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Case report: Whole exome sequencing of circulating cell-free tumor DNA in a follicular thyroid carcinoma patient with lung and bone metastases

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Abstract

Metastatic follicular thyroid carcinoma (FTC), unresectable or resistance to radioactive iodine, is associated with poor survival. It is believed that this kind of FTC is driven by mutated genes. However, what kind of changes of genome and underlying mechanisms are elusive. The aim of this article is to understand whether there are somatic mutations in circulating cell-free tumor DNA (cfDNA) in a FTC patient with lung and bone metastases. A 55-year-old woman was diagnosed with FTC with bone and lung metastases. Appropriate amounts of DNA were extracted from formalin-fixed, paraffin-embedded thyroid tumor, peripheral cell-free plasma, and peripheral blood leukocytes and then sequenced. The significance of DNA sequencing was evaluated. There were 13,519 common variants in both tissue DNA and cfDNA. Fifty-five somatic mutations were identified in tumor, with 5 of them nonsynonymous. Seventy-two somatic mutations were found in cfDNA, with 2 of them causing amino acid change. Sixteen common alterations existed in both samples, that is, 31.3% of all the tissue somatic mutations. This pilot study provided proof that cfDNA represents the genomic characteristics of FTC primary tissue DNA well, but also metastatic tumors. Further studies are needed to better prove the effectiveness of cfDNA in the field of thyroid cancer metastatic mechanism research and real-time monitoring.

Keywords

Follicular thyroid carcinoma, metastasis, circulating cell-free DNA, whole exome sequencing

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Introduction

Most follicular thyroid carcinomas (FTCs) grow slowly after they are first identified at an early stage. It is frequently cured with adequate surgical management and radioactive iodine ablation therapy. However, few cases develop fast with lung and bone metastases. Most metastatic FTCs are unresectable and refractory to radioactive iodine and associated with poor survival.[1] Analysis of carcinogenesis has revealed that gene mutations play a very important role in the progression and development of tumors. The genetic profile of solid tumors is often obtained from surgical or biopsy specimens. However, tumor samples may not be obtained due to the confirmation of metastasis. In that situation, blood samples or biopsy can be used for analysis. Several studies of circulating cell-free DNA (cfDNA) in plasma have been used for analyzing individual loci, genes, or structural variants to quantify tumor burden and detect previously characterized resistance-conferring mutations.[2–4] Individual mutations of cfDNA and SLC5A8 and SLC26A4 hypermethylation

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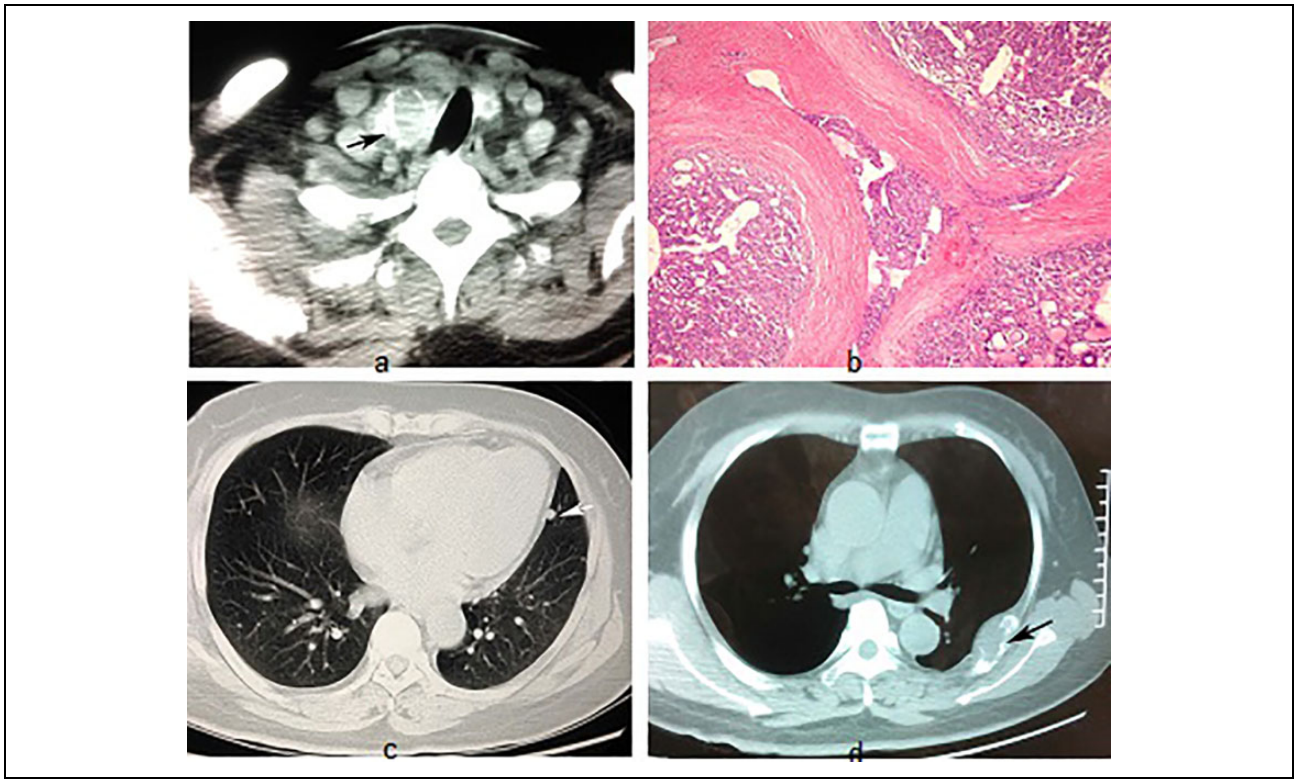

