

Microsatellite Instability in Thyroid Cancer: Hot Spots, Clinicopathological Implications, and Prognostic Significance¹

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ABSTRACT

Purpose: To determine whether microsatellite instability (MSI) in particular loci has clinicopathological significance in thyroid cancer.

Experimental Design: Seventy-six cases of surgically resected thyroid cancer were screened for MSI at nine microsatellites: *THRA1*, *TSHR*, *D2S123*, *D11S912*, *D2S115*, *D2S399*, *p53*, *RET*, or *BAT-26*. Multivariate analysis was performed to test for links between MSI and the clinical parameters of gender, age, histology, stage, nodal involvement, and prognosis.

Results: *THRA1*, residing in the thyroid hormone receptor α gene, displayed the highest levels of MSI at 36.5%. MSI in *TSHR*, located within the thyroid-stimulating hormone receptor gene, was found to be linked to cancer in the elderly (>70 years of age) and with high-grade (N 3, 4) nodal involvement. In follicular cancer, MSI in *D2S123* occurred at a frequency of 100% (7/7) with no (0%) occurrence of MSI at the nearby *D2S115*, *D2S399*, or *BAT-26* loci. Regarding prognosis, patients with MSI-positive cancer showed better long-term survival. *BAT-26*, which is an important marker in colorectal cancer, displayed the lowest frequency of MSI in our panel of thyroid tumors.

Conclusion: Whereas patients with MSI-positive cancer showed better long-term survival, as is the case for colorectal cancer, our finding of the low frequency of MSI in *BAT-26* suggests that the biochemical defects governing the

spectrum of MSI in thyroid and colorectal cancer are different. MSI in *THRA1*, *TSHR*, and *D2S123* appears to be an integral part of thyroid carcinogenesis, as evidenced by the high frequency of MSI and significant correlation to clinical data.

INTRODUCTION

Human thyroid tumors derive from either epithelial follicular cells or from parafollicular C cells (1). Tumors of follicular cell origin range from benign adenomas through papillary and follicular cancers to undifferentiated anaplastic carcinoma. Follicular adenomas and carcinomas often show mutations in one of the three *Ras* genes (2) and occasionally in the *PTEN* tumor suppressor gene (3). PTC³ often show characteristic gene rearrangements, which give rise to the formation of several types of *RET/PTC* chimeric genes (4). Such genomic instability occurs in ~50% of all of the papillary cancers and involves the juxtaposition of the 3'-tyrosine kinase domain of the *RET* proto-oncogene with the 5' domain of a ubiquitously expressed gene, resulting in the constitutive activation of *RET/PTC* proteins (5, 6). Anaplastic carcinoma is associated frequently with mutations of the *p53* tumor suppressor gene (7). Point mutations of the *RET* proto-oncogene are a common feature of medullary thyroid carcinoma, a malignant tumor derived from parafollicular C cells. Despite these beginnings, a conclusive relationship between specific gene alterations and particular forms of thyroid cancer has not yet been established (8).

MSI is a distinct form of genomic instability linked to defects in DNA mismatch repair (9). MSI is characterized by alterations in the length of simple homopolymeric sequences (*i.e.*, microsatellites) that are ubiquitous in the genome. The presence of MSI in tumor tissue has been associated with unique clinical and pathological characteristics (10–13). While most notably associated with hereditary nonpolyposis colon cancer, MSI has been found to be involved in the genesis of many types of cancer (14, 15). Although MSI was not initially observed in thyroid cancer (16), subsequent studies have documented MSI not only in thyroid cancer but also in benign goiters and follicular adenomas of the thyroid (17–19). These more recent studies support the notion that MSI plays an important role in thyroid cancer and disease.

