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Targeted next-generation resequencing in pediatric osteosarcoma patients

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Background: Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents. Recent genomic analysis demonstrated that OS are characterized as a high frequency of structural variations and chromosomal copy number changes. Meanwhile, specific genomic alterations such as RB1 or TP53 have been identified, although driver gene mutation for OS tumorigenesis has yet to be found. The aim of this study is to identify specific somatic mutations in OS patients by the method of comprehensive targeted resequencing.
Patients and Methods: 13 pairs of tumor and non-tumor frozen tissues taken from the patients diagnosed as a primary conventional OS were subjected to this study. All tumor samples were collected at the time of preoperative biopsy and confirmed pathologically the tumor cell content more than 80%. We utilized a next generation sequencing technique with the Ion AmpliSeq Comprehensive Cancer Panel (CCP) containing 409 cancer-related genes. 40 ng of DNAs were used for multiplex PCR amplification and sequencing was run on the Ion Torrent Proton loaded with PIv2 chip. Data analysis including alignment to the hg19 human reference genome and variant calling was done using the Torrent Suite Software. Obtained genomic data was validated by visualizing in Integrative Genomics Viewer and candidate mutations were annotated by wANNOVAR database.
Results: An adequate library was obtained from all samples for subsequent sequencing. Mean read length was 110 base pairs and an average coverage was approx.1500 for tumors and 400 for paired non-tumors. A total of 153 candidate non-synonymous somatic SNVs (median; 13.9 per tumor) were found with the threshold of over 5% frequency. After validation analysis and filtering process of variants, at least 12 SNVs were confirmed in 8 patients. Recurrent SNVs have not been observed among the 13 patients. There was a trend toward increased number of SNVs in dead patients comparing to alive patients (median 0 vs 1), although no significant difference was observed (P=.23, Wilcoxon test).? Pathway analysis of the candidate SNVs showed significant correlations with three gene function categories including cell cycle, ATP binding and ligand-dependent nuclear receptors ($p<0.02$). We also calculated relative copy number changes in tumors using the coverage analysis data and found at least three gene amplification on chromosome 1p, 17p and 19p.
Conclusion: We identified at least 12 non-synonymous somatic SNVs of cancer-related genes on the CCP panel in pediatric OS patients. Targeted sequencing is a useful and cost-effective tool to quickly survey known cancer-related mutations of pediatric OS.