Microsatellite Instability Occurs in a Subset of Follicular Thyroid Cancers

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Background: Inactivation of DNA mismatch repair (MMR) and the resulting microsatellite instability (MSI) are frequently observed in endometrial, stomach, and colorectal cancers, as well as more rarely in other solid tumor types. The prevalence of MSI in thyroid cancer has not been explored in depth, although recent studies utilizing data from large cancer sequencing efforts such as The Cancer Genome Atlas indicate that MSI is absent or at least very rare in the most common and most well studied histologic subtype, papillary thyroid carcinoma. This study aimed to determine the prevalence of MSI in thyroid cancer by using a large series comprising all major histological subtypes.

Methods: A total of 485 thyroid cancer patients were screened for MSI/MMR deficiency, including all major histologic subtypes (195 papillary thyroid carcinoma, 156 follicular thyroid carcinoma [FTC], 50 anaplastic thyroid carcinoma, 65 medullary thyroid carcinoma, and 17 poorly differentiated thyroid carcinomas) by using a combination of polymerase chain reaction–based detection, immunohistochemistry, and next-generation sequencing.

Results: A total of four tumors were MSI-high and had loss of MMR protein expression, all of which were from FTC patients. Whole-exome sequencing was performed on two MSI-high FTCs and revealed a hemizygous loss of function mutation in *MSH2* in one tumor.

Conclusions: Based on these data, it is estimated that the overall prevalence of MSI in FTC is 2.5%, and MSI is either entirely absent or rare in other histology subtypes of thyroid carcinoma. These findings highlight the importance of testing for MSI in FTC.

Keywords: microsatellite instability, follicular thyroid cancer, DNA mismatch repair, Lynch syndrome, papillary thyroid carcinoma

Introduction

THYROID CANCER IS the eighth most common malignancy in the United States, with an estimated 53,990 new cases in 2018 (1). Carcinomas arising from the follicular cells of the thyroid are the most common and include papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), which account for approximately 70–80% and 10–15% of cases, respectively. Less common histologic subtypes include medullary thyroid cancer (MTC), which makes up 5–10% of cases and develops from parafollicular cells, anaplastic thyroid cancer (ATC), a rare and more aggressive undifferenti-

ated subtype present in approximately 1% of cases, and poorly differentiated thyroid cancer (PDTC), which is also rare but distinct from ATC (2).

Microsatellite instability (MSI) is an accumulation of insertion or deletion mutations in repetitive DNA sequences due to underlying defects in DNA mismatch repair (MMR) (3). Tumors with MSI have a greatly increased overall mutational burden in addition to changes in microsatellite lengths (4). Importantly, due to this increased mutation load, dynamic mutational profiles, and continued renewal of tumor neoantigens, tumors that show MSI tumors respond well to anti programmed death-ligand 1 (PD-L1)

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immunotherapy, which has substantially improved patient outcomes in several cancers (5–8).

Defining MSI status by utilizing polymerase chain reaction (PCR) and capillary electrophoresis was standardized in 1997 with the Bethesda panel of five selected microsatellites: the mononucleotide repeats BAT25 and BAT26, and the dinucleotide repeats D17S250, D2S123, and D5S346 (9). Tumors must show instability in at least two of the five markers to be classified as MSI-high (MSI-H), whereas those that show instability for a single locus are considered MSI-low, and those with no instability are microsatellite stable (MSS) (10). More recently, it has become more common to detect MSI using next-generation sequencing (NGS) approaches (11). Several informatics tools are available to detect MSI status using targeted, whole-exome, and whole-genome sequencing data, including the authors' own MonoSeq variant caller and MANTIS tool (12,13). MSI status can also be inferred through immunohistochemistry (IHC)-mediated detection of expression of the MMR proteins MLH1, PMS2, MSH2, and MSH6 because MSI is caused by underlying defects in DNA MMR. Paired loss of expression of either MLH1 and PMS2 or MSH2 and MSH6 is typically observed in MSI-H tumors (4).

While MSI is widely reported in colorectal, endometrial, and stomach cancers, the prevalence of MSI in thyroid cancer appears to be low, but a large detailed study is missing (14,15). Previous investigations of MSI in thyroid cancer have almost exclusively been performed on PTC cases, neglecting less common histologic subtypes, and have reported disparate and conflicting results (Table 1). In this study, a comprehensive effort was undertaken to assess the prevalence of MSI in a large series of malignant thyroid tumors comprising all major histologic subtypes.