Studies on genomic instability in cancer have revealed that MSI occurs much more frequently in certain loci than in others and that the spectrum of MSI varies with cancer type (20). In colorectal cancer, where the majority of past work has focused, a set of five microsatellites (*BAT26*, *BAT25*, *D2S123*, *D5S346*,

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³ The abbreviations used are: PTC, papillary thyroid carcinoma; MSI, microsatellite instability.

and *D17S250*) has been selected by an international consortium of scientists to serve as a reference panel for assessment of MSI status (20). MSI in *BAT-26* appears most strongly linked to the replication error phenotype in colorectal cancers and cell lines (21). The biological significance of the reference panel is still unclear outside of the observation that if MSI is observed in members of the reference panel then it is highly likely that MSI will be observed in a variety of alternative microsatellite loci. Overall, the reference panel of five microsatellites was chosen by consensus vote rather than asserting some defining criteria. As the link between MSI and cancer becomes more clear selection criteria will undoubtedly also be brought into better focus. The character of MSI in noncolonic tumors was reviewed by Boland *et al.* (20), and it was concluded that the reference panel of five microsatellites used to assess MSI in colorectal cancer is not suitable to serve as a reference panel for other types of cancer. Consequently, there is a need to find microsatellites appropriate for assessing MSI status in noncolonic tumors. In this report we present data on particular microsatellites that appear well suited for assessing MSI in thyroid cancer.

We chose to analyze MSI at locations with an association to thyroid cancer, focusing on microsatellites close to the *RET*, *p53*, *hMSH2*, *TSHR*, and *THRA1* genes, and a microsatellite on chromosome 11. To date, mutations of the *RET* gene have the strongest link to thyroid cancer (8), whereas loss of heterozygosity affecting the *p53* gene is often observed in anaplastic thyroid carcinoma (22). Although loss of heterozygosity in *p53* has not been frequently observed in thyroid cancers of other histological types (5, 23, 24), the importance of *p53* in carcinogenesis overall made an assessment of MSI near *p53* prudent. Given the importance of defective DNA mismatch repair in the MSI phenotype, four microsatellites on chromosome 2 near *hMSH2* were tested, namely, *BAT-26*, which is located in an intron of *hMSH2*, and *D2S123*, *D2S115*, and *D2S399*. We also chose to analyze *TSHR*, located within the thyroid stimulating hormone receptor gene, and *THRA1*, which resides in the thyroid hormone receptor α gene (25, 26). We included one additional microsatellite in our panel, namely *D11S912*, chosen because chromosome 11 carries a putative tumor suppressor gene important in thyroid tumorigenicity (27, 28).

MATERIALS AND METHODS

Patients Profile and Clinical Data. Seventy-six normal tumor-matched samples were collected at Fukushima Medical University Hospital from 1993 to 1999. Patient clinical data are shown in Table 1. There were 65 female and 11 male patients. Histopathological classification and staging are according to Union Internationale Contre le Cancer criteria (29). The average of survival follow-up term was 166.8 weeks. All of the cases were sporadic primary thyroid carcinoma except one multiple endocrine neoplasia type 2A case. In one portion of the study dealing with MSI at *D2S123* in follicular carcinoma, 45 cases of benign follicular adenoma (7 male, average age 47.2 years, and 38 female, average age 50.9 years) were analyzed.

Sample Collection and DNA Extraction. After surgical resection, tumor and normal control tissue was classified as to histology and stage by a medical pathologist then immediately frozen to -80°C . To obtain purified DNA the frozen samples

Table 1 Patient and clinicopathological data

Total	76
Gender	
Male	11
Female	65
Age	
Average (range)	55.1 (20–87) years
Histological type	
Papillary carcinoma	65 (85.6%)
Follicular carcinoma	7 (9.2%)
Anaplastic carcinoma	2 (2.6%)
Medullary carcinoma	2 (2.6%)
Stage	
Stage I	30
Stage II	8
Stage III	27
Stage IV	9
Unknown	2
Prognosis	
Survivor	65
Dead	10
Unknown	1
Average of follow-up period (range)	166.8 (7–328) weeks

were homogenized, treated with proteinase K, and the DNA isolated by phenol-chloroform extraction (30).