Figure 1. Imaging of primary and metastasis lesions and pathological examination of primary thyroid lesion. (a) Imaging CT of primary thyroid lesion. Black arrow indicates tumor location. (b) Histological analysis of primary lesion of FTC (×100). (c) Lung metastasis as indicated by white arrow. (d) Rib metastasis as indicated by black arrow. CT: computed tomography; FTC: follicular thyroid carcinoma.

of thyroid carcinoma including follicular carcinoma have been reported.[5] Given that sequencing of entire genes to detect FTC mutations in circulating DNA has not been demonstrated, we report a somatic mutational analysis of cfDNA in a FTC patient with lung and bone metastases by whole exome sequencing (WES).

The results demonstrate that the detection of cfDNA may reflect the mutation of FTC and further develop to be a better way for monitoring the progression of thyroid cancer.

Patient and methods

Patient

A 55-year-old woman was diagnosed with FTC with synchronous bone and lung metastases and then performed total thyroidectomy at Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Figure 1). The informed consent for DNA sequencing was obtained from the patient. Preoperative peripheral cell-free plasma and peripheral blood leukocytes and postoperative formalin-fixed, paraffin-embedded (FFPE) thyroid tumor of this patient were obtained. Procedure and protocol were reviewed and approved by the ethics committee of the Sixth People's Hospital.

DNA extraction

Three different types of DNA were obtained from this present patient, including peripheral blood leukocytes, FFPE thyroid tumor tissue, and cfDNA. Total amount of DNA were extracted according to the manufacturer's instructions using QIAamp DNA Blood Mini Kit (cat# 51106), QIAamp DNA FFPE Tissue Kit (cat# 56404), and cfDNA QIAamp circulating Nucleic Acid Kit (cat# 5514), respectively. DNA concentrations were 81 ng/μl, 85 ng/μl, and 81 ng/μl, respectively, and the optical density 260/280 is 1.86, 1.87, and 1.89, respectively. DNA samples were then subjected to Agilent 2100 for more accurate tests. All three DNA samples passed a strict quality test.

Whole exome sequencing

The three different samples above were proceeded with WES. Briefly, first, DNA libraries were established with Agilent SureSelect Human All ExonV5 kits, covering about 23,000 genes. After the quality test, qualified libraries were sequenced as 100-bp paired-end reads on Illumina HiSeq 2000 platform (Illumina, San Diego, California, USA) according to the manufacturer's instruction. All of the experiments were carried out in Zhangjiang Center for Translational Medicine, Shanghai, China.

Table 1. Outline of the WES of cfDNA, tumor DNA, and peripheral blood DNA.

	Peripheral blood DNA	Tumor DNA	cfDNA
Total effective sequence data (Gb)	11.0	10.0	11.2
Coverage rate on targeted region	99.76	99.67	99.8
Mean depth	140.04	139.59	133.21
No. of SNVs	41,410	41,874	41,206
No. of Indels	3259	3490	3486

cfDNA: cell-free DNA; WES: whole exome sequencing.

Data analysis

Clean data were achieved using FastQC and low quality reads were filtered. Burrows–Wheeler Alignment (0.7.12) methods were adopted to map the clean reads to reference genome (UCSC hg19). Then, Picard (http://picard.sourceforge.net/) and Genome Analysis Toolkit (GATK) methods were used for duplicate removal, local realignment, and base quality recalibration. GATK unified genotyper was used for variants calling.

