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Infrequent Microsatellite Instability in Papillary Carcinomas of the Thyroid

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ABSTRACT

The aim of the present study was to confirm the frequency of microsatellite instability in thyroid cancers. We investigated 26 patients suffering from thyroid cancer (25 papillary carcinomas, 1 follicular carcinoma) using 8 microsatellite markers. Microsatellite instability (MSI) was found in two (7.7%) papillary carcinomas. Our data indicate that MSI is a relatively rare event and may seldom contribute to the carcinogenesis of papillary carcinomas of the thyroid.

Key words: *Microsatellite instability, Papillary carcinomas, Thyroid gland*

Recent studies based on molecular biology have revealed that genetic instability is one of the important predispositions for human multistep carcinogenesis[1,4,8,12,15,18]. Microsatellite loci, widely distributed in the human genome, are comprised of simple repeated nucleotide sequences including repeats of cytosine-adenine (CA) dinucleotides and represent high frequencies of polymorphism. Alterations in the repeat numbers of microsatellites have been reported in cancers with a DNA mismatch repair defect. This phenomenon is called replication error (RER) or microsatellite instability (MSI). MSI reflects an unstable genomic status and is thought to increase the normal mutation rate resulting in multiple mutations of various oncogenes and tumor suppressor genes.

Although many studies have demonstrated MSI in various human malignancies, detailed microsatellite analyses on thyroid cancer have been relatively few and their results inconsistent[11,17,19]. The aim of the present study was to assess the frequency of MSI and to clarify its role in human thyroid carcinogenesis.

MATERIALS AND METHODS

Sample Collection

Twenty-six cases of thyroid cancer were used in this study. They were selected at random from the files of surgically resected thyroid cancers at the Department of Otorhinolaryngology, Hiroshima University School of Medicine, for this study. Among them, 25 cases were papillary carcinomas and one case was follicular carcinoma histopathologically. Representative paraffin blocks from each case were selected and used.

DNA Extraction

DNA from paraffin-embedded tissues was obtained as decribed by Ionov et al[8], with some modifications. Tissues were incubated at 55°C overnight in each 50-μl DNA extraction solution [100 mM Tris-HCl, 2 mM EDTA (pH 8.0), and 400 mg/ml proteinase K]. After extraction, proteinase K was inactivated by boiling for 10 min.

Microsatellite Assay

To assess the RER status, we used oligonucleotide primer sets for 8 microsatellite markers. D1S191, D5S494, D7S486 and D17S855 (Research Genetics, Huntsville, AL) were used as CA repeat markers, and BAT-25, BAT-26, BAT-40 and BAT-RII, whose primers were synthesized to order at TAKARA Biomedicals (Tokyo, Japan), were used as poly (A) markers[16]. BAT-RII alteration indicates mutation of poly (A) tract in *TGF-β RII* (the open reading frame of *TGF-β* type II recetor gene). PCR was performed as described by Semba et al[16]. Briefly, each 15-μl reaction mixture containing about 10–20 ng of DNA, 6.7 mM Tris-HCl (pH 8.8), 6.7 mM EDTA, 6.7 mM $MgCl_2$, 0.33 mM of labeled primer with [γ-^{32}P]ATP, 0.175 mM unlabeled primer, 1.5 mM deoxynucleotide triphosphates, and 0.75 units of recombinant Taq DNA polymerase (Perkin-Elmer, Norwalk, CT) was amplified for 40 cycles with the following regime: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec.

PCR products were electrophoresed in 6% polyacrylamide-8 M urea –32% formamide gels and autoradiographed overnight at –80°C.

RESULTS

The results of microsatellite analysis in this study are summarized in Table 1.