Microsatellite Analysis. PCR-based microsatellite analysis was performed at the eight $(\text{CA})_n$ dinucleotide repeat microsatellite markers, *D2S115*, *D2S123*, *D2S399*, *RET*, *D11S912*, *TSHR*, *THRA1*, and *p53*, and the one $(\text{A})_{26}$ mononucleotide microsatellite marker *BAT-26*. PCR primers were designed using sequence information from GenBank⁴ and Primer3 for primer selection⁵ except *D11S912* and *p53*. For these two loci, we used PCR primers that were reported previously (28, 31). PCR primer sequences are presented in Table 2. The *THRA1* locus exists in an exon, but the other loci exist in intronic regions. PCR amplifications were performed in a 10- μl reaction mixture containing 100 ng of DNA, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 100 μM deoxynucleotide triphosphates, 1.5 mM MgCl₂, 10 pmol of forward and reverse primers, 0.01% gelatin, 0.25 units of Taq DNA polymerase (Perkin-Elmer, Foster City, CA), and 1 mCi of [α -³²P]dCTP (EASYSIDES; NEN Life Science Products Inc., Boston, MA). PCR was performed using an initial denaturing step at 94°C for 3 min, then steps of 94°C for 40 s, 54–60°C for 40 s, 72°C for 40 s, repeated for 30 cycles, followed by a final extension step of 72°C for 5 min. PCR amplification was performed on a PTC-100 PCR machine (MJ Research, Inc., Watertown, MA). For gel electrophoresis the PCR amplification products were first mixed with a formamide loading dye heat denatured and then loaded on 6% SequaGel (National Diagnostic, Atlanta, GA) containing Tris-borate EDTA buffer and 6 M urea. Electrophoresis on a standard vertical DNA sequencing gel apparatus was done at 1300 V for 2–3 h. After electrophoresis, gels were dried and used for

⁴ Internet address: <http://www.ncbi.nlm.nih.gov/>.

⁵ Internet address: http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi.

Table 2 PCR primer sets^a

Locus	Forward primer	Reverse primer
<i>D2S115</i>	CAGCCATATTGACTTGAACGTAAG	GGTACAGCCCATGTGTGAGA
<i>D2S123</i>	AATGGACAAAAACAGGATGC	CCCTTTCTGACTTGGATACC
<i>D2S399</i>	TTCACATGGCAGACCTGATTAT	GCTCAATGGGAAGTTATTGAATG
<i>BAT-26</i>	CAGTCAGAGCCCTTAACCTTTT	GCTCCTTCTAAGCCTTCTTCAC
<i>RET</i>	AGGGCCTTGGTAATGTAGACCT	CATCCTGGCTCAGAATAAACCT
<i>D11S912</i>	TACTGCTTTGGGTATGCATATG	GCTTTTTGTCTAGCCATGATTG
<i>TSHR</i>	CTTGCCCTCCTCAACAAC	CGTGAGAGAAGCTTTCCTGGC
<i>THRA1</i>	CTTAAGCAGTGGGAACCTG	ATAGCATGCTTCCCATGT
<i>p53</i>	ACTGCCACTCCTTGCCCCATTC	AGGGATACTATTTCAGCCCAGGTG

^a Each primers set generated originally based on the sequence information from GenBank except *D11S912* and *p53*. Sequence direction: 5' to 3' (left to right).

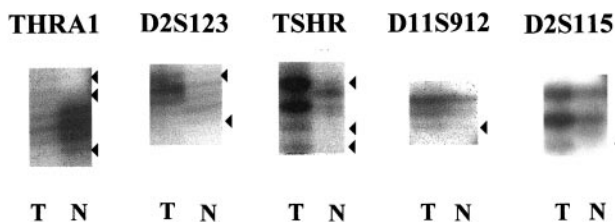


Fig. 1 Autoradiographs of MSI in thyroid samples. PCR product of the indicated loci analyzed by denaturing PAGE (see text for additional details). Arrows, different sized PCR amplification products in tumor samples (T) compared with patient matched normal tissue (N).

autoradiography with Fuji HR-H film (Fuji Photo Film Co., Ltd., Tokyo, Japan) for 12–48 h.