ANNOVAR (2015-03-22) software was used to annotate variants for function (exonic, TR, intronic) reference gene, exonic function (synonymous, nonsynonymous, frameshift, stopgain, unknown), amino acid change, allele frequency (AF) 1000 Genomes Project, and dbSNP reference number.

Somatic mutations were analyzed by subtracting the variants of the peripheral blood DNA from the tumor DNA or cfDNA. *T*-test was used to compare the AF between different types of samples. Pearson correlation coefficient was adopted to calculate the relationship between different types of samples.

Results

Exome sequencing, sequence alignment, and variant calling

In this study, we totally sequenced three different types of DNAs from one patient. More than 10 Gb sequencing data were generated per sample. The average sequencing depth for peripheral blood DNA, tissue DNA, and cfDNA is 139×, 140×, and 133×, respectively. For three samples, more than 98.7% of the exome was covered at least 10×. The coverage rate for the above three samples was larger than 99.6%. More details are shown in Table 1. The mutation types in different samples were nearly the same.

Variations identified in different samples

We compared variations (both SNV and Indel) in different DNA samples. Generally, a large concordance between the three types of samples was observed (Figure 2(a)). There were 13,519 common variants in both tissue DNA and cfDNA. They shared more than 98.6% common variants compared with peripheral blood sample. The coefficient rate was 0.95 (p value $< 10^{-15}$) and 0.96 (p value $< 10^{-15}$), respectively; 1.39% of variants identified in tissue DNA were not found in peripheral blood and 1.13% of variants identified in cfDNA did not exist in peripheral blood. Comparing tissue DNA with cfDNA showed that 99.2% variants were identical (Figure 2(b) to (d)). The Pearson coefficient between cfDNA and tissue DNA is 0.95 (Figure 2(e)).

Somatic mutations were determined by subtracting the variants of the peripheral blood leukocytes DNA from the tumor DNA or cfDNA. Fifty-five somatic mutations were found in tissue DNA, in which 5 mutations were nonsynonymous. Seventy-two somatic mutations were found in cfDNA, in which 2 of them changed amino acid. Sixteen common alterations existed in both samples, that is, 31.3% in all the tissue somatic mutations (Table 2). Most of the common mutations were synonymous. In cfDNA,only GLUD2 (c.G103A: p.G35R) and HLA-B (c.A652G: p.I218 V) were nonsynonymous mutations, of which GLUD2 (c.G103A: p.G35R) was also found in the tumor DNA.

Discussion

Genome-wide sequencing of plasma DNA was first used in prenatal diagnostics, demonstrating comprehensive genome characteristics.[6] Recent studies showed that sequencing of circulating cfDNA from plasma is a potential tool for monitoring advanced cancer. Compared with traditional biopsy, cfDNA sequencing is noninvasive, easy to get samples repeatedly, and to large extent reflects the comprehensive genomic characteristic of the tumor progress.[2–4,7] In our data, cfDNA from FTC was for the first time sequenced together with primary tumor in the advanced thyroid cancer. The results demonstrate that SNV and mutation rate of cfDNA are characterized by the features of FTC, and both tissue DNA and cfDNA shared more than 95% common alterations. It will be helpful for doctors or researchers to use cfDNA as a new method for the diagnosis and monitoring of thyroid carcinoma.

Recent studies showed that cancers arise through a process of somatic evolution that can result in substantial subclonal heterogeneity within tumors, and a large fraction of polyclonal tumors and a larger sub-clonal mutation fraction may be associated with relapse and metastasis.[8,9] It has been established that there were different mutation variants between primary and metastatic tumors. In our results, there were different variants identified in tissue DNA and cfDNA except for common alterations. One of the reasons may be the existence of distinct mutational genes of bone and lung metastatic tumors in cfDNA.[7] In addition, the detection of genomic alteration by sequencing of cfDNA is to some extent dependent on the AF of the mutant alleles in the tissue DNA. AF value of variants which existed in both cfDNA and tissue DNA is significantly larger than that of variants only

