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BRIEF COMMUNICATION

Ewing sarcoma mimicking atypical carcinoid tumor: detection of unexpected genomic alterations demonstrates the use of next generation sequencing as a diagnostic tool

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Increasingly, tumors are being analyzed for a variety of mutations and other genomic changes, with the goals of guiding personalized therapy and directing patients to appropriate clinical trials based on genotype, as well as identifying previously unknown genomic changes in different tumor types and thereby providing new insights into the pathogenesis of human cancers. Next generation sequencing is a powerful research tool now gaining traction in the clinic. In this report, we demonstrate the utility of next generation sequencing assays in providing diagnostic information when evaluating tumor specimens. This is illustrated by a case previously thought to represent an atypical carcinoid tumor, in which an *EWSR1-ERG* translocation was detected during next generation sequencing using a hybrid capture approach, leading to a revised diagnosis of Ewing sarcoma. The role of translocation detection in these assays is also discussed.

Keywords Next generation sequencing, Ewing, EWSR1, ERG, carcinoid

Somatic genetic alterations in oncogenes and tumor suppressor genes contribute to the pathogenesis and evolution of human cancers. These alterations can provide both prognostic and predictive information. Increasingly, tumors are being analyzed for a variety of mutations and other genomic changes, with the hope of effectively guiding personalized treatment and directing patients to appropriate clinical trials based on genotype, as well as recognizing previously unknown genomic changes in different tumor types and thereby providing new insights into the pathogenesis of human cancers (1–5).

In most institutions that offer next generation sequencing assays, the technique is currently used primarily as a research tool but is gaining acceptance as a clinical tool. The Center for Advanced Molecular Diagnostics (CAMD) at Brigham and Women's Hospital and the Center for Cancer Genome Discovery (CCGD) at Dana–Farber Cancer Institute, Boston, MA, have developed a targeted next generation sequencing assay (OncoPanel), performed in a Clinical Laboratory Improvement Amendments-certified laboratory, to detect somatic mutations, copy number variations, and structural variants in 275 known or putative oncogenes and tumor suppressor genes (3). After informed consent is obtained from patients, tumor samples are analyzed by this novel assay, and resulting variants are reported back to the requesting oncologist, who may use these results to adjust therapy or enroll patients in relevant clinical trials. We report herein a case previously thought to represent an atypical carcinoid tumor of lung that was analyzed using the OncoPanel, in which an *EWSR1-ERG* fusion gene was detected. Subsequent genetic and pathologic analysis revealed that the tumor was in fact a Ewing sarcoma with a rare variant fusion gene and histologic appearances mimicking an atypical carcinoid tumor. This case demonstrates the utility of this type of assay in providing diagnostic information when evaluating tumor specimens and illustrates its potential as an ancillary test in future pathology practice.

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Materials and methods

Patient characteristics

The patient was a 42-year-old male of Asian descent who presented with a right upper lobe lung mass detected by routine chest radiograph imaging as part of an annual physical examination. The patient had a light smoking history (approximately 2 pack-years). A right upper lobectomy with regional lymph node dissection was performed at an outside hospital. The lung tumor measured 13 cm and was diagnosed as atypical carcinoid tumor based on histologic appearances, along with expression of the neuroendocrine markers synaptophysin and CD56. The regional lymph nodes were negative for tumor. The patient was treated with four cycles of cisplatin-etoposide. However, restaging scans showed progression of disease with two new nodules in the left lung. The patient underwent wedge resection of one of these nodules at Brigham and Women's Hospital. Histologically, the new tumor was morphologically similar to the initial lung tumor previously resected, and a diagnosis of metastatic atypical carcinoid tumor was rendered. Formalin-fixed paraffin-embedded (FFPE) material from this tumor was analyzed using the OncoPanel.

