12.5
4	-	-	-	-	-	-	+	1	14	+	-	+	+	+	-	-	-	-	4	44	5	31
5	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
6	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
7	-	-	-	-	-	-	-	0	0	+	+	-	-	-	+	-	-	-	3	33	3	19
8	-	ND	-	ND	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
9	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
10	-	-	-	-	-	-	-	0	0	-	-	+	+	-	-	-	-	-	2	22	2	12.5
11	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
12	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
13	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
14	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
15	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
16	-	-	+	-	-	-	-	1	14	-	-	-	-	+	+	-	+	-	3	33	4	25
17	+	-	+	+	+	+	+	6	86	+	-	-	+	-	-	+	-	-	3	33	9	56
18	-	-	-	ND	-	-	-	0	0	-	-	+	-	-	-	-	-	+	2	22	2	13
19	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
20	-	-	-	-	-	-	-	0	0	-	-	-	-	-	+	-	-	-	1	11	1	6
21	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
22	-	-	-	-	-	-	ND	0	0	-	-	-	-	-	+	-	-	+	2	22	2	13
23	-	-	-	+	-	+	+	3	43	+	-	+	+	+	+	-	-	+	6	67	9	56
24	-	-	-	-	-	-	-	0	0	+	-	+	-	+	-	-	-	-	3	33	3	19
25	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
26	-	-	-	-	-	-	ND	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
27	-	-	-	-	-	+	-	1	14	-	-	+	-	-	-	+	-	+	3	33	4	25
28	-	-	-	-	-	+	-	1	14	-	-	-	-	-	-	-	-	-	0	0	1	6
29	-	-	-	-	-	ND	+	1	17	-	-	-	-	-	-	-	-	-	0	0	1	7
30	-	-	-	-	-	ND	-	0	0	-	-	-	-	-	-	+	-	+	2	22	2	13
31	-	-	-	-	-	-	-	0	0	-	-	-	-	+	-	-	-	-	1	11	1	6
32	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
33	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
34	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
35	-	-	+	-	-	+	+	3	43	-	-	-	-	+	+	-	+	-	3	33	6	37.5
36	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
37	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
38	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	+	-	-	1	11	1	6
39	-	-	-	-	-	ND	+	1	17	+	-	-	-	-	+	+	+	-	4	44	5	33
40	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
41	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	+	-	-	1	11	1	6
42	-	-	+	-	+	-	+	3	43	-	+	+	-	+	+	-	+	-	5	56	8	50
43	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
44	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	+	-	1	11	1	6
45	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
46	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	+	-	-	1	11	1	6
47	+	-	-	-	-	+	-	2	29	+	-	+	+	-	+	-	-	-	4	44	6	37.5
48	-	-	-	-	-	-	-	0	0	-	-	-	-	-	+	-	-	+	2	22	2	12.5
49	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
50	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
51	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
52	ND	-	-	+	-	+	-	2	33	-	-	-	-	+	-	-	+	-	2	22	4	27
53	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0
54	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	+	1	11	1	6
	2	0	4	4	2	7	7			7	2	8	5	10	10	7	7	9				
%	3.8	0	7.4	7.7	3.7	13.7	13.5			13	3.7	14.8	9.2	18.5	18.5	13	13	16.7				

ND, notdeterminable.

Table III. Correlations between triplet repeats instability (TRI) and dinucleotide instability (DI) and clinicopathological characteristics in the 54 cases studied.