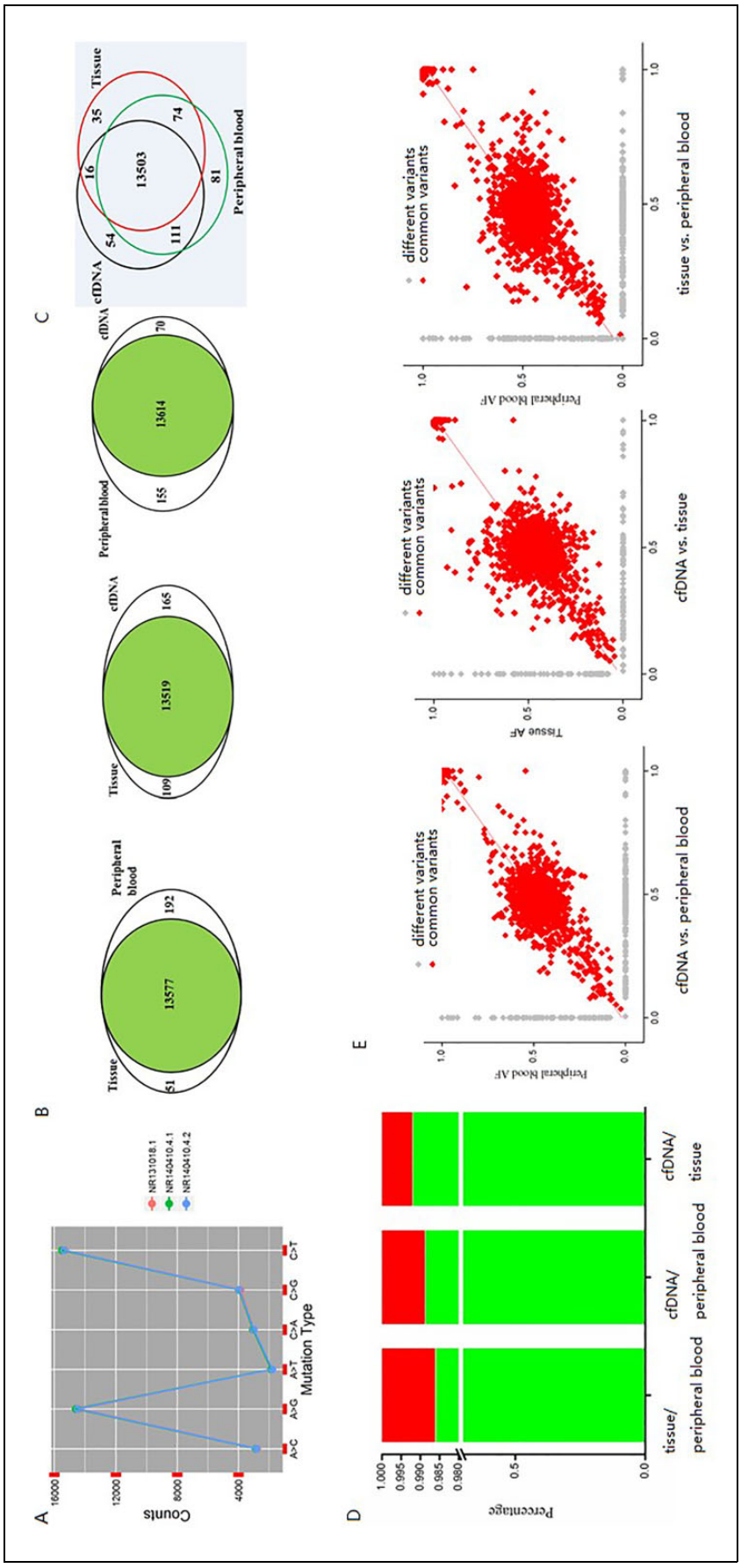

Figure 2. Relationship between the three types of DNA samples. (a) Mutation types of three different DNA samples; (b) and (c) common variants are shown by venny diagram; (d) percentages of common or different variants shown in histogram (green, common variants; red, different variants); (e) correlation among variants in different samples.

Table 2. List of genes with somatic mutations in tumor and cfDNA.