Assessment of MSI. To assess MSI, we compared the band pattern produced after gel electrophoresis of paired PCR reactions in which patient matched normal and tumor DNA was amplified. If the normal and tumor PCR amplification products displayed different electrophoretic mobility we scored the case as positive for MSI. If either of the PCR amplifications failed, we omitted the case from the judgement of MSI. Microdissection of tumor samples was not performed; consequently, the MSI frequencies we report are lower than or equal to MSI frequencies in absolutely pure tumor tissue. MSI was independently judged in a blinded manner by the authors.

Statistical Analysis. To assess the statistical significance of relationships between MSI and clinical parameters, Student's *t* test, χ^2 test, and McNemar's test were used. For survival rate, the Kaplan-Meier survival curve method was used with statistical significance by log-rank test. $P < 0.05$ was considered as statistically significant. All of the tests were performed with Statview version 5.0 software (SAS Institute Inc., San Francisco, CA). Multivariate analysis was performed, and only links supported by a $P < 0.05$ have been presented.

RESULTS

Frequency of MSI. Representative gel electrophoresis band patterns of MSI positive and negative cases are shown in Fig. 1. At the eight (CA)_n dinucleotide microsatellites tested, the following frequencies of MSI were observed: *THRA1* (27/74, 36.5%); *D2S123* (24/74, 32.4%); *D11S912* (21/76, 27.6%); *D2S115* (17/76, 22.4%); *RET* (12/64, 18.8%); *TSHR* (14/76, 18.4%); *p53* (13/76, 17.1%); and *D2S399* (10/70, 14.3%). The

Table 3 Frequency of MSI with significance of correlation to *THRA1* locus

Locus	Positive/tested ^a	Frequency	Comparison to <i>THRA1</i> locus
<i>D2S115</i>	17/76	22.4%	NS ^b ($P = 0.08$)
<i>D2S123</i>	24/74	32.4%	NS ($P = 0.72$)
<i>D2S399</i>	10/70	14.3%	$P = 0.004$
<i>BAT-26</i>	5/60	8.3%	$P = 0.0001$
<i>RET</i>	12/64	18.8%	$P = 0.03$
<i>D11S912</i>	21/76	27.6%	NS ($P = 0.32$)
<i>TSHR</i>	14/76	18.4%	$P = 0.021$
<i>THRA1</i>	27/74	36.5%	—
<i>p53</i>	13/76	17.1%	$P = 0.012$

^a MSI = positive case/tested informative case.

^b NS, not significant.

lowest frequency of MSI was observed at the mononucleotide (A)₂₆ microsatellite *BAT-26* (5/60, 8.3%), which is located in an intron of the DNA mismatch repair gene *hMSH2*.

The *THRA1* (CA)₁₈ microsatellite, residing in exon 9 of the thyroid hormone receptor α gene (25), showed the highest frequency of MSI in our study. The frequency of MSI in *RET*, *TSHR*, *p53*, *D2S399*, and *BAT-26* was calculated to be significantly lower than that measured in *THRA1* based on McNemar's test with 95% confidence (see Table 3). The frequency of MSI in the *D2S123*, *D11S912*, and *D2S115* markers was not significantly different from that measured in *THRA1* based McNemar's test within a 95% confidence interval. As a group, *THRA1*, *D2S123*, *D11S912*, and *D2S115* showed on average MSI in 29.7% of the thyroid cancer tissues tested.