Characteristics	No. of cases (%)	TRI (%)	DI (%)
Age			
≤50 years	18 (33)	5 (28)	12 (77)
>50 years	36 (67)	8 (22)	14 (39)
P-value		0.45	0.05
Tumor size			
≤2 cm	19 (36)	5 (26)	5 (26)
>2 cm	34 (64)	8 (23)	20 (59)
P-value		0.54	0.02
Lymph nodes status			
N^-	23 (44)	3 (13)	7 (30)
N^+	29 (56)	9 (31)	18 (62)
P-value		0.11	0.02
Grading			
G1/G2	6 (14)	2 (33)	2 (33)
G3	36 (86)	7 (20)	17 (47)
P-value		0.38	0.43
Histology			
Ductal	42 (78)	5 (12)	19 (45)
Lobular	12 (22)	4 (33)	7 (58)
P-value		0.09	0.32
Hormonal status			
Negative	11 (22)	1 (9)	5 (46)
Positive	39 (78)	12 (31)	19 (49)
P-value		0.14	0.56
TLI			
Low	22 (61)	5 (33)	8 (36)
High	14 (39)	4 (29)	9 (64)
P-value		0.49	0.097
p185			
Negative	42 (84)	10 (24)	20 (48)
Positive	8 (16)	3 (38)	4 (50)
P-value		0.34	0.6
p53			
Negative	34 (68)	10 (29)	19 (56)
Positive	16 (32)	3 (19)	5 (31)
P-value		0.33	0.09
Clinical stage			
I	19 (37)	2 (10)	4 (21)
II-III	32 (63)	9 (18)	20 (67)
P-value		0.13	0.004

Table II shows the presence (+) and absence (-) of MSI for each dinucleotide or trinucleotide repeat, and the percentages of locus incidence and case instability. MSI (TRI and/or DI) was identified in 28 of the 54 cases (52%). Dinucleotide instability occurred at ≥2 loci in 19/54 (35%) cases and TRI in 6/54 (11%). With regard to single locus instability, DI occurred in 26/54 (48%) of tumors, and TI in 13/54 (24%). The most frequently unstable triplets were DRPLA and X25-GAA (about 14% of cases); SCA2 instability was not detected in our series. The dinucleotide loci more frequently unstable were D5S107 and ACTC (18.5% of the cases), whereas the most stable locus was D2S71 (3.7%). MSI ranged between 0 and 56%.

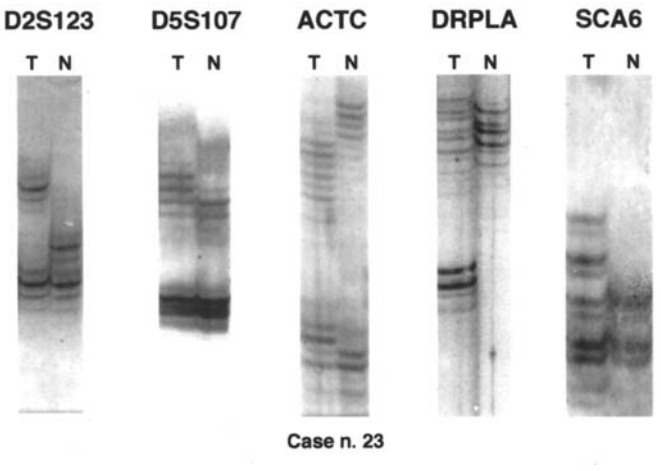

Figure 1. Examples of deranged electrophoretic mobility of PCR-fragments from tumor DNA (T) compared with normal DNA (N) (silver stain). Panel of altered loci in patient no. 23 who had high levels of DNA instability (9/16 loci, 56%).

Table III shows the relationships among TRI, DI and clinicopathological features. Triplet repeat instability was more frequent among patients ≤50 years old, in tumors with lymph-node metastasis, in lobular histotype, in tumors with positive hormonal status, positive p185 expression, negative p53 expression and in advanced clinical stages of the disease, although correlations were not significant. Dinucleotide instability was associated with age ≤50 years (p=0.05), tumors >2 cm (p=0.002), lymph node metastasis (p=0.02), high TLI values (p=0.097), negative p53 expression (p=0.09) and advanced clinical stage (p=0.004). Interestingly, most tumors with TRI showed DI. There was a correlation between TRI and DI in the same tumor (42 vs 7% in DI+ and DI- tumors respectively, p=0.0028). In fact 11/13 (86%) cases with TRI showed DI. Fig. 1 shows several altered loci in the patient no. 23 who had the greatest DNA instability (9/16 loci, 56%).