Tissue somatic mutation genes		cfDNA somatic mutation genes		Intersection (31%)
FAM69A	UBE2L3	TTC39A	CILP2	FAM69A
RPL5	TFAP2E	FAM69A	ZNF431	RPL5
ANKRD45	FBXO28	RPL5	DEFB124	ANKRD45
SLC9A2	RHOA	TMEM56	LOC101927631	RAPGEF4-AS1
RAPGEF4-AS1	LOC101243545	ANKRD45	UBE2L3	EOMES
ATF2	TAPT1-AS1	NUCKS1	FAM9B	TWF2
EOMES	BRD9	DIEXF	SH3KBP1	NCALD
TWF2	NEUROD6	CNRIP1	EFNB1	MPV17L
RUVBL1	TMEM229A	ERMN	PJA1	UBE2L3
TM4SF19-AS1	Unc5D	RAPGEF4-AS1	MOSPD1	Unc5D
MARCH6	DPY19L4	EOMES	MIR4424	FJX1
KCTD20	GLIPR2	TWF2	MAP6D1	SNX20
TMEM248	HRAS	GOLIM4	HLA-B	CDRT15L2
DNAJC2	FJX1	HES1	HLA-DRB1	LOC100287072
NCALD	FAM86C1	PDCL2	PPIA	EID2
HAS2-AS1	RCOR1	ANXA5	NCF1C	GLUD2
RAB1B	PDCD7	CPE	Unc5D	
CXCR5	CHRNA5	NUDCD2	FAM35BP	
LNX2	SNX20	CASC15	FAM35DP	
CTAGE10P	ALOX15P1	EPB41L2	FJX1	
EMC7	CDRT15L2	FZD1	LOC338797	
SNN	LOC100287072	XPO7	SLITRK1	
MPV17L	VAT1	NCALD	MIR4511	
DCXR	EID2	MSANTD3-TMEFF1	IDH3A	
RWDD2B	NCOA3	TMEFF1	GNPTG	
GLUD2		CSGALNACT2	SNX20	
		AMBRA1	PAFAH1B1	
		NAALAD2	CDRT15L2	
		FGFR1OP2	LOC100287072	
		MICU2	CERS1	
		MEIS2	GDF1	
		MPV17L	EID2	
		ZNF771	GNG8	
		TOM1L2	ZNF816-ZNF321P	
		SMARCE1	GLUD2	

cfDNA: cell-free DNA.

existed in tissue DNA, indicating that the higher the AF is in tissue DNA, the easier the cfDNA is to be detected. Thirdly, the sensitivity of cfDNA sequencing was dependent on sequencing depth.[10] High sequencing depth will help to find rare mutations in tissue DNA. In this study, we acquired 100 sequencing depth, so the mutation with less than 1% AF is unable to be identified theoretically.

Except for FAM69A, RAPGEF4-AS1, LOC100287072, and CDRT15L2, most of these common mutation genes are related to many functions of cancer cells, and Unc5D gene inhibits thyroid cancer cell behaviors.[11] As a common non-synonymous mutant gene GLUD2, its activity supports cancer cell proliferation under glutamine depletion.[12] Therefore, these gene mutations may be involved in the metastatic process of FTC.

In summary, we first performed WES from cfDNA of an advanced thyroid cancer patient. This pilot study provided preliminary evidence that cfDNA represents the genomic characteristics of primary FTC, implying the risk of metastasis. Further studies guarantee sequencing of metastatic tumors, increase more cases, and determine the biomarker of cfDNA for metastasis of thyroid cancer.

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Declaration of Conflicting Interests

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References

1. Kapiteijn E, Schneider TC, Morreau H, et al. New treatment modalities in advanced thyroid cancer. *Ann Oncol* 2012; 23(1): 10–18.
2. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 2013; 497(7447): 108–112.
3. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med* 2012; 4(136): 136ra68.
4. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; 6(224): 224ra24.
5. Zane M, Agostini M, Enzo MV, et al. Circulating cell-free DNA, SLC5A8 and SLC26A4 hypermethylation, BRAF(V600E): a non-invasive tool panel for early detection of thyroid cancer. *Biomed Pharmacother* 2013; 67(8): 723–730.
6. Vlkova B, Szemes T, Minarik G, et al. Advances in the research of fetal DNA in maternal plasma for noninvasive prenatal diagnostics. *Med Sci Monit* 2010; 16(4): RA85–RA91.
7. Crowley E, Di Nicolantonio F, Loupakis F, et al. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; 10(8): 472–484.
8. Marusyk A, Tabassum DP, Altrock PM, et al. Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. *Nature* 2014; 514(7520): 54–58.
9. Zhang J, Fujimoto J, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. *Science* 2014; 346(6206): 256–259.
10. Klevebring D, Neiman M, Sundling S, et al. Evaluation of exome sequencing to estimate tumor burden in plasma. *PLoS One* 2014; 9(8): e104417.
11. Zhang MM, Sun F, Cui B, et al. Tumor-suppressive function of UNC5D in papillary thyroid cancer. *Oncotarget* 2017; 8(56): 96126–96138.
12. Takeuchi Y, Nakayama Y, Fukusaki E, et al. Glutamate production from ammonia via glutamate dehydrogenase 2 activity supports cancer cell proliferation under glutamine depletion. *Biochem Biophys Res Commun* 2018; 495(1): 761–767.