様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　２ 年　 ６ 月 １７ 日現在

機関番号：７２６０２
研究種目：若手研究
研究期間：2018～2019
課題番号：１８Ｋ１５３３２

研究課題名（和文）Establishment of Japan circulating tumor DNA cancer screening panel

研究課題名（英文）Establishment of Japan circulating tumor DNA cancer screening panel

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：この研究は、固形がんから循環腫瘍DNA（ctDNA）を検出するためにカスタマイズされたNGSパネルを確立することを目的としています。2つのパネルが確立されました。がんスクリーニングパネルは、TCGAおよびCOSMICデータベースからの変異プロファイルを評価した後に設計された33の癌遺伝子パネルです。 固形がんでは、ctDNA検出率は50-67%です。Actionable mutationパネルは、分子標的薬の変異、CNV、およびfusionをカバーする59遺伝子パネルです。ctDNAの平均検出率は79%です。これらのパネルはリキッドバイオプシーから0.2%の検出限界で変異を検出しました。

研究成果の学術的意義や社会的意義
Next generation sequencing (NGS) of circulating tumor DNA (ctDNA) enables the evaluation of genetic profiles of the primary and metastatic tumor sites. This study established customize ctDNA NGS panels that could be used for detection, monitoring and treatment selection for cancer patients.

研究成果の概要（英文）：This study aims to establish customized NGS panels to detect circulating tumor DNA (ctDNA) from various solid tumors. Two panels were established: JFCR cfDNA screening panel for cancer screening and JFCR cfDNA actionable mutation panel for alterations detection with therapeutic implication.
JFCR screening panel is a 33 cancer gene panel, designed after assessing mutation profiles from TCGA and COSMIC databases. By using reference standard, JFCR cfDNA screening panel was able to detect mutation as low as 0.2% of molecular allele frequency. ctDNA detection rate varies among solid cancers and stages, range between 50-67% by using this panel. JFCR cfDNA actionable mutation panel is a 59 gene panel that covers actionable mutations, copy number variations and fusions. The average ctDNA detection rate is 79% in various solid tumor with limit of detection at 0.1%. This study include only very small sample size, further studies are needed to evaluate the feasibility of these panels.

研究分野：cancer genetics

キーワード：liquid biopsy　ctDNA　cell-free DNA　NGS　actionable mutation

1. **Scientific background**

It is foreseen that the incidence of cancer will rise to 22 million within the next two decades urging the needs to explore methods for early cancer screening, prognostic determination, and monitoring. Cancer is a genetic disease that caused by the accumulation random somatic alterations. Typically, these alterations could be evaluated through needle biopsy obtained from the primary tumor sites for the determination of therapeutic strategy. Nevertheless, there are shortcomings of this approach. Cancers are highly heterogeneous and mutation profiles of tumor evolves over time, biopsy taken from the primary tumor reflects only the genomic alteration of the location and the time when the biopsy was taken. In addition, given the difficulties in repeatedly obtaining tissue biopsies through invasive method, liquid biopsy that include circulating tumor DNA (ctDNA), provides an alternative non-invasive way to evaluate mutation profile of tumor via blood in which ctDNA could be extracted from the plasma.

ctDNA are short DNA fragments (~150-200 bp) that break away from the primary and metastatic tumor site(s) during cell death, which subsequently enter into the bloodstream. These ctDNA carry the information of tumor-specific genetic alterations from all tumor site(s) and hence could provide an overview mutation profiles of a patient. Importantly, mutation status in ctDNA from plasma is highly concordant with the corresponding tumor tissue and the level of ctDNA increase corresponds to the stage of cancer.[1] In addition, it is known that ctDNA has superior sensitivity compared to conventional biomarkers, such as CA-153, and has a greater dynamic range that correlates with changes in tumor burden.[2] The evaluation of ctDNA provide a cancer "snapshot", which is therefore relevant not only for risk stratification, but for guiding treatment. Detection of ctDNA could serves as better biomarkers for early detection of cancer and monitoring.

2. Research objectives

(1) The current research proposal aims to establish customized ctDNA panels for next generation sequencing (NGS). Two panels were established:

(a) JFCR screening panel was designed after evaluating mutation profiles of the top 12 solid tumors that shows highest incidence and mortality in Japan.

(b) JFCR actionable mutation panel was designed focusing on actionable mutations for possible selection of targeted therapy.

(2) To optimize NGS methodology to obtain desirable depth of sequencing.

(3) To evaluate the efficacy, limit of detection, sensitivity and specificity of the panel with commercial controls and samples from cancer patients.

