Detection of Actionable Lung Cancer Fusion Genes with Known and Novel Partners from Highly Degraded FFPE Material

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INTRODUCTION

Chromosomal rearrangements resulting in expression of oncogenic chimeric proteins drive tumor progression. More than 10,000 gene fusions have been identified in cancers and many of them are strong driver oncogenes.[1] Gene fusions are promising targets for cancer therapy in various types of cancers.[2] Therefore, faithful detection of gene fusions is essential in precision medicine. Previously, fusions have been detected using FISH, RT-PCR and RNA-seq. Targeted sequencing provides better sensitivity as only the regions associated with the driver genes are sequenced, increasing possible coverage depth, and reducing cost.[3]

METHOD

We developed a targeted RNA-based OptiSeq™ lung cancer fusion panel. It utilizes 5′ RACE technology to construct a NGS library for detection of fusion transcripts present in total RNA from highly degraded samples, such as blood or FFPE samples (Figure.1). The panel is deliberately designed to cover 63 known actionable fusion genes as well as novel gene fusions. Using an in-house fusion RNA mix comprising of 52 synthetic fusion transcripts, the panel was optimized and balanced. A total of 20 lung cancer patient FFPE samples, one 6-fusion FFPE reference standard and three fusion positive and negative cell line samples were used for validating the panel.

Figure 1. Overview of OptiSeq™ lung cancer fusion NGS panel workflow

RESULTS

I. Fusion Gene Targets

63 known fusion genes were used for this NGS panel (**Table 1**)

Table1. OptiSeq™ Lung cancer fusion NGS panel targets

Actionable Target Genes	ALK AXL BRAF BRD4 CIT EGFR ERBB2 ERBB4 ETFBKMT FGFR1 FGFR2 FGFR3 LTK MET NRG1 NTRK1 NTRK2 NTRK3 PDGFRA PIK3CA METTL21A RET ROS1
Expression Control Genes	BPIFA1 CXCL17 SFTA3 SFTPA1 SFTPA2 SLC34A2

II. Analytical Sensitivity

In vitro transcribed fusion transcripts were mixed with 10 ng of normal lung tissue RNA. The amounts of fusion transcripts were correlated with the reads obtained in the fusion NGS assay. Most of targets were detected at the level of 1.0 fg (50/52, **Table2**).

Table 2. Analytical Sensitivity of OptiSeq™ Lung Cancer Fusion Panel

Amount of each target				Amount of each target				Amount of each target			
Fusion Gene	2.0 fg	1.0 fg	0.2 fg	Fusion Gene	2.0 fg	1.0 fg	0.2 fg	Fusion Gene	2.0 fg	1.0 fg	0.2 fg
KIF5B-ALK	V	V		ST7-MET	V			GOPC-ROS1	V	V	
EML4-ALK	V	V		MET e14D	V	V		SDC4-ROS1	V	V	
FGFR3-BAIAP2L1	V	V	V	RET-NCOA4	V	V		CD74-ROS1	V	V	
SLC45A3-BRAF	V	V		CD74-NRG1	V	V		EGFR-SEPTIN14	V	V	
FGFR2-CCAR2	V	V		THAP7-NRG1	V	V	V	EGFR-SHC1	V	V	
FGFR2-CIT	V	V		TFG-NTRK1	V	V		FGFR2-SHTN1	V	V	
APPL2-CIT	V	V	V	TPM3-NTRK1	V	V		FGFR1-SLC20A2	V	V	
EGFR vIII	V	V		LMNA-NTRK1	V	V	V	BRAF-SLC26A4	V	V	
ERBB2 e16D	V	V		TRIM24-NTRK2	V	V	V	FGFR3-TACC3	V	V	V
EZR-ERBB4	V	V		ETV6-NTRK3	V	V		MET-WNT2	V	V	V
KDM6A-ETFBKMT	V	V		BRD4-NUTM1	V	V					
CIT-FAM222A	V	V		CLOCK-PDGFRA	V	V		Expression Control Gene			
BAG4-FGFR1	V	V	V	SCAF11-PDGFRA	V	V		BPIFA1	V	V	V
NSD3-FGFR1	V	V	V	TBL1XR1-PIK3CA	V	V		CXCL17	V	V	V
CCAR2-FGFR2	V	V		METTL21A-PLEKHM3	V	V	V	SFTA3	V	V	V
CLIP1-LTK	V	V		CCDC6-RET	V	V		SFTPA1	V	V	V
AXL-MBIP	V	V		KIF5B-RET	V			SFTPA2	V	V	V
KIF5B-MET	V	V		NCOA4-RET	V	V		SLC34A2	V	V	V