Relationship between MSI at One Locus and Average MSI. To determine whether MSI status in one locus was indicative of an elevated overall frequency of MSI, we compared the frequency of MSI at each locus to the average MSI for our panel (Table 4). For example, in the 27 tumors displaying MSI at *THRA1*, the average MSI for our panel was 38.2% (see Table 4). In the 47 tumors that did not display MSI at *THRA1*, the average MSI for our panel was 16.1%. Therefore, if a tumor displayed MSI in *THRA1*, then MSI was more likely to be observed in other microsatellites in our panel compared with those tumors that did not exhibit MSI in *THRA1*. *D2S123*, *D11S912*, *TSHR*, *p53*, *D2S115*, and *D2S399* also showed this trait (see Table 4). Interestingly, *BAT-26* and *RET* did not display this characteristic. Tumors displaying MSI in *BAT-26* or *RET* were not significantly different from the tumors not dis-

Table 4 Relationship between MSI at one locus and average MSI

Locus	MSI ^a	Cases	Average MSI (%)	<i>t</i> test ^b
D2S115	-	59	19.9	$P = 0.0004$
	+	17	38.4	
D2S123	-	50	17.3	$P < 0.0001$
	+	24	38.6	
D2S399	-	60	20.8	$P = 0.0058$
	+	10	36.3	
BAT-26	-	55	21.4	NS ^c
	+	5	28.5	
RET	-	52	22.9	NS
	+	12	28.9	
D11S912	-	55	18.4	$P < 0.0001$
	+	21	39.0	
TSHR	-	62	20.2	$P = 0.0001$
	+	14	41.2	
THRA1	-	47	16.1	$P < 0.0001$
	+	27	38.2	
p53	-	63	20.4	$P = 0.0002$
	+	13	41.8	

^a MSI status of each locus, +: MSI positive, -: MSI negative.

^b Student's *t* test, $P < 0.05$ is statistically significant.

^c NS, not significant.

Table 5 Relationship between MSI and clinicopathological factors

Locus	Factors	<i>P</i>	
D2S123	histological type	$P = 0.0003$ **	
	follicular type		7/7 ^a (100%)
	other type		17/67 (25.3%)
	follicular adenoma	3/45 (6.6%) $P < 0.0001$	
TSHR	age	$P = 0.05$	
	>70 years		4/8 ^b (50%)
	<70 years	10/68 (14.7%)	
	nodal involvement	$P = 0.04$	
N0-2	9/54 ^c (14.3%)		
	N3, 4	5/13 (38.5%)	

^a D2S123 MSI = positive cases/total follicular type cases.

^b TSHR MSI = positive cases/total >70 years of age cases.

^c TSHR MSI = positive cases/total N 0-2 cases.

playing MSI in *BAT-26* or *RET* in regard to the frequency of MSI at the other microsatellites tested in this study.

Relationship between MSI and Clinicopathological Factors. To clarify the relationship between MSI and various standard clinical parameters, we compared MSI status at each microsatellite with the following clinical parameters: gender; age; TNM classification; stage; histological type; recurrence; and nodal involvement (see Table 5). Significant correlation between MSI in certain microsatellite markers and histological type, patient age, nodal involvement, and tumor stage were observed. As to histology, all seven of the follicular carcinomas displayed MSI in the *D2S123* locus, whereas in nonfollicular tumors MSI in *D2S123* was observed at a frequency of 25.3% (17 of 67). The link between MSI in *D2S123* and follicular carcinoma was calculated to be statistically significant ($P = 0.0003$). To test if the link between MSI in *D2S123* extended to follicular adenoma, we screened for MSI at *D2S123* in 45 cases of benign follicular adenoma (7 male, average age 47.2 years, and 38 female, average age 50.9 years). We found 7% (3/45) of