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Table 1. Microsatellite alterations in thyroid cancers

Case No.	Sex	Age	Histological type	Microsatellite loci							
				BAT25	BAT26	BAT40	BATRII	D1S191	D5S494	D7S486	D17S855
1	F	44	PC[a]	–[b]	–	–	–	–	–	–	–
2	M	69	PC	–	N.D.[e]	–	–	N.D.	–	–	–
3	F	72	PC	–	–	–	–	–	–	–	–
4	F	34	PC	–	–	–	–	–	–	LOH[d]	LOH
5	M	51	PC	–	–	–	–	RER[c]	–	–	–
6	F	60	PC	–	–	–	–	–	–	N.D.	N.D.
7	M	57	PC	–	–	–	–	N.D.	–	N.D.	N.D.
8	M	57	PC	–	–	–	–	N.D.	–	–	–
9	F	24	PC	–	–	–	–	–	–	–	–
10	M	51	PC	–	–	–	–	N.D.	N.D.	–	–
11	F	51	PC	–	N.D.	–	–	N.D.	–	–	–
12	F	65	PC	–	–	–	–	N.D.	N.D.	–	–
13	F	57	PC	–	–	–	–	N.D.	–	–	–
14	F	41	PC	–	N.D.	–	–	–	–	–	N.D.
15	F	63	PC	–	–	–	–	–	–	–	–
16	F	57	PC	–	–	N.D.	–	N.D.	–	–	–
17	M	25	PC	–	–	–	–	–	–	–	RER
18	F	39	PC	–	–	N.D.	–	–	–	–	–
19	F	28	PC	–	–	N.D.	–	–	–	N.D.	N.D.
20	M	54	PC	–	–	–	–	–	–	–	N.D.
21	F	49	PC	–	–	N.D.	–	–	–	–	N.D.
22	F	42	PC	N.D.	–	N.D.	N.D.	N.D.	–	N.D.	N.D.
23	F	21	PC	N.D.	–	N.D.	N.D.	–	–	N.D.	N.D.
24	F	53	follicular	–	–	–	–	–	–	–	
25	F	21	PC	–	–	–	–	–	–	N.D.	N.D.
26	M	64	PC	–	–	–	–	–	–	–	N.D.

a) PC,papillary carcinoma; b)–, no microsatellite alteration ; c) RER, replication error;
d) LOH, loss of heterozygosity; e) N.D., not done

Two cases (case 5 and case 17; 7.7%) demonstrated MSI in their thyroid cancer. In both cases, only one microsatellite locus was altered. In addition, loss of heterozygosities at D7S486 and D17S855 was observed in case 4.

Figure 1 shows the representative results of microsatellite assays on case 5 and 17. D1S191 revealed MSI in case 5, and D17S855 revealed MSI in case 17.

Table 2 shows the comparisons between the microsatellite status and clinicopathological features of the patients in this study. The two MSI tumors were papillary carcinomas histologically. No microsatellite alterations was found in the follicular carcinoma. The one case with MSI-positive tumor was a young man (25-year-old), and the other was a middle-aged man (51-year-old). Both MSI cases were accompanied by paratracheal lymph node metastases. No correlation was observed among local invasion, recurrence, familial deposition and microsatellite status in the present cases studied.

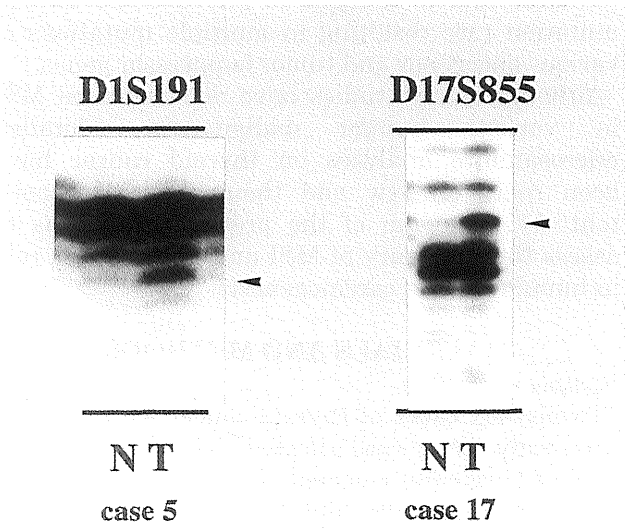

Fig. 1. Result of microsatellite analysis on two cases of papillary carcinoma of the thyroid. N, normal control; T, tumor tissue. Extra-peaks (arrow) are observed in PCR products of D1S191 microsatellite (left: case 5), and D17S855 microsatellite (right: case 17) from tumor DNA.