Methods

Patients

An initial sample set of 301 patients were recruited from a multidisciplinary thyroid tumor clinic at The Ohio State University Wexner Medical Center (OSUWMC), and all cases were histologically confirmed as either PTC, FTC, MTC, or ATC. All patients provided written informed consent, completed a questionnaire that included demographic, medical, and family history information, and donated a blood sample. Relevant clinicopathologic data for cases were extracted from the electronic medical records. All patients provided written informed consent, and the experimental protocols were approved by the Institutional Review Board at The Ohio State University. These samples were assessed for MSI using PCR-based detection and IHC of a tissue microarray (TMA).

A validation set of 188 tumor samples from 184 advanced thyroid carcinoma patients, defined as progressive recurrent and/or with distant metastases, were analyzed by targeted NGS at M.D. Anderson Cancer Center as part of patient care for planning additional therapies. Tumors with matched normal tissue or paired blood were analyzed, allowing for germline sequence variants to be excluded in the analysis. All patients provided written informed consent, and the experimental protocols and data sharing were approved by the Institutional Review Board at M.D. Anderson Cancer Center. These samples were assessed for MSI using NGS-based detection programs on targeted sequencing NGS data.

DNA extraction

The DNA samples from primary thyroid tumor tissue were snap frozen in liquid nitrogen after biopsy, digested with proteinase K, and extracted using phenol-chloroform methods. Twenty MTC cases were prepared using the same methods. For six ATC cases, formalin-fixed paraffin-embedded (FFPE) samples were deparaffinized using xylene, and DNA was extracted with phenol-chloroform methods. Thirty FTC samples were deparaffinized using xylene and extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). In the case of samples where adjacent normal thyroid tissue was unavailable, blood or mouthwash DNA was used. In the three cases that lacked adjacent normal thyroid tissue, skeletal muscle tissue was used for two cases and lymph node tissue for one.

PCR-based MSI testing

Marker-specific PCR primers for amplification of five microsatellites were used, with a fluorescein amidite (FAM) M13 universal forward primer, as previously described (16). Capillary electrophoresis was performed with an ABI PRISM[®] 3730 DNA Analyzer (Thermo Fischer Scientific, Waltham, MA) to detect strand-slippage mutations indicative of MSI. Malignant samples were compared with their germline DNA, a negative experimental control, and three MSI-H colorectal cancer control samples.

Whole-exome sequencing

Libraries were prepared using the KAPA Hyper Prep Kit (Roche, Basel, Switzerland) and were enriched using the xGEN[®] Exome Research Panel v1.0 (Integrated DNA Technologies, Coralville, IA). Sequencing was performed on a HiSeq4000 (Illumina, Inc., San Diego, CA) at The Genomics Services Laboratory at Nationwide Children's Hospital (Columbus, OH). Sequencing reads were aligned to hg19 using bwa v0.7.14 (17). Deduplication was performed using Picard v2.3.0 (18). Quality recalibration and local realignment around indels was performed with Picard and GATK v3.5 (19). Somatic single-nucleotide variations (SNVs), somatic indels, and germline SNVs were called using VarScan2 v2.3.9 (20).

TMA preparation

Thyroid resection specimens from 110 patients who were diagnosed with FTC were selected for the TMA. Hematoxylin and eosin slides of resection specimens were reviewed to select FFPE tissue blocks. All specimens included in this study contained a tumor surface area of >1 cm² and had multiple available tumor blocks. TMAs were performed using duplicate 1 mm diameter cores. IHC of MLH1, PMS2, MSH2, and MSH6 was evaluated for all specimens with control reactivity of selected normal thyroid tissue. MMR protein expression was considered intact if convincing nuclear staining was present in >1% of tumor cells. IHC was repeated on whole tissue sections from nine cases that showed weak or absent staining in the presence of inadequate available internal controls on TMA slides.

