Identification of Clinically Relevant Variants in Formalin-Fixed Paraffin-Embedded Colorectal Cancer Tissue Samples Using Targeted Next Generation Sequencing

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Colorectal Cancer (CRC) is the third most commonly diagnosed cancer with over 1.9 million new cases worldwide in 2020. Accurate patient classification is imperative for the identification of the group of patients likely responding to treatment while conserving others from ominous treatment. The Next Generation Sequencing (NGS) technology can provide rapid genetic investigation and facilitate routine diagnosis. In this study, we performed targeted NGS to examine CRC tumours for clinically relevant variants among the genes commonly predictive of resistance to anti-EGFR therapies (*KRAS, NRAS, BRAF*) and among the DNA mismatch repair (MMR) genes (*MLH1, PMS2, MSH2, MSH6*) which could guide clinicians for further evaluation of Lynch syndrome and the Fluorouracil regime treatment option. A total of 87 formalin-fixed paraffin-embedded (FFPE) colorectal cancer tissue samples were collected. The DNA was extracted with GeneRead DNA FFPE kit, followed by library preparation with the QIASeq Human CRC Panel. The samples were sequenced using NovaSeq 6000 and a median coverage of 634.5x was achieved for all samples. Variants were identified using CLC Genomics Workbench and annotated using QCI Interpret software. In summary, *KRAS, NRAS, BRAF, MLH1, PMS2, MSH2* and *MSH6* non-synonymous variants were detected in 42.5% (37/87), 4.6% (4/87), 9.2% (8/87), 16.1% (14/87), 81.6% (71/87), 8.0% (7/87) and 9.2% (8/87) of the CRC cases, respectively. *KRAS, NRAS* and *BRAF* non-synonymous variants were absent in 47.1% (41/87) of the CRC cases while *MLH1, PMS2, MSH2* and *MSH6* non-synonymous variants were absent in only 12.6% (11/87) of the CRC cases. *PMS2* c.89A>C p.Gln30Pro was the most common variant detected among the MMR genes (80.5%, 70/87), with allele frequency ranging from 0.5% to 30.9%. Work is ongoing to evaluate the concordance of microsatellite instability with the variants detected among the MMR genes. In conclusion, the targeted NGS method performed in this study had superior detection sensitivity and is capable of rapidly identifying the clinically relevant variants in multiple genes.

Keywords: Colorectal Cancer (CRC), Next Generation Sequencing (NGS)

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