Mutation detection sensitivity in matched FFPE tissue and liquid biopsy samples

Assay measurement using optimized high-throughput workflows

Introduction

Molecular analysis of tumor tissue biopsies has become standard practice in clinical oncology for cancer management, allowing detection of genetic abnormalities, which guides treatment options. In the last few years, liquid biopsy using circulating tumor DNA (ctDNA), a fraction of circulating cell-free DNA (cfDNA) present in blood, has emerged as a promising noninvasive companion diagnostic in cases where traditional tissue biopsy is challenging. ctDNA may enable assessment of genetic alterations in tumors for early detection of cancer, personalized therapy, and treatment monitoring. Liquid biopsy, however, has certain limitations: secretion of ctDNA into body fluids is incompletely understood, the success of liquid biopsy largely depends on the type and stage of cancer, and the method does not allow for determination of the location of tumors [1-4].

Here we report on concordance in genomic profiling between formalin-fixed, paraffin-embedded (FFPE) tissue from tumor biopsies and plasma ctDNA from liquid biopsies. Samples were obtained from 10 donors with colon cancer, 6 with breast cancer, and 2 with lung cancer. Next-generation sequencing (NGS) analysis was performed for both tissue-derived DNA and matched ctDNA, and compared.

Materials and methods

A total of 18 matching FFPE tissue blocks and plasma samples from the same donors were obtained from Discovery Life Sciences (Table 1).

Table 1. Cohort demographics.

Subject/sample description		
Mean age of donor	n = 18	62.6 yr
Sex		
Male	n = 3	16.7%
Female	n = 15	83.3%
Type of cancer		
Colon	n = 10	55.6%
Breast	n = 6	33.3%
Lung	n = 2	11.1%
Cancer stage		
<III	n = 9	50.0%
III	n = 8	44.4%
IV	n = 1	5.6%
Smoking history		
Current or former	n = 2	11.1%
Nonsmoker	n = 16	88.9%
Alcohol history		
Current or former	n = 1	5.6%
Nondrinker	n = 17	94.4%

An overview of the standardized workflows utilizing optimized Applied Biosystems™ MagMAX™ kits and downstream analysis instrumentation is shown in Figure 1.

For each FFPE tissue block, one 5 µm section was deparaffinized using Applied Biosystems™ AutoLys M Tubes, followed by protease digestion. DNA was then isolated from these samples using the Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit (Cat. No. A31881) and Thermo Scientific™ KingFisher™ Presto system integrated with the Applied Biosystems™ NIMBUS™ automation system, allowing for hands-free preparation.

Corresponding matched cfDNA from plasma (2–4 mL) was purified using the Applied Biosystems™ MagMAX™ Cell-Free DNA Isolation Kit (Cat. No. A29319). DNA was eluted in a low elution volume of 15 µL to maximize sample input towards NGS library preparation.

The obtained nucleic acid from FFPE tissue and blood samples was quantified using the Invitrogen™ Qubit™ 4 Fluorometer, and integrity was analyzed using the Agilent™ 2100 Bioanalyzer™ system. DNA from both sample types was then used towards NGS library preparation with the Ion Torrent™ Oncomine™ Breast and Colon cfDNA Assays and the Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay.

Final libraries were quantified by qPCR using the Ion Library TaqMan® Quantitation Kit. Template-positive Ion Sphere™ particles were prepared from the library samples by clonally amplifying the DNA onto the Ion Sphere particles using the Ion Chef™ Instrument. Automated enrichment of the template-positive Ion Sphere particles was performed using Invitrogen™ Dynabeads™ MyOne™ Streptavidin C1 beads. The clonally amplified DNA fragments bound to the Ion Sphere particles are biotinylated, and during enrichment they bind to streptavidin-coated magnetic beads. All “empty” Ion Sphere particles are washed away. Enriched template-positive Ion Sphere particles were then sequenced on the Ion S5™ instrument using the Ion 530™ or Ion 540™ Chip. Upon completion of sequencing runs, data were analyzed with the Torrent Variant Caller plugin on Torrent Suite™ Software using the appropriate Ion Torrent™ Oncomine™ liquid biopsy or tumor analysis plugin.

