（博士課程医学領域）　　（別記様式第５号）
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論　文　内　容　要　旨

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論文題目	Fractionated small cell-free DNA raised possibilities to detect cancer-related gene mutations in advanced colorectal cancer（進行大腸癌患者の血清を用いた腫瘍由来循環DNA濃縮による癌関連遺伝子検出向上の試み）		

論文内容要旨

（研究の目的）

Colorectal cancer (CRC) is the second leading global cause of cancer death in men and the third leading cause of cancer in women, respectively. Advanced CRC patients who cannot be completely resected receive systemic chemotherapy. Recent advancements in therapeutic options including molecular targeted therapies and immunotherapies have enabled the prescription of more precise medications in accordance to the molecular profile of the patients' tumor. In other words, and for precision medicine to be accurate and efficient, it necessitates the acquisition of tumor tissues that reveal patients' genetic profiles. However, this process can be a very difficult task in advanced cancer patients with decreased activities of daily living.
Liquid biopsy is a method that can efficiently detect tumor genetic abnormalities from body fluids such as blood and urine. Detection sensitivity and the available number of mutations in cell-free DNA (cfDNA) are limited. In this study, we develop a highly sensitive and comprehensive method to detect mutations from cfDNA by concentrating tumor fractions of small-sized cfDNA in advanced CRCs.

（方法）

Biopsied specimens and 37 serum samples were collected from 27 patients with advanced colorectal carcinoma. A serum-extracted cfDNA was divided into enriched fractionated small cfDNA and unfractionated cfDNA. Both cfDNAs were subjected to digital polymerase chain reaction (PCR) to evaluate their *KRAS*, *BRAF*, and *TP53* status. Consequently, their mutant allele frequencies (MAFs) were compared and analyzed by next-generation sequencing (NGS) in conjunction with tissue-derived DNA.

（結果）

NGS analyses revealed mutations in *TP53* (63%), *KRAS* (63%), *APC* (30%), and *PIK3CA* (22%). Digital PCR could detect *KRAS* mutations in 17 out of 19 samples (89%) of unfractionated cfDNA, a rate that increased to 100% when samples were enriched with fractionated small cell-free (cf) DNA (6.8 vs. 10.7%, $p < 0.001$). NGS also showed increased MAFs in fractionated small cfDNA compared to unfractionated cfDNA (16.3 vs. 18.8%, $p = 0.012$), and a tendency to detect a gretaer number of cancer-related genes in fractionated cfDNA.

備　考

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（考察）

In our study, the sensitivity in detecting mutations in cfDNA from serum using dPCR was 84% and 89% when cut-off values of MAFs were set at >0% and >0.1%, respectively. These results are consistent with current literature findings. On the contrary, the sensitivity in detecting cfDNA mutations from the serum was 14% and 25% when cut-off values of MAFs were set at >2% and >1%, respectively. Therefore, NGS sensitivity directly relies on MAFs cut-off. In fact, a great number of sequences with incorrect variants was identified when variants with MAFs below or around 1% are investigated.

To improve the sensitivity of cfDNA mutation detection, we enriched fractionated small nucleic acids from cfDNA. This process facilitated a greater number of MAFs of driver mutations by dPCR and NGS, and hence higher possibilities to detect cancer-related gene alterations by NGS. Despite the fact that dPCR is highly sensitive, it can only detect a few targets, whereas although NGS is less sensitive, it facilitates a comprehensive gene analysis. CfDNA from tumor cells has been reported to own altered fragmentation profiles compared to cfDNA from healthy individuals. Moreover, the proportion of small, fragmented DNA in cfDNA has been found to be significantly higher in patients with lung cancer, colorectal cancer, and cholangiocarcinoma. Our results are consistent with these previous reports and demonstrate a distinct possibility to detect a greater number of cancer-related genes that can, in turn, be targeted by related molecular agents. Multiple clinical implications are fostered by the findings of this study. Firstly, our sensitive liquid biopsy can facilitate efficient monitoring of the therapeutic outcomes in a more rapidly and accurate manner compared to ordinary tumor markers such as CEA and CA19-9. In fact, and as shown earlier, conventional tumor marker responses during the systemic therapy in our two cases were very slow despite the fact the change observed in their CT images. On the other hand, very rapid responses were observed when using liquid biopsy especially in fractionated small cfDNAs. Secondly, efficient mutations detection in *KRAS*, *BRAF*, *NRAS* and *PIK3CA* by liquid biopsy with our sensitive method can be a resistance marker for molecular targeted drugs which are widely used in conjunction with systemic chemotherapy in CRC. Therefore, and with this method, it is possible to obtain a genetic profile of the tumor even in advanced cancer patients with decreased physical strength. Thirdly, fractionated small-sized cfDNA increased the possibility of detecting cancer-related gene mutations, including actionable gene mutations, which can be a potential a molecular target for drugs.

（結論）

Fractionated small cell-free cfDNA increased MAFs of gene mutations, and increases the possibilities to detect cancer-related genes even in advanced cancer patients who are difficult to obtain tissue samples.

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