Targeted sequencing

Sequencing libraries were prepared using the OncoPrint v1 Assay, which amplifies 2530 target regions in 143 genes, and

TABLE 1. SUMMARY OF PUBLISHED INVESTIGATIONS OF MICROSATELLITE INSTABILITY IN THYROID CANCER

<i>Study</i>	<i>Subtype</i>	<i>No. of cases</i>	<i>% MSI-H</i>	<i>% MSI-L</i>
Santos <i>et al.</i> (25)	PTC	70	54	30
	FTC	12	75	17
	Benign thyroid neoplasia	14	0	43
Mitmaker <i>et al.</i> (53)	PTC	14	64	36
	FTC	16	62	38
Vaish <i>et al.</i> (54)	PTC	12	25	42
	FTC	9	33	44
	MTC	3	0	66
	PDTC	2	0	50
	Benign thyroid neoplasia	10	10	0
Vaish <i>et al.</i> (55)	Malignant thyroid tumors ^b	12	33 ^a	—
	Benign thyroid neoplasia	8	63 ^a	—
Bauer <i>et al.</i> (26)	PTC	16	0	0
Onda <i>et al.</i> (56)	Malignant thyroid tumors ^b	76	37 ^a	—
Dobosz <i>et al.</i> (57)	FTC	12	17 ^a	—
	Multinodular thyroid hyperplasia	17	18 ^a	—
Richter <i>et al.</i> (58)	PTC	112	8 ^a	—
	FTC	5	0 ^a	—
	FTA	2	0 ^a	—
	MTC	2	50 ^a	—
	Undefined thyroid carcinoma	8	13 ^a	—
Yamamoto <i>et al.</i> (59)	PTC	25	8	0
	FTC	1	0	0
Lazzereschi <i>et al.</i> (60)	PTC	29	3	17
	FTC	4	50	0
	FTA	12	8	0
	ATC	2	50	0
	Follicular nodule	3	0	33
Nikiforov <i>et al.</i> (27)	PTC	37	0	3
Schäffler <i>et al.</i> (28)	PTC	23	0	0
	FTC	3	0	0
	MTC	1	0	0
Soares <i>et al.</i> (61)	PTC	12	8	8
	FTC	4	0	25
	FTA	15	7	7
	Nodular goiters	13	8	8
Vermiglio <i>et al.</i> (29)	DTC	8	0	0
	ATC	1	0	0

^aStudy did not distinguish between MSI-H and MSI-L.

^bHistology not specified.

ATC, anaplastic thyroid carcinoma; CE, capillary electrophoresis; DTC, differentiated thyroid carcinoma; FTA, follicular thyroid adenoma; FTC, follicular thyroid carcinoma; IHC, immunohistochemistry; MSI-H, microsatellite instability high; MSI-L, microsatellite instability low; MTC, medullary thyroid carcinoma; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PDTC, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma.

sequenced on an Ion Torrent platform. In brief, 20 ng of DNA extracted from FFPE or fine-needle aspirate tumor samples and matched normal controls was utilized per assay. Library preparation and subsequent analytical steps were completed, as previously described (21,22). Microsatellites contained in the Oncomine panel were identified and examined for strand-slippage mutations using MonoSeq, as previously described (23). MSI was determined for each microsatellite in each sample by comparing the percentages of reads containing strand-slippage mutations between paired tumor and normal DNAs.

Results

A set of 191 thyroid cancer tumor samples from 122 PTC, 35 FTC, 20 MTC, and 14 ATC patients was screened for MSI using PCR followed by capillary electrophoresis of four

microsatellites from the Bethesda panel used for MSI detection (BAT25, BAT26, D17S250, and D2S123). Two out of 35 FTC samples showed clear instability at multiple microsatellites (Fig. 1A, Supplementary Fig. S1, and Supplementary Table S1). None of the PTC, MTC, or ATC tumor samples displayed MSI, although two ATC samples had single base pair insertions or deletion mutations for BAT26 (Supplementary Table S1). Loss of MMR protein expression was validated in the two MSI-H FTC cases by IHC. Tumor FTC-19 showed loss of expression of MSH2 and MSH6, while the staining of FTC-34 for MLH1 and PMS2 was equivocal (Fig. 1B and Supplementary Fig. S2).