Discussion

Most studies of TRI in neoplasms concern the androgen receptor and its modification in testicular germ cell cancer (22), prostate cancer (23) and breast cancer (26,27). Mutations in the trinucleotide repeat ARP (arginine-rich protein) gene (3p21) have been reported in renal carcinomas (19), and in lung, breast, prostate, pancreatic and head and neck cancer (20). King *et al* (21) studied the trinucleotide loci SBMA, SCA1, SCA3 and DRPLA in 20 cell lines from 9 familial testicle tumors (21), and found germinal expansion of CAG repeats in 5/11 cell lines and in 1/11 sporadic testicle cancer. More recently, Giwercman *et al* (22) reported that the androgen receptor (AR) polymorphism was associated with both histological type and the progression rate of testicular germ cell

cancer. In prostate carcinoma, the AR has a polyglutamine region encoded by CAG repeats in the aminoterminal domain; a reduced number of these repeats is associated with an earlier disease onset age (23).

Data on TRI in breast cancer are limited to a few studies conducted with a small number of cases (17,24-27). Here, we demonstrate that in primary breast cancer TRI is a molecular marker associated to DI and earlier onset age, and not merely caused by incidental mutations. Breast cancer is characterized by genetic anticipation and more aggressive disease in younger patients (2,16,17,26-30). These two features are typical of breast cancer, as they are of neurodegenerative triplet disease (6,16,17,28-30).

In our series, MSI was due mostly to DI, and with a frequency similar to that of other studies (26,28,30). Furthermore, loci D2S123, D5S107 and ACTC, which are recommended markers of MSI (2), are more frequently altered in primary breast cancer (2,30).

All triplet repeats examined in our study were unstable in breast cancer except SCA2. But interestingly, TRI and DI were significantly associated (p=0.0028). In fact, 11 of the 13 patients with TRI also had DI. This result coincides with the finding of DM-1 CAG repeat instability in 4 of 78 cases (5%) where two of the positive cases had MSI in 9 of the 10 loci studied (17).

Our results indicate that TRI, like DI, can be considered a marker of reduced genomic stability triggered by alterations in genes involved in DNA replication and repair mechanisms (4,5,30). Unstable cells will culminate in a cascade of genomic mutations that overwhelm mechanisms of cell proliferation control. TRI was identified in the E2F-4 gene, which is a transactivating factor promoting the cell cycle G1-S phase. Yoshitaka *et al* (18) examined 20 colorectal cancers and found alterations in the copy number of 13 consecutive trinucleotide repeats in the encoding exon E2F-4 in the two cases with MSI.

Several studies have demonstrated that MSI in primary breast cancer is predictive of recurrent and more aggressive disease (28-30). Here we confirm the relationship between DI and lymph node metastasis and larger tumor size, as well as the correlation between DI and an advanced clinical stage of the disease. DI was also more frequent in patients ≤50 years old. Similar results, albeit not statistically significant, were obtained with TRI. TRI was more frequently associated with lobular carcinoma, lymph node metastases and more advanced clinical stages. It was also more frequent in patients ≤50 years old, with positive steroidal hormone receptor status, positive p185 and negative p53.

In conclusion, we extend the finding of TRI, already reported in neurodegenerative disease, to primary breast cancer. We demonstrate a relationship between these mutations and the clinicopathological characteristics of cancer aggressiveness and an association with DI. Furthermore, the association of TRI and DI in the same tumor indicates that alterations in the DNA repair gene could culminate in selective breast cancer progression in a considerable number of patients. Further studies need to verify whether TRI respect to DI (or MSI) correlates to outcome or response to treatment.

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