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Quantitative cell-free circulating *EGFR* mutation concentration is correlated with tumor burden in advanced NSCLC patients

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ABSTRACT

Objectives: Droplet digital polymerase chain reaction (ddPCR) has shown sufficient concordance in detecting plasma epidermal growth factor receptor (*EGFR*) status in non-small cell lung cancer (NSCLC), compared to tumor tissues. However, the clinical significance of the quantitative plasma mutated *EGFR* concentration remains unknown. The purpose of this study was to explore the relationship of plasma mutated *EGFR* concentration with tumor burden in advanced NSCLC patients.

Materials and methods: Using ddPCR, plasma DNA samples prior to administration of therapies from 113 consecutive NSCLC patients were analyzed for *EGFR* L858R substitution and deletion of exon19 (ex19del). Plasma *EGFR* status was compared to tumor *EGFR* status to determine concordance. Then, we assessed the correlation of plasma mutated *EGFR* concentrations with tumor burden and other tumor characteristics.

Results and conclusion: Compared to tumor *EGFR*, the concordance rate of plasma and tissue *EGFR* status was 86.73%. Of the 64 patients who harbored tumor *EGFR* mutation, plasma mutated *EGFR* concentrations significantly correlated with number of metastatic sites (Spearman's $r = 0.4954$, $p < 0.0001$), number of lesions (Spearman's $r = 0.4484$, $p = 0.0002$), and sum of measurable lesions' diameters (Spearman's $r = 0.3539$, $p = 0.0048$). Number of metastatic sites was independently associated with mutated *EGFR* concentration in multiple linear regression. Besides, plasma mutated *EGFR* concentrations were significantly higher in those with extensive tumor burden (median concentration, 386.9 vs. 13.4 copies/mL; $p < 0.0001$) and stage IV disease (median concentration, 244.2 vs. 0 copies/mL; $p = 0.0252$). In conclusion, mutated plasma *EGFR* concentration determined by ddPCR analysis significantly correlated with tumor burden.

1. Introduction

Recently, circulating cell-free DNA (cfDNA) has attracted greater attention for its minimally invasive nature, and droplet digital polymerase chain reaction (ddPCR) is a quantitative analysis technology that can be used to detect mutated cfDNA allele concentration. Studies have reported that ddPCR could achieve an accordance of 80–90% in detecting plasma epidermal growth factor receptor (*pEGFR)* status, compared with tumor *EGFR* (*tEGFR*) status, in non-small cell lung cancer (NSCLC) [1]. However, as a quantitative assay, the clinical significance of *pEGFR* mutation *(pEGFRmut)* concentration is still unknown. In this study, we evaluated using ddPCR, the quantitative *EGFR* L858R mutation and exon19 deletion (ex19del) status of cfDNA from NSCLC patients' plasma, aiming to explore the relationship of *pEGFR* mutation concentration with tumor burden.

2. Materials and methods

2.1. Patients and treatments

We prospectively studied all prior untreated NSCLC patients, who were admitted in the Department of Oncology of Guangdong Provincial Hospital of Chinese Medicine from October 2014 to May 2016. Patients who had uncontrolled other malignant tumors, uncontrolled infection or *Mycobacterium tuberculosis*, or severe mental disease were excluded from this study. If the patients' *tEGFR* status had been tested in other qualified hospitals, we recorded only the results. Otherwise, the *EGFR* status in formalin-fixed, paraffin-embedded specimens was detected in

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our hospital's Department of Pathology with the amplification refractory mutation system (ARMS). All patients provided written informed consent for this study and the *EGFR* gene test. This study was approved by the Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine.

2.2. Plasma collection and ddPCR workflow

For each patient, plasma was collected before first-line therapy. 6–10 mL of whole venous blood were collected into EDTA-containing vacutainers, and centrifuged for 10 min at 1800*g* and 4 °C within 6 h of collection. Plasma was frozen at −80 °C until use. Before DNA extraction, plasma was further cleared by centrifugation for 10 min at 3000*g* and 4 °C. Circulating cell free DNA was isolated using the QIAmp circulating nucleic acid kit (Qiagen) according to the manufacturer's protocol. DNA was eluted in AVE buffer (100 uL) and stored at −80 °C until use. The ddPCR workflow, detailed in another paper by our group [2], was conducted at WuXi AppTec Medical Testing Institute (Shanghai) Co., Ltd.

2.3. Statistical analysis

First, we compared *pEGFR* status with matched tumors to determine the concordance. Second, we assessed the relationship between the *pEGFRmut* concentration and number of metastatic sites, number of lesions, and measurable lesions burden, using the Spearman rank correlation coefficient, with an alpha < 0.05. Metastatic sites were defined as pleura, contralateral lung, extrathoracic lymph nodes, bone, skin, omentum, brain, and any visceral organ, according to the American Joint Committee on Cancer (AJCC) staging manual, 7th edition. All of measurable and non-measurable lesions were included for the counting of number of lesions. Those with more than 10 lesions were recorded as ten. Measurable lesions burden were measured as the sum of the longest diameters, according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Multiple linear regression was used to define the independent correlated factor for *pEGFRmut* concentrations.

3. Results

3.1. Concordance of pEGFR with matched tEGFR status

A total of 113 patients with pathologically diagnosed NSCLC were enrolled, including 64 who harbored mutated *tEGFR* and 49 who harbored wild *tEGFR (Supplementary* Table 1). Compared to *tEGFR* status, the sensitivity and specificity of *pEGFR* status by ddPCR were 75.61% (31/41) and 98.61% (71/72) for L858R and 82.61% (19/23) and 100% (90/90) for ex19del, respectively. One sample with a false-positive result was detected with a very low concentration of mutant L858R in plasma (5.8 copies/mL).