To investigate underlying mutations in *MMR* genes and to validate MSI in these cases orthogonally, whole-exome sequencing was performed. FTC-34 was confirmed to be MSI-H by both the MANTIS tool and MSISensor (24), whereas FTC-

FIG. 1. (A) An example of a capillary electrophoretogram for the D2S123 dinucleotide marker showing microsatellite instability (MSI) for sample FTC-19. (B) Microphotographs of immunohistochemistry for the indicated DNA mismatch repair (MMR) proteins on sample FTC-19. MSH2 and MSH6 (brown) were absent in the nuclei (blue) of the tumor sample, and present in the nuclei of the adjacent normal tissue. Scale bar = 100 μm; insert is an additional 4× zoom. (C) Integrated genomics viewer screenshot of whole-exome sequencing data performed on paired tumor and normal DNA from FTC-19 shows a nonsense mutation in *MSH2* (NM_000251:c.1035G>A, variant allele fraction = 0.76). Color images are available online.

19 was not sequenced to adequate depth for evaluation with these tools (Supplementary Table S2). In FTC-19, a nonsense mutation was detected in *MSH2* (NM_000251:c.1035G>A, p.W345*) with a variant allele fraction (VAF) of 0.76, which is indicative of loss of heterozygosity. As the corresponding adjacent normal tissue did not harbor the *MSH2* mutation, Lynch syndrome can be ruled out (Fig. 1C). In the other MSI-H case, FTC-34 did not have any loss-of-function mutations in the *MMR* genes, and it is speculated that MSI is a result of epigenetic silencing of *MLH1*.

To determine the overall frequency of MSI in FTC, an additional 110 FTC tumor samples from a different cohort of patients were assembled into a TMA, and IHC microscopy for the four MMR proteins was performed. Two of 110 cases were MMR deficient, with both lacking expression of MSH2 and MSH6 (Supplementary Fig. S3 and Supplementary Table S3).

Finally, as a validation of these experiments, the study sought to use available targeted NGS data from an additional 188 thyroid carcinomas (74 PTC, 45 MTC, 37 ATC, 18 PDTC, 11 FTC, and 3 Hürthle cell carcinomas) to determine MSI status in these tumors using the MonoSeq variant caller. Since previous studies using MonoSeq to investigate MSI status relied on data generated from Illumina sequencers (12,23) and the thyroid carcinoma NGS data were generated using an Ion Torrent platform, first the accuracy of MonoSeq to determine MSI status of samples sequenced with an Ion Torrent correctly was examined. MonoSeq was used to determine the MSI likelihood scores for each of 19 colorectal cancers of known MSI status (9 MSI-H and 10 MSS), and there was a clear separation of the two groups (Supplementary Fig. S4A). Therefore, MonoSeq was applied to the thyroid carcinoma samples, and 10 outliers were identified that were the most likely to be MSI-H (3 PTC, 3 FTC, 2 ATC, 1 PDTC, and 1 MTC; Supplementary Fig. S4B). Five of these cases had available paired tumor and normal DNA, and PCR-based MSI detection was performed using the five Bethesda panel microsatellites. None of these cases showed MSI for any of the assessed markers (Supplementary Table S4), making it unlikely that any of the samples in this cohort were MSI-H.

Discussion

This study found that 2.5% (4/156) of FTC cases displayed MSI. MSI was not detected in the 327 thyroid cancers of other histologies. The MSI-H FTC cases were evaluated for expression of MMR proteins using IHC, and three were deficient in MSH2/MSH6, while the remaining case showed equivocal expression of MLH1/PMS2 (Supplementary Figs. S2 and S3). Additionally, one of these cases harbored a somatic hemizygous loss-of-function mutation in *MSH2* (Fig. 1).

Previous investigations of MSI in thyroid cancer have produced controversial results (Table 1). One study reported MSI is present in >80% of cases (25), whereas others have reported it to be entirely absent (26–29). It is speculated that the elevated MSI rates in many of the early investigations can be attributed to frequent false-positives from the use of polyacrylamide gels for determination of microsatellite lengths, which can confound accurate determination of MSI status. In a report where the more accurate method of measuring microsatellite lengths using capillary electrophoresis was employed, MSI was found to be completely absent in thyroid cancer (26). More recently, investigations have used NGS data to determine the rate of MSI. Those that were limited to PTC showed a complete absence (14,30), whereas a report by Le *et al.* including other thyroid cancer histologies showed approximately 2% of cases were MSI-H (5). The present study highlights the importance of examining molecular phenotypes in uncommon subtypes of cancers that are not represented well in large data sets.