III. Verification of Clinical Samples

First, we tested FFPE reference and cell line with this NGS panel and confirmed it worked well (**Figure 2**); Second, we verified this panel with patient FFPE samples, two fusion genes and one splicing variant were found in these samples (**Figure 3.**)

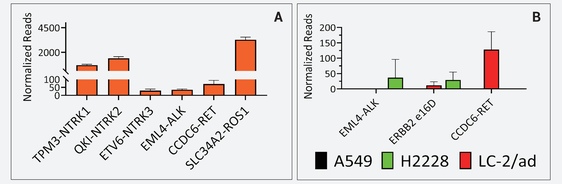

Figure 2. Detection of Fusion genes in FFPE reference standard and cell lines.(A) Fusion detection in Pan-cancer 6 fusion panel FFPE RNA reference standard (HD834) (B) Fusion detection in lung cancer cell lines. (A549: fusion negative, LC-2/ad: CCDE-RET, and H2228: EML4-ALK)

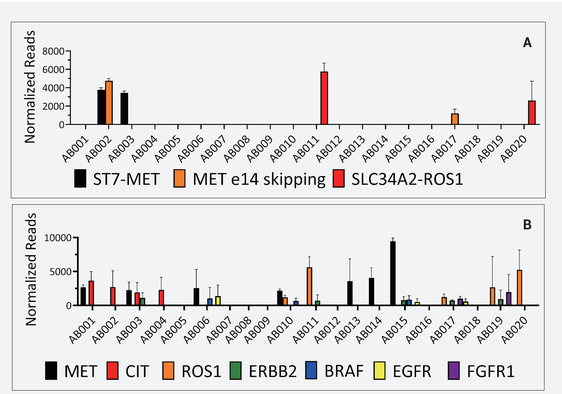

Figure 3. Detection of Fusion genes in Patients FFPE samples (A) Among twenty patient samples, 5 samples showed ST7-MET, MET exon14 skipping, or SLC34A2-ROS1 fusions. (B) Overexpression of driver oncogenes compared to normal patients. Seven different receptor tyrosine kinases were highly expressed in patient FFPE samples.

CONCLUSIONS

- Limit of detection (LoD) was determined using the in-house RNA mix and normal lung tissue RNA. The minimum RNA input to detect all 52 fusion transcripts was 2.0 fg of fusion transcripts in the background of 10 ng of normal lung tissue RNA.
- The panel successfully detected all six fusion transcripts with 10 ng of RNA from a 6-fusion reference FFPE sample.
- With this confirmation, we tested clinically relevant sample types which are damaged RNAs from FFPE materials. The result shown that fusion genes were identified in five patients.
- This study demonstrates the welly performance of our newly developed NGS OptiSeq™ Lung Cancer fusion gene panel with clinical samples.
- It accommodates highly degraded RNA samples with low amounts of RNA input.
- The next step would be to expand the testing to include more samples and cell-free RNAs.

REFERENCE

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2. Advanced-stage non-small cell lung cancer: Advances in thoracic oncology. Remon et al. J. Thorac. Oncol. 2019, 14, 1134-1155.
3. Overview of fusion detection strategies using next-generation sequencing. Murray, S.S. In Tumor Profiling, 2019, 1908, 125-138.

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