OncoPanel next generation sequencing

The OncoPanel assay surveys DNA sequences of all coding exons of 275 cancer genes and 91 selected introns across 30 genes for rearrangement detection. DNA was isolated from tissue containing at least 20% tumor nuclei using a QIAamp DNA mini kit (Qiagen, Valencia, CA), sheared into fragments of 250 bp in average size with a sonicator (Covaris, Woburn, MA), and made into a library with the TrueSeq sample preparation kit (Illumina, San Diego, CA). Pools of single-stranded RNA probes were designed and synthesized as part of the Agilent SureSelect hybrid capture kit (Agilent, Santa Clara, CA) and used as baits to select DNA of interest from the sample library. The selected DNA was quantified, normalized, pooled, and sequenced with an Illumina HiSeq 2500 sequencer. Sequence data was demultiplexed and aligned using the Burrows-Wheeler Aligner software tool (17). MuTect (6) and GATK (7) were used to detect single nucleotide variants and small insertion-deletions (indels); a tool developed in-house based on the calculation of the $\log_2$ ratio of read counts of individual specimens against a panel of 72 normal tissues (VisCapCancer) was used to detect copy number variations (CNVs). Another tool developed in-house, cTReX, was used to detect translocations. The aligned BAM files were filtered for reads with soft-clipped sequences, and the filtered reads were grouped by the start coordinate of the soft-clipped sequences and sorted by the count of each group. Groups with more than four reads that shared the same soft-clipped start coordinate and at least one read with more than 30 soft-clipped bases were candidates for rearrangement. The soft-clipped sequences were used to Basic Local Alignment Search Tool for potential chimeric reads. At least one discordant read (two ends mapped to different chromosomal locations) is used to support the finding of a rearrangement.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) evaluation for *EWSR1* rearrangement was performed on 4 -μ tissue sections with an LSI EWSR1 Dual Color, Break Apart Rearrangement Probe (Abbott Laboratories, Abbott Park, IL) at 22q12, consisting of a mixture of two FISH DNA probes. The first probe, an approximately 500 kb probe labeled in SpectrumOrange, flanks the 5′ side of the *EWSR1* gene and extends inward into intron 4. The second probe, an approximately 1,100 kb probe labeled in SpectrumGreen, flanks the 3′ side of the *EWSR1* gene. There is a 7 kb gap between the two probes.

Immunohistochemistry

Immunohistochemical analysis for CD99 (clone: O−13; dilution: 1:150; retrieval: citrate buffer pressure cooker; CoVance, Dedham, MA), synaptophysin (polyclonal; dilution, 1:50; retrieval, none; Abbott Laboratories, Abbott Park, IL), and chromogranin (clone, LK2H1; dilution: 1:4000; retrieval, citrate buffer pressure cooker; Thermo Fisher Scientific, Waltham, MA), was performed on 4- μ-thick sections from FFPE tissue.

Results

Histologically, the tumor was composed of sheets and nests of a relatively monotonous population of round, ovoid and focally spindled cells with moderate amounts of palely eosinophilic cytoplasm. The nuclei had fine chromatin with a speckled pattern, similar to that seen in neuroendocrine tumors. The tumor cells expressed synaptophysin and CD56, and were negative for chromogranin (Figure 1).

Targeted sequence analysis by OncoPanel (see Materials and Methods) demonstrated a near-normal profile with no copy number gain or loss of any target sequences. However, cTReX identified 14 chimeric reads and seven discordant reads involving the *EWSR1* and *ERG* genes, at 22q12.2 and 21q22.3 respectively, consistent with a fusion event (Figure 2). The single nucleotide variant (SNV) and Indel analyses found two SNVs of unknown significance: *EZH2* c.1922A>C p.Y641S and *PIK3C2B* c.4829G>A p.R1610.

On the basis of the OncoPanel findings, FISH analysis for *EWSR1* was performed, and the results were interpreted as abnormal, with a presumptive *EWSR1* rearrangement observed in 36 of 50 nuclei (72%). The abnormal cells contained two normal-appearing red-green *EWSR1* FISH signals; in addition, these cells contained a separate diminished 5′ *EWSR1* red FISH signal (Figure 1). The extra but diminished 5′ *EWSR1* FISH signal was consistent with a break within one of the 5′ *EWSR1* regions, with the 5′ *EWSR1* region inverted and inserted into the *ERG* locus to create a functional *EWSR1-ERG* fusion. This finding is typical for the types of rearrangement associated with an *EWSR1-ERG* oncogenic fusion.

Immunohistochemical analysis for CD99 was performed on unstained slides from both the primary and metastatic tumors. The tumor cells showed diffuse membranous nuclear expression of CD99, the pattern typically seen in Ewing sarcoma. Based on the cumulative findings, a revised diagnosis of Ewing sarcoma was rendered. The