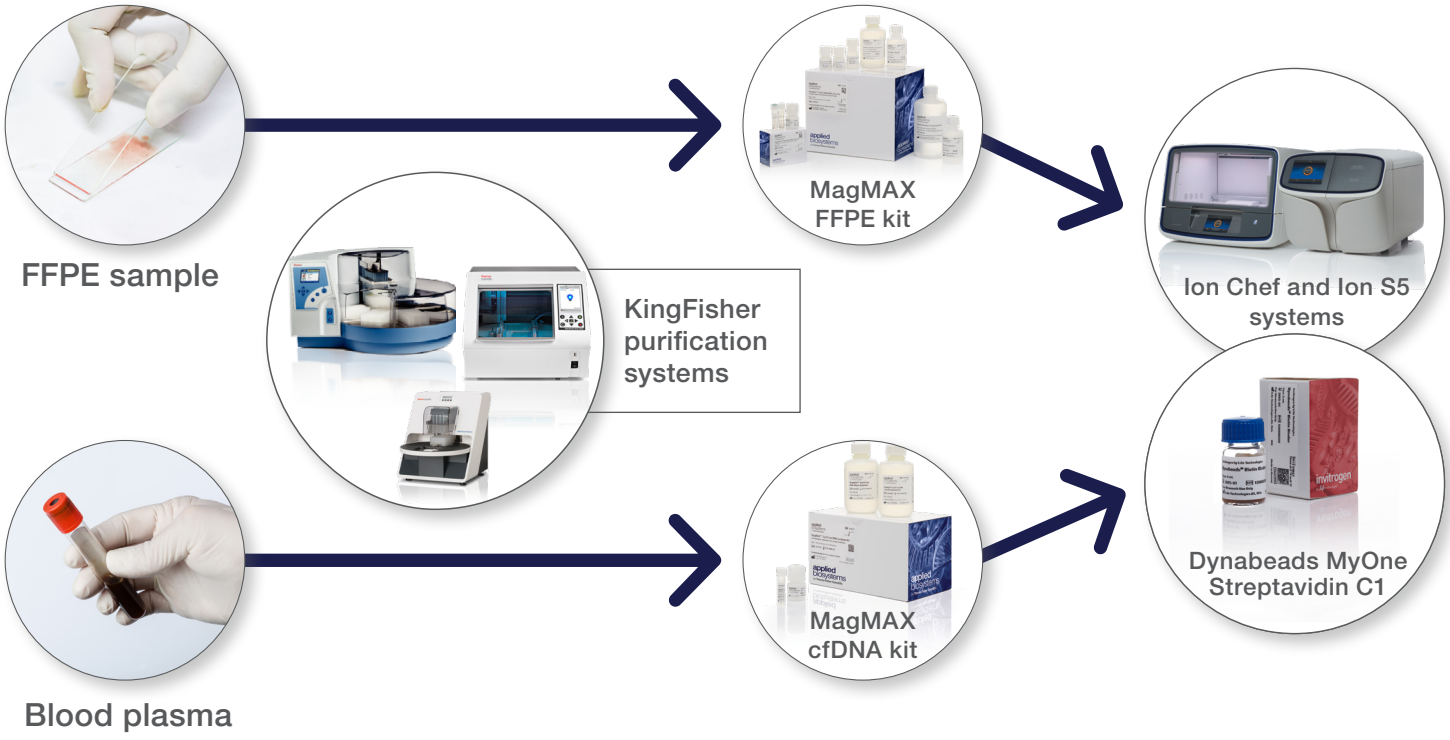

Figure 1. Workflows for processing FFPE and plasma samples for genomic profiling. DNA was isolated from the matched FFPE and blood plasma samples from a total of 18 donors, using the corresponding MagMAX kits. Next, DNA was quantified and NGS libraries were prepared using the Ion Torrent™ Oncomine™ Focus Assay panel with 10 ng of sample input. The library preps were templated with the Ion Chef instrument, and automated enrichment of template-positive Ion Sphere particles was carried out using the Dynabeads MyOne Streptavidin C1 beads. After enrichment, template-positive Ion Sphere particles were sequenced on the Ion S5 System using an Ion 530 or 540 Chip. Analysis of NGS data was performed with an Oncomine liquid biopsy or tumor analysis plugin.

Results

The samples were processed and analyzed as shown in Figure 1, and results of the study are shown in Tables 2 and 3. In total, 29 alterations were detected in the FFPE tissue samples, and 25 in the plasma-derived cfDNA samples. The number of unique FFPE tissue mutations not detected in cfDNA was 18. The number of true positive concordant mutations (exact same gene and nucleotide change detected in FFPE and cfDNA) was 11, 25% of the total of 44 mutations detected (Table 3).

Table 2. Concordance analysis. Allele frequencies in FFPE vs. plasma samples from 18 donors were analyzed by sequencing. True positive concordant mutations are highlighted in green.