It is not unexpected that MSI was found in FTC but not in PTC, since the genetic events that are typically found in these two histologic subtypes are distinct (31,32). The most commonly mutated gene in PTC is *BRAF*, which is mutated in about 45% of tumors, but *BRAF* mutations are almost never seen in FTC patients (33–38). In addition, FTCs often display aneuploidy, which is rare in PTCs, and have a higher mutational burden compared to PTCs (38,39). Although both FTC and PTC patients typically have favorable prognoses (40), a

small subset of patients display widely invasive disease, and for these patients, the five-year survival rate drops to 38% (41).

In cancer types that show high rates of MSI, such as endometrial and colorectal cancer, MMR deficiency is widely regarded as an early event in carcinogenesis (42–48). In contrast, whole-exome sequencing data in this study from FTC-19 showed that the somatic, hemizygous loss of function mutation in *MSH2* had a VAF of 0.76, which is consistent with a subclonal mutation that occurred late in tumor development (a fully clonal hemizygous mutation would have a VAF of 1). Further supporting the concept that MMR inactivation is a late event and does not contribute to carcinogenesis are two independent reports of Lynch syndrome patients who presented with thyroid carcinomas that were not MSI-H (49,50). Additionally, in the study by Le *et al.*, who reported ~2% of thyroid carcinomas were MSI-H, all of the MSI-H thyroid cancers were late stage (5). It was previously reported that MSI is absent in diagnostic samples of acute myeloid leukemia (AML) (23). However, MSI has been found in smaller, subclonal populations of AML, indicating it can occur as a late event in cancer evolution (51).

Tumor MSI status has become more clinically relevant because of the remarkable response of patients with MSI-H cancers to treatment with programmed cell death protein 1/PD-L1 immune checkpoint inhibitors such as nivolumab and pembrolizumab. In one report, an FTC patient who presented with MSI-H disease and a somatic frameshift mutation in *MSH2* was treated with pembrolizumab and had an ongoing partial response (52).

In conclusion, the present results indicate that at least a small percentage of FTC patients have MSI/MMR defects. This suggests that in some instances, clinical MSI testing and potential use of checkpoint inhibitor therapy should not be overlooked for FTC patients.

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
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Supplementary Table S4

References

1. Siegel RL, Miller KD, Jemal A 2018 Cancer statistics, 2018. *CA Cancer J Clin* **68**:7–30.
2. Fagin JA, Wells SA 2016 Biologic and clinical perspectives on thyroid cancer. *N Engl J Med* **375**:1054–1067.
3. Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P 1993 Hypermutability and mismatch repair deficiency in RER+ tumor-cells. *Cell* **75**:1227–1236.
4. de la Chapelle A, Hampel H 2010 Clinical relevance of microsatellite instability in colorectal cancer. *J Clin Oncol* **28**:3380–3387.
5. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Lubner BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Gretchen TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA 2017 Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**:409–413.
6. Herbst RS, Baas P, Kim D-W, Felip E, Pérez-Gracia JL, Han J-Y, Molina J, Kim J-H, Arvis CD, Ahn M-J, Majem M, Fidler MJ, de Castro G, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB 2016 Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* **387**:1540–1550.
7. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Lubner BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA 2015 PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* **372**:2509–2520.
8. Hamid O, Robert C, Daud A, Hodi FS, Hwu W-J, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Ellassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumei PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A 2013 Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *New Engl J Med* **369**:134–144.
9. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschhoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomäki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HFA, Hawk ET, Barrett JC, Freedman AN, Srivastava S 2004 Revised Bethesda Guidelines for hereditary non-polyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* **96**:261–268.
10. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S 1998 A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* **58**:5248–5257.

11. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC 2014 Microsatellite instability detection by next generation sequencing. *Clin Chem* **60**:1192–1199.
12. Walker CJ, Miranda MA, O'Hern MJ, Blachly JS, Moyer CL, Ivanovich J, Kroll KW, Eisfeld AK, Sapp CE, Mutch DG, Cohn DE, Bundschuh R, Goodfellow PJ 2016 MonoSeq variant caller reveals novel mononucleotide run indel mutations in tumors with defective DNA mismatch repair. *Hum Mutat* **37**:1004–1012.
13. Kautto EA, Bonneville R, Miya J, Yu L, Krook MA, Reeser JW, Roychowdhury S 2017 Performance evaluation for rapid detection of pan-cancer microsatellite instability with MANTIS. *Oncotarget* **8**:7452–7463.
14. Hause RJ, Pritchard CC, Shendure J, Salipante SJ 2016 Classification and characterization of microsatellite instability across 18 cancer types. *Nat Med* **22**:1342–1350.
15. Kim TM, Laird PW, Park PJ 2013 The landscape of microsatellite instability in colorectal and endometrial cancer genomes. *Cell* **155**:858–868.
16. Schuelke M 2000 An economic method for the fluorescent labeling of PCR fragments. *Nat Biotechnol* **18**:233–234.
17. Li H, Durbin R 2009 Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**:1754–1760.
18. Picard Tools—By Broad Institute. Available at <https://broadinstitute.github.io/picard/> (accessed February 17, 2017).
19. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA 2010 The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* **20**:1297–1303.
20. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L, Wilson RK 2012 VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res* **22**:568–576.
21. Hovelson DH, McDaniel AS, Cani AK, Johnson B, Rhodes K, Williams PD, Bandla S, Bien G, Choppa P, Hyland F, Gottimukkala R, Liu G, Manivannan M, Schageman J, Ballesteros-Villagrana E, Grasso CS, Quist MJ, Yadati V, Amin A, Siddiqui J, Betz BL, Knudsen KE, Cooney KA, Feng FY, Roh MH, Nelson PS, Liu C-J, Beer DG, Wynngaard P, Chinnaiyan AM, Sadis S, Rhodes DR, Tomlins SA 2015 Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia* **17**:385–399.
22. Luthra R, Patel KP, Routbort MJ, Broaddus RR, Yau J, Simien C, Chen W, Hatfield DZ, Medeiros LJ, Singh RR 2017 A targeted high-throughput next-generation sequencing panel for clinical screening of mutations, gene amplifications, and fusions in solid tumors. *J Mol Diagn* **19**:255–264.
23. Walker CJ, Eisfeld A-K, Genutis LK, Bainazar M, Kohlschmidt J, Mrózek K, Carroll AJ, Kolitz JE, Powell BL, Wang ES, Stone RM, Bundschuh R, de la Chapelle A, Bloomfield CD 2017 No evidence for microsatellite instability in acute myeloid leukemia. *Leukemia* **31**:1474–1476.
24. Niu B, Ye K, Zhang Q, Lu C, Xie M, McLellan MD, Wendl MC, Ding L 2014 MSIsensor: Microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* **30**:1015–1016.
25. Santos JC, Bastos AU, Cerutti JM, Ribeiro ML 2013 Correlation of MLH1 and MGMT expression and promoter methylation with genomic instability in patients with thyroid carcinoma. *BMC Cancer* **13**:1–7.
26. Bauer AJ, Cavalli LR, Rone JD, Francis GL, Burch HB, Tuttle RM, Ringel MD, Stratakis CA, Haddad BR 2002 Evaluation of adult papillary thyroid carcinomas by comparative genomic hybridization and microsatellite instability analysis. *Cancer Genet Cytogenet* **135**:182–186.
27. Nikiforov YE, Nikiforova M, Fagin JA 1998 Prevalence of minisatellite and microsatellite instability in radiation-induced post-Chernobyl pediatric thyroid carcinomas. *Oncogene* **17**:1983–1988.
28. Schäffler A, Palitzsch KD, Seiffarth C, Höhne HM, Riedhammer FJ, Hofstädter F, Schölmerich J, Rüschhoff J 1998 Coexistent thyroiditis is associated with lower tumour stage in thyroid carcinoma. *Eur J Clin Invest* **28**:838–844.
29. Vermiglio F, Schlumberger M, Lazar V, Lefrère I, Bressac B 1995 Absence of microsatellite instability in thyroid carcinomas. *Eur J Cancer Oxf Engl* **31A**:128.
30. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen H-Z, Reeser JW, Yu L, Roychowdhury S 2017 Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol* **2017**.
31. Lui W-O, Foukakis T, Lidén J, Thoppe SR, Dwight T, Höög A, Zedenius J, Wallin G, Reimers M, Larsson C 2005 Expression profiling reveals a distinct transcription signature in follicular thyroid carcinomas with a *PAX8-PPARγ* fusion oncogene. *Oncogene* **24**:1467–1476.
32. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW, Tallini G, Kroll TG, Nikiforov YE 2003 *RAS* point mutations and *PAX8-PPARγ* rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab* **88**:2318–2326.
33. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggs GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JWC, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA 2002 Mutations of the *BRAF* gene in human cancer. *Nature* **417**:949–954.
34. Soares P, Trovisco V, Rocha A, Feijão T, Rebocho A, Fonseca E, Vieira de Castro I, Cameselle-Teijeiro J, Cardoso-Oliveira M, Sobrinho-Simões M 2004 *BRAF* mutations typical of papillary thyroid carcinoma are more frequently detected in undifferentiated than in insular and insular-like poorly differentiated carcinomas. *Virchows Arch* **444**:572–576.
35. Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, Ohtsuru A, Saenko VA, Kanematsu T, Yamashita S 2003 Clinical implication of hot spot *BRAF* mutation, V599E, in papillary thyroid cancers. *J Clin Endocrinol Metab* **88**:4393–4397.
36. Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA, Nikiforov YE 2003 *BRAF* mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *J Clin Endocrinol Metab* **88**:5399–5404.
37. Xing M 2005 *BRAF* mutation in thyroid cancer. *Endocr Relat Cancer* **12**:245–262.

