Genomic Profiling of Tumors Using Liquid Biopsy to Match Patients to Best Treatment Options

液態切片基因定序提供癌症最佳治療選擇

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Purpose: Next generation sequencing (NGS) based cancer tissue profiling has demonstrated clinical utility for therapy selection. Cell-free tumor DNA (cftDNA) in blood circulation harbors genomic alterations originated from tumor cells. Thus, genomic profiling of cftDNA from blood (liquid biopsy) can be used as a non-invasive method in real time to select personalized therapies for metastatic or refractory patients – especially when remaining tissue is insufficient for analysis.The major challenge of using NGS to analyze cftDNA is the extremely small fraction of cftDNA present within background levels of normal cell-free DNA (cfDNA). Standard NGS is too error prone to reliably detect mutations at low tumor fraction. We have developed a Single Molecule Sequencing (SMSEQ) platform that dramatically improves detection sensitivity and specificity at low tumor DNA inputs.

Materials & Methods: We tested 30 cancer patients from Chang Gung Memorial Hospital, Taoyuan, Taiwan, with pathologically confirmed metastatic colorectal cancer. 21 healthy patients were included as controls. cfDNA was extracted from >5mL blood, and converted to libraries and sequenced with the SMSEQ platform. Reads were aligned and filtered, and high-quality variants called are called with a custom variant caller.

Results: We developed a Single Molecule Sequencing (SMSEQ) assay, which allows confident calling of variants down to 0.25% tumor fraction with analytical specificity > 99.999%. Somatic mutations were identified in 29 CRC cases, yielding a clinical sensitivity of 97% (29/30). The most frequently mutated genes were *TP53*, *APC*, and *KRAS*. 87% of patients had at least one mutation in a gene known to have an effect on treatment efficacy. Three patients were identified with wild-type KRAS and mutated BRAF. Mutated BRAF, which occurs in 5%-10% of Wild-Type KRAS patients and is not commonly tested in mCRC, and may confer resistance to anti-EGFR treatment.

Conclusion: cftDNA analysis (liquid biopsy) with SMSEQ is shown to be a reliable method of detecting tumor mutations for metastatic CRC. Liquid biopsy's non-invasiveness as compared to tissue sampling, and ability to track mutations in real time expands the treatment options for patients without sufficient tissue sample.