

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

Wen-Sy Tsai¹, Ying-Chih Chang², Shan-Fu Wu³, Julian Lucas³, Gangwu Mei³, Mana Javey³, Rui Mei^{3*}

1 Chang Gung Memorial Hospital, #5, Fuxing St, Guishan Dist, Taoyuan, Taiwan 333

2 Academia Sinica Genomics Research Center, No. 128, Section 2, Academia Rd, Nangang Dist, Taipei, Taiwan 115

3 CellMax Life Taiwan, 18F-1, #3, Park St, Nangang Dist, Taipei, Taiwan 115

* Corresponding author: rui@cellmaxlife.com

蔡文司¹, 張瑛芝², 巫善福³, Julian Lucas³, 梅剛武³, Mana Javey³, 梅蕊^{3*}

1 長庚紀念醫院大腸直腸肛門外科, 桃園市龜山區復興街5號

2 中央研究院基因體研究中心, 台北市南港區研究院路二段 128號

3 合度精密生物科技, 台北市115南港區園區街3號18樓之一

* 通訊作者: rui@cellmaxlife.com

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.