

## Comprehensive genomic profiling of a rare thyroid follicular dendritic cell sarcoma

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### Abstract

We previously reported an extremely rare case of follicular dendritic cell sarcoma (FDCC) presented as a thyroid mass. Given the rarity of this disease, there are no personalized and molecularly targeted treatment options due to the lack of knowledge in the genomic makeup of the tumor. A 44-year-old white woman was diagnosed with an extranodal FDCC in thyroid. The patient underwent a total thyroidectomy, central compartment dissection, parathyroid re-implantation, and adjuvant radiation therapy. Tumor DNA sequencing of 236 genes by FoundationOne panel found truncating mutations in PTEN and missense mutations in RET and TP53. However, patient-matched germline DNA was not sequenced which is critical for identification of true somatic mutations. Furthermore, the FoundationOne panel doesn't measure genomic rearrangements which have been shown to be abundant in sarcomas and are associated with sarcoma tumorigenesis and progression. In the current study, we carried

out comprehensive genomic sequencing of the tumor, adjacent normal tissues, and patient-matched blood, in an effort to understand the genomic makeup of this rare extranodal FDCC and to identify potential therapeutic targets. Eighty-one somatic point mutations were identified in tumor but not in adjacent normal tissues or blood. A clonal truncating mutation in the *CLTCL1* gene, which stabilizes the mitotic spindle, was likely a driver mutation of tumorigenesis and could explain the extensive copy number aberrations (CNAs) and genomic rearrangements in the tumor including a chr15/chr17 local chromothripsis resulted in 6 expressed fusion genes. The fusion gene HDGFRP3→SHC4 led to a 200-fold increase in the expression of oncogene SHC4 which is a potential target of the commercial drug Dasatinib. Missense mutations in *ATM* and splice-site mutation in *VEGFR1* were also detected in addition to the TP53 missense mutation reported by FoundationOne.

### Introduction

Originally described by Monda *et al.* in 1986,<sup>1</sup> follicular dendritic cell sarcoma (FDCC) originates from follicular dendritic cells at germinal center of lymphoid follicles that bind and retain immune complexes.<sup>2</sup> FDCC is an rare disease and accounts for less than 0.4% of all soft tissue sarcomas,<sup>3</sup> and around two thirds of FDCC<sup>4</sup> are localized within the lymph nodes. Extranodal sites of FDCC include the oral cavity, spleen, liver, bowel and thyroid. Extranodal thyroid FDCC is an exceedingly rare tumor with only three case reports to date.<sup>5-7</sup> There is a lack of genomic understanding of this disease resulting in the lack of personalized and molecularly targeted treatment options.

We previously reported the 3<sup>rd</sup> case of thyroid FDCC.<sup>7</sup> A 44-year-old white woman was diagnosed with an extranodal FDCC in thyroid.<sup>7</sup> The patient underwent a total thyroidectomy, central compartment dissection, parathyroid re-implantation, and adjuvant radiation therapy. Tumor DNA sequencing of 236 genes by FoundationOne panel found truncating mutations in PTEN and missense mutations in *RET* and *TP53*. However, patient-matched germline DNA was not sequenced which is critical for identification of true somatic mutations.<sup>8</sup> Furthermore, the FoundationOne panel doesn't measure genomic rearrangements which have been shown to be abundant in sarcomas and are associated with sarcoma tumorigenesis and progression. In the current study, we carried out comprehensive

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Key words: genomic sequencing, thyroid FDCC, genomic rearrangement, fusion.

Acknowledgements: We would like to acknowledge the expert help from Mayo Cytogenetics Core and its Director Patricia T. Greipp, as well as Bruce Eckloff from Mayo Sequencing Core and Chris Kolbert from the Mayo Gene Expression Core. Support for this work was provided by generous gifts from Everett and Jane Hauck to the Center for Individualized Medicine at Mayo Clinic Jacksonville Florida; and by Donna Foundation.