Cancer sample	Mutation	Gene	Allele frequency in FFPE sample	Allele frequency in plasma sample
Colon 1	p.V600E	*BRAF*	12.69	13.59
	p.R273C	*TP53*	2.33	0.59
	p.G244D	*TP53*	–	8.35
Colon 2	p.R213Q	*TP53*	29.30	–
	p.R361H	*SMAD4*	32.66	–
Colon 3	p.R1114Ter	*APC*	17.89	–
	p.E1309fs	*APC*	35.57	0.50
	p.G244D	*TP53*	51.88	–
	p.R273H	*TP53*	0.57	–
	p.G245S	*TP53*	0.40	–
Colon 4	p.E1309fs	*APC*	31.78	–
	p.G12D	*KRAS*	26.41	–
Colon 5	p.E1379Ter	*APC*	58.60	0.16
	p.G245S	*TP53*	46.88	0.05
Colon 6	p.G12V	*KRAS*	34.06	0.09
Colon 7	p.E545K	*PIK3CA*	4.63	–
	p.Q546K	*PIK3CA*	11.45	–
	p.R479Q	*FBXW7*	15.08	0.34
	p.Q1291Ter	*APC*	16.41	0.09
Colon 8	p.R248W	*TP53*	50.15	0.05
	p.G12V	*KRAS*	38.15	–
Colon 9	p.R465C	*FBXW7*	–	0.05
	p.H179Y	*TP53*	–	0.05
	p.R175H	*TP53*	–	0.13
	p.R201H	*GNAS*	–	1.46
Colon 10	p.G13D	*KRAS*	23.73	0.28
	p.R248W	*TP53*	38.67	0.35
	p.Q61K	*NRAS*	–	0.06
	p.E1306Ter	*APC*	–	0.13
	p.R273L	*TP53*	–	0.07
	p.R213L	*TP53*	–	0.07
	p.R175L	*TP53*	–	0.05
	p.G510V	*SMAD4*	–	0.06
Breast 1	p.E542K	*PIK3CA*	3.12	–
	p.H1047R	*PIK3CA*	1.51	–
Breast 2	No mutations			
Breast 3	p.H1047R	*PIK3CA*	52.09	–
Breast 4	p.C238Y	*TP53*	–	0.09
	p.H1047R	*PIK3CA*	20.91	–
Breast 5	p.H1047R	*PIK3CA*	44.25	–
Breast 6	p.R273H	*TP53*	–	0.30
	p.L194R	*TP53*	–	0.19
	p.C176Y	*TP53*	40.32	–
Lung 1	CNV	*NKX2-1*	NA	NA
Lung 2	p.G12C	*KRAS*	8.99	–

Legend

CNV = copy number variation
NA = not applicable
– = not detected
True positive concordance at nucleotide change

Table 3. Composite NGS data comparing FFPE tissue vs. cfDNA isolated from plasma.

Total number of alterations detected in the FFPE tissue samples	29
Total number of alterations detected in the cfDNA samples	25
Number of unique FFPE tissue mutations not detected in cfDNA	18
Number of unique cfDNA mutations not detected in FFPE tissue	14
Number of true positive concordant* mutations	11, 25% of 44 mutations detected

* Exact same gene and nucleotide change detected in both sample types; n = 44 total alterations detected for both sample types.

NGS analysis with Oncomine panels resulted in robust detection of multiple cancer-associated mutations in all samples. However, notable discordance in the genomic alterations of the matched tumor tissue and cfDNA samples was also observed (especially with low allele frequencies). The extent of discordance varies with the cancer type, stage, and treatment status of the donors; the intrinsic heterogeneous mutation pattern in tumor tissues contributes as well. Importantly, most of the donors in this study had early stages (I–III) of cancer, and the amount of ctDNA released by tumors into circulation at earlier stages is relatively small, limiting detection.

The sensitivity of alterations detected in plasma could be further increased by isolating cfDNA from a larger plasma volume, for instance, 8–12 mL instead of 2–4 mL. Utilization of additional body fluid–derived analytes—cfRNA or protein—should be advantageous compared to analysis of cfDNA alone. Also, exploration of exosomes and other extracellular vesicles, as well as certain nucleic acid–protein complexes found in circulation, can further boost sensitivity and efficiency of the tests.

The main value of cfDNA towards diagnostics is tumor detection at an early stage, and based on the above results this will be possible for certain types of cancer, while for others it will be challenging or even impossible. At the same time, by using this method, monitoring of the effectiveness of cancer treatment (e.g., by monthly blood draws) and recurrence of cancer is straightforward. Overall, liquid biopsy will serve as an important and informative companion to solid tumor biopsy, and it has the potential to significantly change the way the medical field operates in the future.

Conclusions

- A complete workflow enabling efficient molecular profiling using clinical research samples was developed.
- Robust sample preparation tools are key to success—the MagMAX kits enable isolation of high-quality DNA, with automation on KingFisher instruments.
- Dynabeads MyOne Streptavidin C1 beads enable the enrichment of template-positive particles, improving NGS analysis and enhancing detection of rare genetic abnormalities.
- Liquid biopsy based on ctDNA/RNA has tremendous potential; however, additional studies are required to determine robust applications for various types of cancer.

References

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