38. Sobrinho-Simões M, Eloy C, Magalhães J, Lobo C, Amaro T 2011 Follicular thyroid carcinoma. *Mod Pathol* **24**:S10–S18.
39. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, Carter SL, Stewart C, Mermel CH, Roberts SA, Kiezun A, Hammerman PS, McKenna A, Drier Y, Zou L, Ramos AH, Pugh TJ, Stransky N, Helman E, Kim J, Sougnez C, Ambrogio L, Nickerson E, Shefler E, Cortés ML, Auclair D, Saksena G, Voet D, Noble M, Dicara D, Lin P, Lichtenstein L, Heiman DI, Fennell T, Imielinski M, Hernandez B, Hodis E, Baca S, Dulak AM, Lohr J, Landau DA, Wu CJ, Melendez-Zajgla J, Hidalgo-Miranda A, Koren A, McCarroll SA, Mora J, Lee RS, Crompton B, Onofrio R, Parkin M, Winckler W, Ardlie K, Gabriel SB, Roberts CWM, Biegel JA, Stegmaier K, Bass AJ, Garraway LA, Meyerson M, Golub TR, Gordenin DA, Sunyaev S, Lander ES, Getz G 2013 Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **499**:214–218.
40. Pstrąg N, Ziemnicka K, Bluysen H, Wesoły J 2018 Thyroid cancers of follicular origin in a genomic light: in-depth overview of common and unique molecular marker candidates. *Mol Cancer* **17**:116.
41. D'Avanzo A, Treseler P, Ituarte PHG, Wong M, Streja L, Greenspan FS, Siperstein AE, Duh Q-Y, Clark OH 2004 Follicular thyroid carcinoma: histology and prognosis. *Cancer* **100**:1123–1129.
42. Tibiletti MG, Furlan D, Taborelli M, Facco C, Riva C, Franchi M, Cossu A, Trubia M, Taramelli R, Capella C 1999 Microsatellite instability in endometrial cancer: relation to histological subtypes. *Gynecol Oncol* **73**:247–252.
43. Duggan BD, Felix JC, Munderspach LI, Tourgeman D, Zheng J, Shibata D 1994 Microsatellite instability in sporadic endometrial carcinoma. *J Natl Cancer Inst* **86**:1216–1221.
44. Mutter GL, Boynton KA, Faquin WC, Ruiz RE, Jovanovic AS 1996 Allelotype mapping of unstable microsatellites establishes direct lineage continuity between endometrial precancers and cancer. *Cancer Res* **56**:4483–4486.
45. Heinen CD, Shivapurkar N, Tang Z, Groden J, Alabaster O 1996 Microsatellite instability in aberrant crypt foci from human colons. *Cancer Res* **56**:5339–5341.
46. Beggs AD, Domingo E, Abulafi M, Hodgson SV, Tomlinson IPM 2013 A study of genomic instability in early preneoplastic colonic lesions. *Oncogene* **32**:5333–5337.
47. Giraldez MD, Balaguer F, Bujanda L, Cuatrecasas M, Munoz J, Alonso-Espinaco V, Larzabal M, Petit A, Gonzalez V, Ocana T, Moreira L, Enriquez-Navascues JM, Boland CR, Goel A, Castells A, Castellvi-Bel S 2010 MSH6 and MUTYH deficiency is a frequent event in early-onset colorectal cancer. *Clin Cancer Res* **16**:5402–5413.
48. Bacher JW, Sievers CK, Albrecht DM, Grimes IC, Weiss JM, Matkowskyj KA, Agni RM, Vyazunova I, Clipson L, Storts DR, Thliveris AT, Halberg RB 2015 Improved detection of microsatellite instability in early colorectal lesions. *PLoS One* **10**:e0132727.