Contributions: JID, JSS, SA, JDC, DMM, RWJ, JAC, EAR, RCS, and YWA, contributed to the conception and design of the project; JID, CW, RAK, BMN, VS, ZS, YR, GRO, and YWA contributed to the acquisition, analyses, and interpretation of the data; JID and YWA drafted the manuscript; ASP and JAK reviewed and made critical suggestions; and all authors approved the final version of the paper.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 11 August 2016.

Accepted for publication: 30 March 2017.

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Rare Tumors 2017; 9:6834

doi:10.4081/rt.2017.6834

genomic sequencing of the tumor, adjacent normal tissues, and patient-matched blood, in an effort to understand the genomic makeup of this rare extranodal FDCC and to identify potential therapeutic targets.

In the current study, we performed comprehensive genomic profiling of this thyroid FDCC using the massive parallel Next Generation Sequencing (NGS) technology that include: i) exome sequencing of tumor, adjacent normal tissue, and patient-matched blood DNAs for the detection of small point mutations; ii) whole genome mate-pair sequencing of tumor, adjacent normal tissue, and blood DNAs to identify large genomic rearrangements; and iii) transcriptome sequencing of the tumor and adjacent

normal tissue RNAs to quantify gene expression and identify expressed fusion genes. Our goal is to study the genomic makeup of this rare tumor and also hopefully provide insights into potential personalized treatment options.

## Case Report

The exome sequencing identified 81 somatic mutations (Supplementary Table S1) in tumor but not in blood or adjacent normal tissues, giving a mutation rate of 1.2 per million base-pairs (Mbp) compared to the average rate of 0.41 per Mbp in papillary thyroid cancer.<sup>9</sup> Interestingly, out of the three mutated genes reported by FoundationOne: i) the *RET* mutation is a germline variant (Supplementary Figure 1) because it's clearly also present in the blood DNA as well as in tumor adjacent normal

tissue; ii) two *PTEN* mutations that were supported by very low percentage of reads (1% and 3% respectively) in FoundationOne Panel were not observed in our data; iii) the *TP53* mutation was detected by both FoundationOne and us. In addition, the FoundationOne Panel didn't report mutations in *CLTCL1*, *VEGFR1*, or *ATM*, which are of particular interests due to their established roles in cancer (Table 1, Supplementary Figure 2A-D).

The clonal heterozygous truncating mutation L433\* in *CLTCL1* was found in tumor exome sequencing and was consequently validated by Sanger sequencing (data not shown). While majority of the mutations detected in the tumor were subclonal [alternative allele concentrations (AAC)<0.4], the *CLTCL1* mutation with AAC of 0.63 is clearly clonal (Table 1; Supplementary Figure 3A) and happened early during tumorigenesis as a potentially

driver events. *CLTCL1* is involved in stabilization of the mitotic spindle<sup>10</sup> and the mutation L433\* truncated the VPS functional domain leading to faulty assembly of clathrin complex in tumor (Supplementary Figure 3B-C).