3.2. Association with tumor characteristics

The median cfDNA, *pEGFRmut*, mutant L858R, and ex19del concentrations in plasma of the 64 patients who harbored mutated *tEGFR* are shown in Table 1. Cell-free DNA, *pEGFRmut,* and L858R concentrations were significantly higher in patients with bone metastasis, while a similar trend was also shown in ex19del (Table 1). No significant relationship was found for cfDNA or *pEGFRmut* concentration with demographic or other clinical characteristics, except that patients with poorer ECOG performance status and brain metastasis had higher cfDNA concentrations, and those with contralateral lung metastasis had higher ex19del concentrations (Table 1).

Table 1
Relationship of baseline mutant *pEGFR* concentration with demographic and clinical characteristics.

Characteristics	cfDNA (ng/ul) (n = 64) median(25%–75% percentile)	*pEGFR* (copies/ml,n = 64) median(25%–75% percentile)	L858R (copies/ml, n = 41) median(25%–75% percentile)	ex19del (copies/ml, n = 23) median(25%–75% percentile)
Total (n = 64)	0.36 (0.20–0.57)	186.9 (4.2–494.25)	200 (4.6–477.4)	75 (3.8–607.1)
Gender	p = 0.7779 [a]	p = 0.3835 [a]	p = 0.4273 [a]	p = 0.7631 [a]
Male (n = 25)	0.34 (0.18–0.55)	244.2 (28–477.4)	252.1 (28–477.4)	75 (7–607.1)
Female (n = 39)	0.38 (0.23–0.76)	123.3 (0–625.7)	184.2 (0–625.7)	87.05(3.35–568.55)
Smoking history	p = 0.5767 [a]	p = 0.2271[a]	p = 0.3264 [a]	p = 0.7144 [a]
Yes (n = 16)	0.43(0.23–0.56)	230 (68.2–439.4)	230 (106.55–428.7)	238.2 (37.5–2560.7)
No (n = 48)	0.33(0.20–0.70)	92.85 (0–559.1)	184.2 (0–511.1)	50.8(3.8–607.1)
ECOG performance status	**p = 0.0022a**	p = 0.1284[a]	**p = 0.0144a**	p = 0.6805[a]
0-1 (n = 56)	0.31 (0.19–0.52)	113.35 (3.35–449.4)	167.95 (0–409.15)	62.9 (5–693.55)
2–4 (n = 8)	0.50 (0.99–1.55)	368.55 (183.75–2975.05)	2409.1 (380–3541)	123.3 (0–357.1)
Contralateral lung metastasis	p = 0.1180[a]	p = 0.3707[a]	p = 0.4781[a]	**p = 0.0109a**
Yes (n = 27)	0.42 (0.30–0.64)	260 (6.2–625.7)	222.1 (0–386.9)	780(123.3–4750)
No (n = 37)	0.28 (0.17–0.57)	61.4 (3.8–401.4)	184.2 (14.2–872.7)	38.55(1.45–283.95)
Bone metastasis	**p = 0.0007a**	**p = 0.0001a**	**p = 0.0005a**	p = 0.0632[a]
Yes (n = 33)	0.51 (0.28–1.21)	380(244.2–1222.2)	378.45 (244.2–872.7)	607.1 (6.2–4720)
No (n = 22)	0.25 (0.14–0.4)	34.15 (0–184.2)	12.6 (0–184.2)	50.8 (0–300)
Brain metastasis	**p = 0.0078a**	p = 0.1318[a]	p = 0.1800[a]	p = 0.4243[a]
Yes (n = 23)	0.55 (0.32–0.83)	357.1 (12.6–780)	353.45(101.1–866.65)	357.1 (6.2–780)
No (n = 35)	0.28 (0.17–0.45)	151.7 (0–380)	167.95(0–380)	75 (3.8–300)
Liver metastasis	p = 0.9706[a]	p = 0.1679[a]	p = 0.3473[a]	p = 0.3314[a]
Yes (n = 11)	0.28(0.17–1.33)	267.9 (75–2409.1)	270 (151.7–2409.1)	267.9 (75–780)
No (n = 51)	0.34(0.21–0.57)	123.3 (0–421.4)	189.6 (0–421.4)	45.55 (3.8–401.4)
Clinical stage	**p = 0.0053a**	**p = 0.0252a**	p = 0.2167[a]	**p = 0.0381a**
Non-IV stage (n = 7)	0.14 (0.06–0.3)	0 (0–184.2)	28(0–184.2)	0 (0–150)
IV stage (n = 57)	0.4 (0.25–0.64)	244.2 (7–607.1)	252.1(4.6–511.1)	123.3 (7–780)
Baseline tumor burden	**p = 0.0001a**	**P < 0.0001a**	**p = 0.0002a**	**p = 0.0243a**
Extensive [b] (n = 36)	0.51 (0.29–1.07)	386.9(194.8–1047.45)	386.9 (222.1–846.35)	482.1 (64.75–3255)
Non-extensive (n = 28)	0.25 (0.14–0.38)	13.4 (0–61.9)	12.6(0–61.4)	36.8 (0–75)

pEGFR, Epidermal growth factor receptor gene status in plasma; cfDNA, cell free DNA; ex19del, exon19 deletion.

[a] Rank sum test.

[b] Extensive tumor burden, diffuse involvement of one organ (≥5 lesions) or ≥3 organs involved.