Rapid diagnosis of liquid biopsy in non-small cell lung cancer by the EGFR-LAMP assay

2021 ESMO congress 1794P 16-21 SEPTEMBER 20..

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Abstract

Background: Liquid biopsy has been adopted into one of diagnostic tests of EGFR mutation for patients with advanced or metastatic non-small cell lung cancer (NSCLC). Loop-mediated isothermal amplification (LAMP) has been widely used for the rapid detection in virology or bacteriology, which allows quick amplification of DNA (60 min) with certain cost-effectiveness (WHO recommendations on the use of TB-LAMP presents the price per test is 7 euros). Theoretically, LAMP could enable us to make a quick diagnosis of oncogene as well. Here we developed a set of unique primers for detecting EGFR mutations, and investigated the efficacy of EGFR-LAMP assay using plasma samples of patients with resected NSCLC tumor.

Methods: All samples were collected from patients with suspected primary lung cancer at Saitama Cardiovascular and Respiratory Center between January 2019 and September 2020. All tumor tissues were taken by surgery or surgical biopsy, and the EGFR status of them were investigated by the Therascreen EGFR PCR Kit. Among them, only cases with EGFR mutated tumors were selected for further investigation. The LAMP and NGS assays were conducted for DNA products extracted from preoperative plasma samples. The detection rates of both assays were calculated and compared each other.

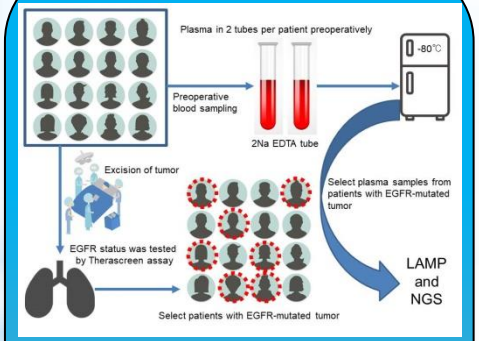
Results: Among 57 EGFR mutated tumors or metastatic lymph nodes, 51 preoperative plasma specimens were available for investigation of EGFR status by the NGS and the LAMP assays. The NGS assay detected only 2 EGFR-mutated samples (2/51), one of which showed EGFR mutation by the LAMP assay (1/51). The detection rates of EGFR mutation were extremely low in both assays (1.9% in the LAMP assay, and 3.9% in the NGS assay, respectively). The two cases were advanced papillary adenocarcinoma showing IIIA and IVA in pathological stage, while most of remaining cases consisted of stage I-II lung cancer (44/49).

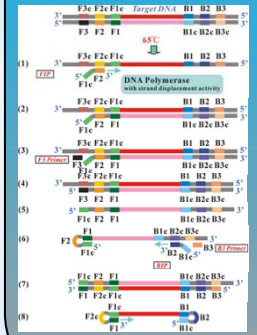
CONCLUSIONS: This is the first report of LAMP liquid biopsy detecting oncogene in a plasma sample. The EGFR-LAMP assay similar performance to the NGS assay in terms of detecting EGFR mutation in NSCLC tumors. The LAMP assay has advantage of time-saving, cost-effective, and simple test compared to the NGS assay. However, further investigation is required for development of more sensitive assay.

Disclosures

Atsuka Matsui and Satoru Michiyuki are employees of Eiken Chemical Co., Ltd.

Methods





LAMP assay

The LAMP reaction takes place isothermally in the three steps shown, 1) starting material production step, 2) cycling amplification step, and 3) elongation and recycling step, by using of polymerase with strand displacement activity.

Tsugunori Notomi et al. Nucleic Acids Research, Vol.28, No.12, e63, 2000

Results

Figure 1 Basic performance of EGFR-LAMP assay

LAMP can detect ctDNA over 5% VAF* in 10,000 copies, certainly

*VAF; variant allele frequency

Table 1 Characteristics of patients

Characteristic	N (%)
Age, years	68.7 ± 8.4
Gender	
Male	20 (39.2)
Female	31 (70.8)
Smoking Status	
Never smoker	22 (43.1)
Former smoker	23 (45.1)
Current smoker	6 (11.8)
Subtype of adenocarcinoma	
Papillary predominant	45 (88.0)
Lepidic predominant	4 (8.0)
Micropapillary predominant	1 (2.0)
Solid predominant	1 (2.0)
Pathological stage	
pIA1	12 (24.0)
pIA2	21 (41.0)
pIA3	6 (12.0)
pIB	2 (4.0)
pIIB	3 (6.0)
pIIIA	4 (8.0)
pIIIB	1 (2.0)
pIVA	1 (2.0)
pIVB	1 (2.0)

Data on age: mean ± SD.

Figure 2 Amount of Plasma cfDNA

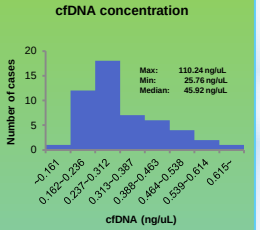


Table 2 Liquid biopsy

LAMP \ NGS	positive	negative	sum
positive	1	0	1
negative	1	49	50
sum	2	49	51

Sensitivity=2.0%
Specificity=98.0%
Accuracy=2.0%

Table 3 Positive cases

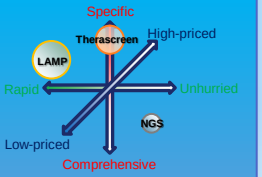
	Case 1	Case 2
Tumor sample		
Therascreen	L858R	L858R
LAMP	L858R	negative
Plasma sample		
LAMP	L858R	negative
NGS	L858R	L858R
P-stage	IVB	IIIA

Discussions

LAMP assay provides considerable features, below

1. High specificity and sensitivity (Biology 2020, 9, 182)
2. Rapidity (Examination time is about 60 min)
3. Simplicity and effortless (in low-resource laboratory settings)
4. Cost-effectiveness (TB-LAMP is recommended by WHO guideline)

Main disadvantage
the complexity of primer design



- Low sensitivity is a major problem of both LAMP and NGS liquid biopsy.
- It is necessary to investigate adaptation conditions of liquid biopsy.
- NGS assay could be suitable for comprehensive screening of oncogene at the time of initial.
- LAMP assay could be useful for monitoring patient therapy and changing it because of it's low-price and rapidity.

Conclusions

- **This is the first report of EGFR-LAMP liquid biopsy**
- **Both LAMP and NGS assays demonstrated similar performance of liquid biopsy**
- **Time-saving, cost effectiveness, and simple procedure are expected in LAMP assay**
- **Further study is required for improvement of the sensitivity**

Acknowledgements

This study is sponsored by Eiken Chemical Co., Ltd.

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