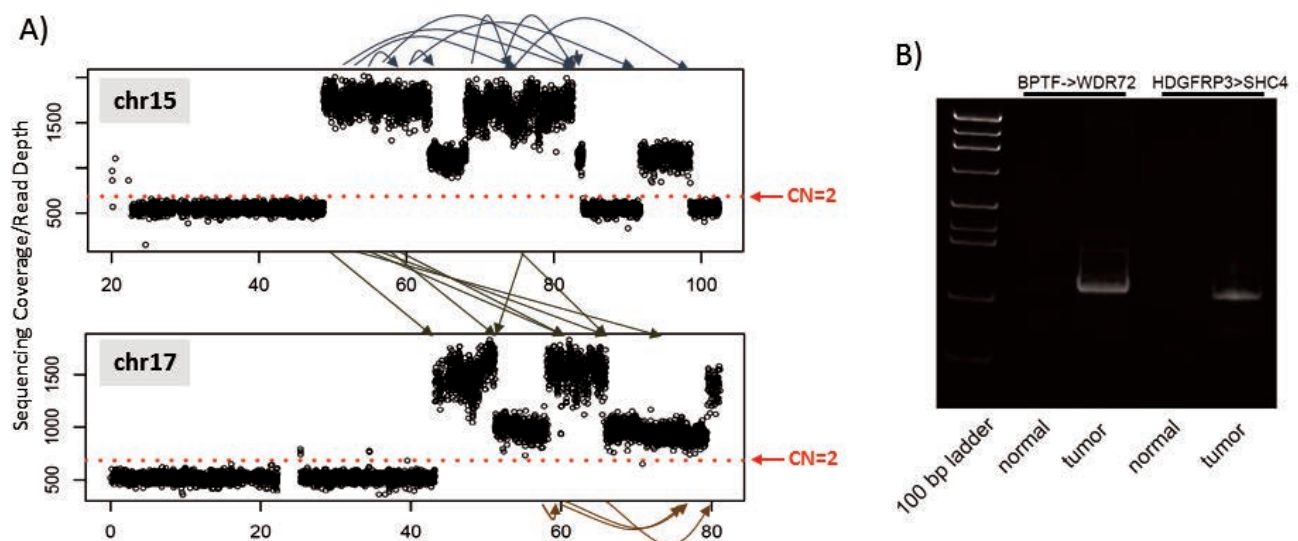
The subclonal splice site mutation (AAC=0.22) in *VEGFR1* detected in the exome located at an intronic splice-site 3bp from the exon boundary led to intron retention in expressed mRNA (Supplementary Figures 2D and 4) which consequently introduced an early stop codon in the kinase domain of *VEGFR1*. *VEGFR1* could act as a decoy receptor to sequester VEGF away from *VEGFR2*,<sup>11</sup> hence a loss of function in *VEGFR1* might lead to activation of the *VEGFR2* pathway and drive angiogenesis.

In addition, subclonal missense mutations were identified in *ATM* (ACC=0.3; Supplementary Figure 2C) and *TP53* (ACC=0.11; Supplementary Figure 2B)

**Table 1. Sequencing evidence of somatic point mutations in CLTCL1, VEGFR1, ATM and TP53.**

Gene	Mutation	Germline exome sequencing	Tumor exome sequencing	Normal tissue RNA-Seq	Tumor tissue RNA-Seq	Gene function
<i>CLTCL1</i>	L433*	0/80 (0.00)	35/58 (0.63)	NA	NA	Spindle formation
<i>VEGFR1</i>	E1128EM*	0/77 (0.00)	10/46 (0.22)	NA	2/2 (1.0)	Angiogenesis
<i>ATM</i>	D1413N	0/21 (0.00)	3/10 (0.30)	0/144 (0.00)	9/33 (0.27)	DNA damage response; cell cycle regulation
<i>TP53</i>	I254V	0/26 (0.00)	1/9 (0.11)	0/123 (0.00)	35/72 (0.49)	Cell cycle regulation

The data is represented as numbers reads supporting mutation/total read depth (alternative allele fraction); or NA indicating no sequencing coverage. All mutations were observed in tumor DNA or RNA sequencing but not in germline or tumor adjacent normal tissue DNA/RNA sequencing.



**Figure 1. Local chromothripsis intra- and inter-chromosome 15 and 17 resulted in extensive copy number aberrations and expressed fusion transcripts. A) Whole genome mate-pair sequencing detected extensive genomic rearrangements intra- and inter- chr15 and chr17. The Y axis is the sequencing depth of the regions. The red dotted line indicates the sequencing depths representing normal copy number of 2 which is calculated across the whole genome from the mate-pair sequencing data. Arrows within and between chr15/chr17 showed the locations of rearrangements (detail in Supplementary Table S2); B) RT-PCR validation of the two expressed fusion transcripts. Both fusions were detected in tumor but not adjacent normal tissues.**

genes by both exome and RNA sequencing.