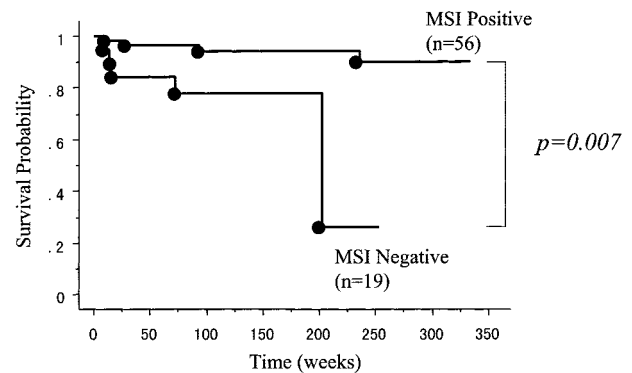


Fig. 2 The relationship between MSI phenotype and survival rate. MSI-positive group showed the better prognosis than the MSI negative group ($P = 0.007$). Survival curve was made with Kaplan-Meier method and tested by log-rank test.

the benign follicular adenoma cases displayed MSI at *D2S123*. A χ^2 test as to the significance of the difference in MSI frequency yielded a P of 0.0001, which strongly supports the notion that MSI in *D2S123* is linked solely to the follicular cancer phenotype.

Regarding patient age, in tumors from patients >70 years of age, MSI in *TSHR* was observed at a frequency of 50% (4 of 8), whereas for patients <70 years of age, the percentage of MSI in *TSHR* was 14.7% (10 of 68). The correlation between MSI in *TSHR* and patient age was significant ($P < 0.05$, χ^2 test). In regard to nodal involvement, the N 3, 4 group of tumors showed a higher frequency of MSI in *TSHR* with 5 of 13 cases or 38.5% versus 9 of 63 cases or 14.3% in the N 0, 1, 2 group. The correlation between MSI in *TSHR* and nodal involvement was calculated to be statistically significant ($P < 0.05$, χ^2 test).

Prognostic Value of MSI in Thyroid Carcinoma. Survival data for 75 of the 76 thyroid cancer cases used in this study is known. To determine whether MSI was linked to survival the 75 cases were divided into two groups: a MSI-negative group with no MSI at any microsatellite tested and a MSI-positive group with MSI found in at least one microsatellite. For these two groups we compared survival (see Fig. 2). The MSI-negative group showed a much poorer prognosis than the MSI-positive group ($P = 0.007$, log-rank test). In the MSI-positive group the survival rate did not depend on whether additional loci displayed MSI (data were not shown).

DISCUSSION

The *BAT-26* locus showed the lowest frequency of MSI in our panel and had the lowest predictive value as to the occurrence of MSI in other loci. For colorectal cancer, MSI in *BAT-26* has been found to be a reliable predictor as to the occurrence of MSI in other loci (21) and has strong prognostic value (12). The significance of MSI in the *BAT-26*, located in intron 5 of the *hMSH2* gene, has been taken as evidence of the importance of defective hMSH2-mediated mismatch repair in MSI-positive colorectal cancers (13, 21). This does not appear to be the case in thyroid cancer.

Our data on *BAT-26* suggests that the biochemical defects giving rise to MSI in thyroid cancer are different from those

responsible for MSI in colorectal cancer. Whereas the biochemistry governing the spectrum of MSI in cancer is unknown, our data indicate that *BAT-26* is not a primary target of MSI in thyroid cancer. Interestingly, whereas thyroid and colorectal cancer appear quite different in regard to *BAT-26*, our observation that patients with MSI-positive tumors had significantly better prognosis parallels what has been found in colorectal cancer. Although exactly why survival is better in the case of MSI-positive tumors is still unclear, it does seem that a MSI-positive phenotype carries with it genetic alterations that manifest a less aggressive phenotype (12, 14). For thyroid cancer, MSI events outside of *BAT-26* would appear to govern prognosis. The lower than expected frequencies and weak prognostic value of MSI in *RET* and *p53* is taken as evidence that these loci are also not primary MSI targets in thyroid cancer and that other microsatellite markers should probably be used to evaluate MSI in thyroid cancer.