It is hopeful that these panels could be used for cancer detection, monitoring therapeutic response and early detection for cancer recurrence. This research grant will support the proof-of-concept of this panel before it could be implemented in the clinical settings.

(3) Research Method

3.1 Establishment of JFCR screening panel

According to cancer statistics Japan 2016, Japan top 12 cancers (considering incidence and mortality), which include cancer of colon, stomach, lung, prostate, breast, liver, pancreas, gallbladder, esophagus uterine, ovarian and prostate were selected. All the mutation and copy number alteration information of the cancer genome atlas project (TGCA) and other publicly available cancer genomics datasets were assessed from COSMIC (Catalogue of somatic mutation in cancer) database. The number of mutations per gene of

each cancer were evaluated and summarized. Hotspots of the most commonly mutated genes were selected to establish the panel.

3.2 Establishment of JFCR actionable mutation panel

This panel was established by selecting the potential actionable alterations (mutations, copy number variations and fusions) compiling from several databases that include OncoKB, My Cancer Genome, CanDL, Clinical knowledgebase, CIVIC and FDA databases. The alterations that were classified in the following level of evidences: FDA/NCCN guidelines approved, early and late clinical trials as well as case reports, were included in the panel.

3.3 Establishment of NGS methodologies

The panels were developed by Ampliseq HD technology from Thermofisher Scientific. This amplicon-based NGS technology has integrated molecular barcode system that are known to reduce error and could accurately detects low-frequency variants. Both of the panels were firstly evaluated using commercially reference standard (OncoSpan reference standard and Horizon Discovery cell-free reference standard) and subsequently with cell-free DNA (cfDNA) extracted from cancer patients. In brief, cfDNA was extracted using MagMax cell-free total nucleic acid kit. The extracted cfDNA was subjected Ampliseq HD library kit to generate libraries for targeted sequencing based on the panel designed. The libraries of the samples were pooled for templating on the Ion Chef System and subsequently sequenced using the Ion S5 Prime System. Ion Reporter was used for data analysis including variant calling and annotation.

(4) Results

4.1 Detection rate of JFCR screening panel

JFCR screening panel covers 1,542 hotspot mutations from 33 most commonly mutated genes in solid tumor. Among the genes, *TP53* is the most frequently mutated in all types of solid tumors followed by *PIK3CA* for breast cancer, *APC* and *KRAS* for colorectal cancer, *EGFR* for lung cancer.

NGS was performed with overall depth at 65,000x and median molecular depth at 3,300x. By using OncoSpan reference standard, JFCR screening panel was able to detect mutation as low as 0.2% of molecular allele frequency. In addition, R2-value of 0.94 indicating highly concordance by comparing OncoSpan reported frequency and the NGS data. With these findings, this panel is considered high sensitivity to detect very low allele frequency variants and high specificity (Figure 1). Subsequently, 16 cell-free DNA from solid tumors were evaluated, 8 (50%) were detected to harbored at least one mutation. Among the detected mutations are located in *TP53, PIK3CA, BRAF, GNAS* and *KRAS.*

CHR	POS	REF	ALT	GENE	AMINO_ACID	OncoSpan_ allelefreq(%)	JFCR screening panel		
							MDP	MAO	Mol freq (%)
chr1	115256531	G	T	*NRAS*	p.Q61K	12	2940	344	11.7
chr3	41266102	C	A	*CTNNB1*	p.S33Y	32	2334	465	19.9
chr3	41266137	CCTT	C	*CTNNB1*	p.S45del	10	1342	147	11
chr3	178936092	G	A	*PIK3CA*	p.E545K	9	1156	96	8.3
chr3	178952086	A	G	*PIK3CA*	p.H1047R	17	3300	569	17.2
chr5	112162855	T	C	*APC*	p.Y486Y	26	3314	533	16.1
chr5	112175771	G	A	*APC*	p.T1493T	35	1557	511	32.8
chr7	55241708	G	A	*EGFR*	p.G719S	24	1693	434	25.6
chr7	55242479	AGGAATTAA GAGAAGC	A	*EGFR*	p.Glu746_Ala750del	2	3643	8	0.2
chr7	55249064	G	A	*EGFR*	p.Q787Q	15	3832	313	8.2
chr7	55249072	C	T	*EGFR*	p.T790M	1	3841	15	0.4
chr7	55259516	T	G	*EGFR*	p.L858R	3	3984	108	2.7
chr7	140453137	A	T	*BRAF*	p.V600E	10	3216	258	8
chr12	25398282	C	T	*KRAS*	p.G13D	15	2892	286	9.9
chr12	25398285	C	T	*KRAS*	p.G12D	6	4310	86	2
chr15	66727452	A	C	*MAP2K1*	p.Q56P	26	2238	401	17.9
chr15	66729148	C	T	*MAP2K1*	p.H119Y	35	2770	540	19.5
chr17	7579473	G	C	*TP53*	p.P72R	93	1994	1841	92.3
chr17	37879589	A	G	*ERBB2*	p.I655V	32	1071	352	32.9
chrX	66765143	G	A	*AR*	p.A52T	38	164	51	31.1