Extensive genomic structural changes were detected by whole genome mate-pair sequencing including CNAs and translocations/inversions (Supplementary Figure 5 and Supplementary Table S2). Two selected CNA regions with either moderate (chr8q) or high (chr17q) CN gains were experimentally validated using FISH (Supplementary Figure 5). Mostly interestingly, we observed a local chromothripsis event intra- and inter- chr15 and chr17 (Figure 1A), including multiple CNAs and rearrangements, resulted in 6 expressed fusion genes detected in RNA-Seq (Supplementary Table S3). Soft tissue sarcomas are known for high frequencies chromothripsis-like patterns in ~80% of cases.<sup>12</sup> Of the 6 expressed fusion RNAs, two were of particular interest which we will discuss in detail below.

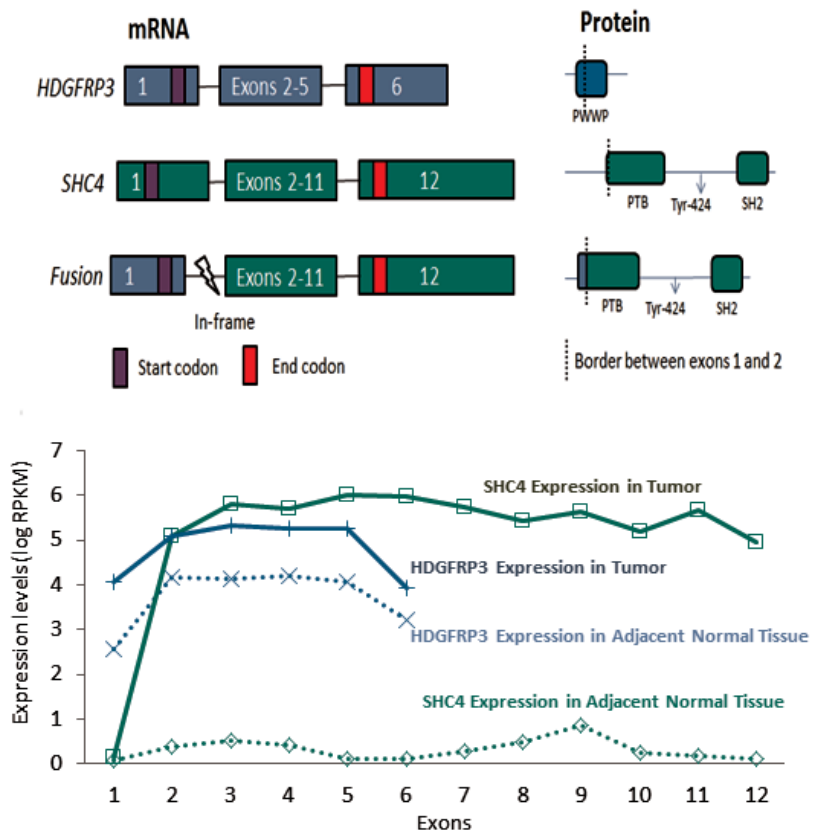
Evidence for the fusion between *HDGRFP3* and *SHC4* was found in mate pair as well as RNA-Seq data (Supplementary Tables S2 and S3) and was confirmed with RT-PCR at the mRNA level (Figure 1B). The fusion gene is in-frame with the exon1 of *HDGRFP3* fused in front of exon 2 of *SHC4*, and the putative fusion protein contains the first 28 amino acids of *HDGRFP3* and *SHC4* from amino acid 196 onwards (Figure 2). Expression of *HDGRFP3* is much higher than *SHC4* in tumor adjacent normal tissues based on the RNA-Seq data (Figure 2). However, the expression of *SHC4* in tumor was over 200-fold higher which is clearly due to fusion because only the fused-in exons had this drastic increase of expression (Figure 2). *SHC4* is an oncogene and a member of the mammalian Src homology and collagen (SHC) family and works as the phosphotyrosine-binding docking molecule. Phosphorylation of *SHC4* at the conserved tyrosine induces Ras and MAPK up-regulation.

