Impact of RNA-sequencing analysis in refining diagnosis in pediatric neuro-oncology

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Background and demographics

The Nationwide Children’s Hospital Institute for Genomic Medicine (IGM) translational research protocol entails pediatric patients with high-risk, relapsed, refractory, or difficult to classify tumors. The goal of this protocol is to refine the patient diagnosis, identify targeted therapeutics relevant to the individual tumor biology and eliminate unsuitable treatments, and determine eligibility for clinical trials. Findings are reported and new cases identified for enrollment with multidisciplinary tumor boards, represented by oncology, surgery, pathology, radiation oncology, and IGM researchers. Medical actionable findings are CLIA validated to allow for return of results to the medical record. To date, 56 unique pediatric patients with central nervous system (CNS) tumors have undergone comprehensive genomic and transcriptomic analyses (Figure 1A). A majority of sequenced patients are male (62%) and between the ages of 1-5 and 11-17 years-old (Figure 1B). Multiple tissue sections or time points were sequenced from some patients. Thus, a total of 70 unique tissues were evaluated, with 63% of tissue from the primary tumor (Figure 1C).

Methods

Transcriptomic analysis was performed on 70 tissue sections collected from 56 patients (snap frozen (48), formalin-fixed paraffin-embedded (FFPE) tissue (n=21), or dissociated cells (n=1)). When possible, total RNA-sequencing was performed (60); however, when the sample was poor quality or low input, DNA capture was utilized to enrich for exonic regions (n=8). RNA was unavailable for 2 patients. We aimed for a total of 180 million mapped reads per sample, with 4 samples falling below that threshold (average mapped reads: 197,344,031 ± 148,456,775 reads). The analysis pipeline is described in Figure 3.

Case 1: Refine diagnosis

A 12-year-old was diagnosed with medulloblastoma, suggestive of Group 3 or Group 4, due to presence of isochromosome 17q. The patient survived 3 years later. Six years after the initial diagnosis, the patient presented with a cerebellar lesion ‘most consistent with recurrent medulloblastoma’, with comment recommending genomic studies to confirm the morphologic impression.

Case 2: Identify targeted therapy

A 13-year-old with choroid plexus carcinoma was treated with chemotherapy and radiotherapy for 2 years then relapsed with recurrent implants. Genomic analysis identified a somatic TP53 mutation and 1p13 loss of heterozygosity. Comprehensive transcriptomic analysis revealed overexpression of multiple pathways (mTOR, FGF, and PDGF signaling) consistent with other IGM choroid plexus carcinoma cases and a 2017 case report (Cornelis et al. 2017 Front. Pharmacol.). Treatment on sunitinib (PDGF inhibitor), everolimus (mTOR inhibitor), and thalidomide (FGF inhibitor) was initiated.

Case 3: Confirm diagnosis

A 10-month-old was diagnosed with a soft/solid neoplasm, most consistent with a CNS antiportional tumor. No constitutional or somatic variations or copy number alterations were noted. The tissue was also sent to an outside institution for the Infinium MethylationEPIC 850k array, reporting a classification of pediatric group [Alutransecral retinoblastoma] (score=0.99).

We are grateful to the patients and their families for their participation.