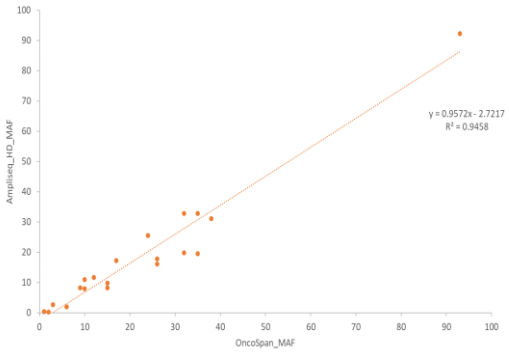

Figure 1: NGS results using JFCR screening panel.

4.2 Detection rate of JFCR actionable mutation panel

JFCR actionable mutation panel covers mutations, copy number variations and fusions of 59 genes that showed to have therapeutic implications. NGS was performed to achieve overall depth at 50,000x and median molecular depth at molecular depth 4,000x. By using Oncospan reference panel spike-in to healthy plasma, mutation as low as 0.1% was detected indicating its high sensitivity. A total of 48 clinical samples were evaluated, 38 (79%) were detected to harbor at least one alteration. Among the 38 mutations detected using this panel, 33 (87%) were actionable mutations indicating therapeutic implications (Figure 2).

Spiked in with HDx Multiplex: OncoSpan				JFCR actionable panel		
Gene	Amino Acid	Genotype	Type	Molecular depth	Count	Frequency (%)
BRAF	p.V600E	ACTG/TCTG	SNV	2726	61	2.2377
BRCA1	p.R1443*	G/A	SNV	4352	242	5.5606
CTNNB1	p.S33Y	C/A	SNV	4607	261	5.6652
CTNNB1	p.S45del	CCTT/C	INDEL	4593	64	1.3934
EGFR	p.E746_A750del	AGGAATTAAGAGAA GCAAC/AAAC	INDEL	5560	28	0.5035
EGFR	p.G719S	G/A	SNV	5523	245	4.4359
EGFR	p.L858R	TG/GG	SNV	4738	16	0.3376
EGFR	p.T790M	C/T	SNV	4306	5	0.1161
ERBB2	p.I655V	A/G	SNV	4604	242	5.2563
KIT	p.D816V	A/T	SNV	4526	88	1.9443
KRAS	p.G12D	CC/TC	SNV	2748	16	0.5822
KRAS	p.G13D	CC/TC	SNV	2751	86	3.1261
MAP2K1	p.H119Y	C/T	SNV	5145	258	5.0145
MAP2K1	p.Q56P	A/C	SNV	4652	215	4.6216
NRAS	p.Q61K	GT/TT	SNV	2628	72	2.7397
PIK3CA	p.E545K	G/A	SNV	2964	57	1.923
PIK3CA	p.H1047R	ATCA/GTCA	SNV	2788	125	4.4835

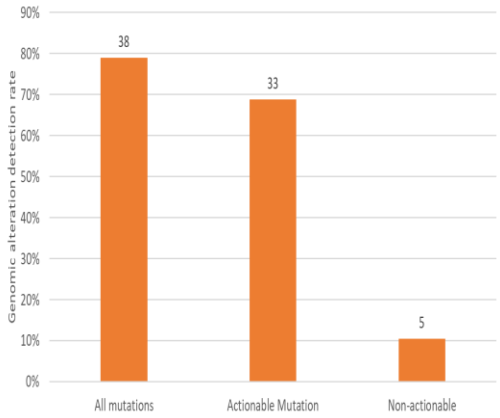

Figure 2: NGS results using JFCR actionable mutation panel

Conclusion

The customized JFCR screening panel will be useful for cancer screening/detection by using liquid biopsy owing to its high sensitivity and specificity. The JFCR actionable mutation panel will be useful for treatment selection using liquid biopsy as this panel covers alterations that shown to have therapeutic implication.

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３．雑誌名 Molecular Oncology	６．最初と最後の頁 印刷中
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/1878-0261.12727	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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