The *THRA1* locus displayed the highest frequency of MSI (36.5%). The *THRA1* gene is a homologue of *v-erba*, which was one of the first examples of a dominant-negative oncogene (32). Immunohistochemical analysis of cancer cell lines (33) has substantiated that MSI in the *THRA1* microsatellite, residing in exon 9 of the gene, affects *THRA1* protein expression (data not shown). Whereas no clinicopathological relation with *THRA1* was found, MSI in *THRA1* was indicative of MSI in other loci with significant relation to clinical data. It is conceivable that MSI in *THRA1* is linked to a clinical parameter we did not test or that MSI in this locus is associated with an obscure phenotype not easily characterized in a clinical setting. The same may be true for *D2S115* and *D11S912*, because high MSI frequencies were also observed in these loci, but no statistically significant connection to a clinical parameter was found. Being such notable targets of MSI is consistent with the notion that MSI in these loci is a hallmark of biochemical events that advance thyroid cancer.

There is good reason to suspect that MSI in *D2S123* is fundamentally connected to follicular cancer, given the level of statistical significance associated with the high (100%) occurrence of MSI in *D2S123*. From our analysis of the four microsatellites on chromosome 2, it appears noteworthy that the spectrum of MSI is quite sharp, because there was no (0%) occurrence of MSI at the nearby *BAT-26*, *D2S399*, or *D2S115* loci in the seven follicular cancer cases we analyzed. For the entire panel of 76 thyroid tumors, the MSI frequencies at *BAT-26* (8.3%), *D2S123* (32.4%), *D2S399* (14.3%), and *D2S115* (22.4%) compared with their chromosome 2 position of 49330585, 53180120, 174313142, and 206995452, respectively (UCSC Genome Browser October 7, 2000 Freeze),⁶ indicates that in sporadic thyroid cancer MSI varies nonuniformly in this portion of chromosome 2. Given the need to better define genetic differences in various forms of thyroid cancer, additional work on the genetic defect(s) that focus MSI at *D2S123* in follicular cancer is, we feel, warranted.

The frequency of MSI in *TSHR* was approximately half that measured in *THRA1*; however, MSI in *TSHR* had stronger

ties to clinical data than *THRA1*, being connected to patient age and nodal involvement. The *TSHR* gene appears to be important in thyroid cancer, because point mutations in *TSHR* are often found in thyroid carcinomas (34). The *TSHR* microsatellite we analyzed resides in a noncoding segment of the gene; consequently, it is unclear exactly how MSI in this locus would manifest itself clinically. We postulate that MSI in the *TSHR* microsatellite could affect gene expression in a number of ways, for example, by affecting changes in the degree of supercoiling and therein altering transcriptional regulation of the gene (35, 36) or by precipitating alternatively spliced variants of the gene (37). It is also reasonable to expect that MSI in a microsatellite such as *TSHR*, residing in an intron, occurs in concert with MSI in distant microsatellites that reside in exons. There are a host of tumor suppressor genes with coding microsatellites, where MSI produces frameshift mutations that knock out gene expression (33, 38) Much additional work is clearly needed to delineate the effects and spectrum of MSI in cancer to determine how MSI in noncoding microsatellites manifests a clinical phenotype.

In our study *THRA1*, *TSHR*, and *D2S123* distinguished themselves as being of special significance for an assessment of MSI in thyroid cancer. In the case of *THRA1*, the highest frequency of MSI was observed, and MSI in *THRA1* was also highly indicative of MSI being observed in other microsatellites. In the case of *TSHR* and *D2S123*, MSI proved to be correlated with important clinical parameters. For these reasons any assessment of MSI in thyroid cancer should ideally include *THRA1*, *TSHR*, and *D2S123*. From our data MSI appears to be an integral part of thyroid carcinogenesis as evidenced by the high frequency on MSI and the strong correlation to clinical and prognostic data we observe. As the link between MSI and thyroid cancer becomes clearer the genetics of thyroid cancer will also be brought into better focus. This, in turn, will aid in pinpointing genetic targets on which to center potent anticancer therapies.

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