A fusion between *BPTF* and *WDR72* was found in both RNA-Seq and mate pair data (Supplementary Table S2 and S3) and confirmed by RT-PCR (Figure 1B). The first 18 exons of *BPTF* fused into the middle of exon 21 of *WDR72* which introduced an early stop codon with the loss of the second PHD zinc finger and Bromo domains in *BPTF* (Figure 3). *BPTF* is part of the NURF complex which is associated with chromatin remodeling, and the second PHD zinc finger binds to H3K4me3,<sup>13</sup> associated with transcription start sites. The loss of this PHD zinc finger may result in transcriptional deregulation.

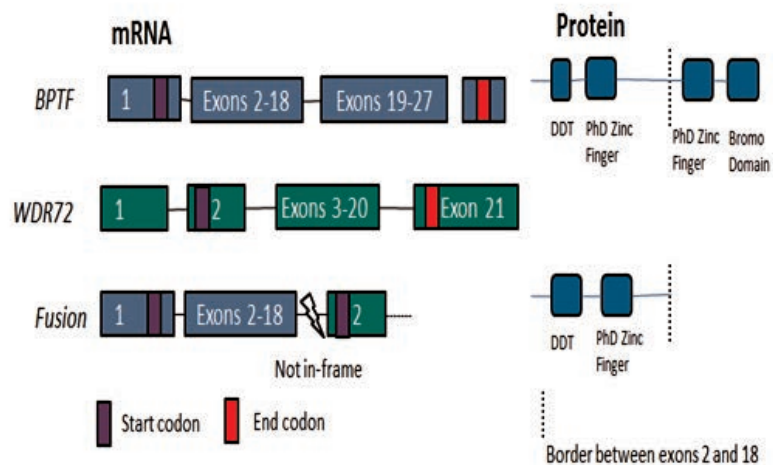
## Discussion and Conclusions

We presented the first extensive genomic characterization of an extranodal thyroid FDCS case using the genomic sequencing approach. This tumor was previously

assayed by FoundationOne Panel which sequenced 236 cancer related genes in tumor tissue only without paired patient normal samples.<sup>7</sup> Our comprehensive genomic profiling of the tumor, tumor adjacent normal and patient germline DNA



**Figure 2.** Structural details of the fusion gene *HDGRFP3->SHC4*. The upper panel shows that the fusion gene consists of the 1st exon from *HDGRFP3* and exons 2-12 from *SHC4*. The structure of the fusion protein is also illustrated. The lower panel shows the expression of *HDGRFP3*, *SHC4*, as well as the *HDGRFP3->SHC4* gene in the RNA-Seq data of tumor and adjacent normal tissues.



**Figure 3.** Structural details of the fusion gene *BPTF->WDR72*, where exons 1-18 from *BPTF* fused in front of the 2<sup>nd</sup> exon of *WDR72*.

identified additional site mutations not reported by and large genomic rearrangements not targeted by the FoundationOne Panel. Specifically we detected mutations in *CLTCL1*, *ATM* and *VEGFR1* in addition to the TP53 mutations reported by FoundationOne. Furthermore, the global approach allowed for detection of extensive copy number aberrations and genomic rearrangements including a chr15/chr17 local chromothripsis event with over 24 structural variants which resulted in 6 expressed fusion genes. The fusion gene of *HDGFRP3*→*SHC4* led to the overexpression of a potential oncogene, *SHC4*, and the fusion between *BPTF*→*WDR72* resulted in the truncation of *BPTF* functional domains involved in chromatin remodeling.

The most intriguing discovery from this study was the *HDGFRP3*→*SHC4* fusion which resulted in a 200-fold overexpression of the oncogene *SHC4*, a member of the Src family. The Src inhibitor *Dasatinib* has been successful treating soft tissue sarcoma<sup>14</sup> and papillary and anaplastic thyroid cancer<sup>15</sup> and might be a potential drug treating this tumor. Furthermore, *SHC4* is downstream of the EGFR signaling pathway and a previous study<sup>16</sup> found that seven out of eight nodal FDOS cases showed moderate to high levels of EGFR; hence the EGFR pathway might be involved in the pathogenesis of this disease.

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