DISCUSSION

Human thyroid cancers display genetic abnormalities involving several oncogenes and tumor suppressor genes. Mutation of *ret* proto-oncogene has been postulated as the primary genetic lesion

Table 2. Clinical and Pathological Characteristics of Microsatellite Instability-negative and -positive Patients

	microsatellite instability negative (n=24)	microsatellite instability positive (n=2)	total
Gender (mean age ; range)			
female	18 (46; 21–72)	0	18 (46; 21–72)
male	6 (59; 51–69)	2 (38; 25–51)	8 (54; 25–69)
total	24 (49; 21–72)	2 (38; 25–51)	26 (48; 21–72)
Family history			
yes	2	0	2
no	22	2	24
Histology			
papillary carcinoma	23	2	25
follicular carcinoma	1	0	1
Lymph node metastasis			
yes	16	2	18
no	8	0	8
Radical neck dissection			
yes	9	1	10
no	15	1	16
Atomic bomb exposure			
yes	2	0	2
no	22	2	24
Operation method			
hemithyroidectomy	12	1	13
near-total thyroidectomy	5	0	5
total thyroidectomy	7	1	8
Extracapsular invasion			
yes	11	2	13
no	13	0	13
Recurrence (Observation period, 9 years)			
yes	3	0	3
no	21	2	23

of multiple endocrine neoplasia as well as of sporadic medullary thyroid carcinoma[6].

In addition, activating rearrangements of this oncogene, *RET/PTC1–3*, have been detected in about 20% of cases of papillary carcinomas[20].

Point mutations of H-ras oncogene were found in papillary carcinomas[13], whereas those of N-ras were specifically detected in follicular carcinomas[14]. Mutations of *p53* tumor suppressor gene were reported to be found in undifferentiated carcinoma progressed from papillary thyroid carcinoma[9]. To explain the accumulation of these genetic abnormalities, the concept of genetic instability has been postulated. Defects in DNA mismatch repair, represented by MSI, can increase the normal mutation rate in multiple mutations in oncogenes and tumor suppressor genes.

Three reports concerning MSI in sporadic human thyroid cancers have been published to date. Vermigio et al[19] failed to detect any MSI in 9 thyroid cancer cases with D2S123, D2S119 and D2S147 microsatellites. Soares et al[17] found MSI in 3 of 16 (18.8%) thyroid cancers, one follicular and two papillary carcinomas, using 8 microsatellites with one tumor showing MSI at three or more loci. Recently, Lazzereschi et al[11] found MSI in 1 of 29 (3.4%) papillary thyroid carcinomas.

In the present study, 2 (7.7%) MSI tumors out of 26 thyroid cancers were found with 8 microsatellite markers. Tumors with MSI at multiple loci were not detected. These observations indicate that MSI is generally a rare genetic event and may not contribute to the accumulation of gene abnormalities in the papillary carcinomas of the thyroid. In addition, LOH was detected in only one case (case 4) at two loci (D7S486 and D17S855). Therefore, loss of the chromosomal segment detected by microsatellites in this study may also not contribute to the carcinogenesis of papillary thyroid cancers.

Both MSI positive cases in the present study had paratracheal lymph node metastasis. It was also reported that MSI was significantly correlated with lymph node involvement in breast cancer[6]. Further investigation of larger series of the thyroid is required.

In conclusion, MSI may be a relatively rare event in papillary carcinomas of the thyroid. Although MSI may possibly contribute to the progression of thyroid cancer, it might not play a main role in the genetic instability of human thyroid carcinogenesis.

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