49. Stulp RP, Herkert JC, Karrenbeld A, Mol B, Vos YJ, Simons RH 2008 Thyroid cancer in a patient with a germline MSH2 mutation. Case report and review of the Lynch syndrome expanding tumour spectrum. *Hered Cancer Clin Pract* **6**:15–21.
50. Broaddus RR, Lynch PM, Lu KH, Luthra R, Michelson SJ 2004 Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. *Mod Pathol* **17**:981–989.
51. Shlush LI, Chapal-Ilani N, Adar R, Pery N, Maruvka Y, Spiro A, Shouval R, Rowe JM, Tzukerman M, Bercovich D, Izraeli S, Marcucci G, Bloomfield CD, Zuckerman T, Skorecki K, Shapiro E 2012 Cell lineage analysis of acute leukemia relapse uncovers the role of replication-rate heterogeneity and microsatellite instability. *Blood* **120**:603–612.
52. Hale-Wallace N, Deshpande HA, Holt EH, Callender GG, Gibson C, Lee G, Carling T, Ho AL, Eder JP, Finberg KE 2018 Genetic variants including microsatellite instability in metastatic Hürthle cell carcinoma of the thyroid. *J Clin Oncol* **36**:e18110 (abstract).
53. Mitmaker E, Alvarado C, Bégin LR, Trifiro M 2008 Microsatellite instability in benign and malignant thyroid neoplasms. *J Surg Res* **150**:40–48.
54. Vaish M, Mishra A, Kaushal M, Mishra SK, Mittal B 2004 Microsatellite instability and its correlation with clinicopathological features in a series of thyroid tumors prevalent in iodine deficient areas. *Exp Mol Med* **36**:122–129.
55. Vaish M, Mishra SK, Mandhani A, Mittal RD, Mittal B 2003 Assessment of microsatellite instability in bladder and thyroid malignancies. *Teratog Carcinog Mutagen* **255**–265.
56. Onda M, Nakamura I, Suzuki S, Takenoshita S, Brogren CH, Stampanoni S, Li D, Rampino N 2001 Microsatellite instability in thyroid cancer: hot spots, clinicopathological implications, and prognostic significance. *Clin Cancer Res* **7**:3444–3449.
57. Dobosz T, Lukieniczuk T, Sasiadek M, Kuczyńska A, Janowska E, Blin N 2000 Microsatellite instability in thyroid papillary carcinoma and multinodular hyperplasia. *Oncology* **58**:305–310.
58. Richter HE, Lohrer HD, Hieber L, Kellerer AM, Lengfelder E, Bauchinger M 1999 Microsatellite instability and loss of heterozygosity in radiation-associated thyroid carcinomas of Belarussian children and adults. *Carcinogenesis* **20**:2247–2252.
59. Yamamoto T 1999 Infrequent microsatellite instability in papillary carcinomas of the thyroid. *Hiroshima J Med Sci* **48**:95–98.
60. Lazzereschi D, Palmirotta R, Ranieri A, Ottini L, Veri MC, Cama A, Cetta F, Nardi F, Colletta G, Mariani-Costantini R 1999 Microsatellite instability in thyroid tumours and tumour-like lesions. *Br J Cancer* **79**:340–345.
61. Soares P, Dos Santos NR, Seruca R, Lothe RA, Sobrinho-Simões M 1997 Benign and malignant thyroid lesions show instability at microsatellite loci. *Eur J Cancer* **33**:293–296.

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