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Identification of Mutations Associated with Response to Pemetrexed-Based Chemotherapy in Lung Adenocarcinoma Using Next-Generation Sequencing

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Abstract

Purpose: Pemetrexed, an inhibitor of Thymidylate Synthase (TS) and other folate-dependent enzymes, is used as standard of treatment for patients with advanced non-squamous non-small-cell lung cancer. We tried to identify SNPs associated with response to pemetrexed-based chemotherapy in lung adenocarcinoma.

Patients and Methods: We retrospectively reviewed the metastatic lung adenocarcinoma patients who received pemetrexed-based chemotherapy at thoracic surgery department of PLA general hospital between February 2013 and December 2018. The patients with Complete Response (CR) or Partial Response (PR) were sorted to the good response group, while those with Stable Disease (SD) or Progression Disease (PD) to the no response group. Illumina sequencing system was applied to sequence the genome of tumor tissues.

Results: Of 19 enrolled patients, 14 patients showed PR and were sorted to the good response group, and the 5 patients experienced SD or PD and were regarded as having no response. Eleven SNPs in nine genes are significantly associated with good response to pemetrexed-based chemotherapy, while twenty-two SNPs in fifteen genes with no response. Eleven SNPs in nine genes are significantly associated with good response to pemetrexed-based chemotherapy, while twenty-two SNPs in fifteen genes with no response. Seven SNPs in gene UGT1A5 (UDP glucuronosyltransferase family 1 member A5) were demonstrated to associated with no response simultaneously.

Conclusion: Few mutations were found to be associated with response to pemetrexed-based chemotherapy in lung adenocarcinoma. Perspective clinical research was warranted to obtain the sensitivity and specificity of these mutations as predictive biomarkers.

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Introduction

In China, lung cancer has evolved to be the leading cause of cancer death both in men and women [1]. Although, the past two decades have witnessed the great progresses in the field of lung cancer treatment, such as advent of molecularly targeted therapies and immune check point inhibitors, there is still a remarkable portion of non-small-cell lung cancer patients who do not harbor the druggable mutation or response to the immune check point inhibitors. Herein, to these patients and to those who response firstly to molecularly targeted therapies and get resistance subsequently, chemotherapy is still the footstone treatment.

Pemetrexed, an inhibitor of Thymidylate Synthase (TS) and other folate-dependent enzymes, such as dihydrofolate reductase and glycinamide ribonucleotide formyltransferase, is used as standard of treatment for patients with advanced non-squamous non-small-cell lung cancer. For patients with non-squamous lung cancer, cisplatin-pemetrexed combination is superior to cisplatin-gemcitabine in term of median Overall Survival (OS) [2]. Both switch and continuation maintenance treatment with pemetrexed after induction chemotherapy was proved to improve PFS and OS compared to placebo in NSCLC patients [3-5]. Recently, the Keynote 021 and 189 trial, which explored the activity of pembrolizumab (an anti-PD-1) in addition to pemetrexed and platinum compounds doublet, demonstrated that the combination can translated into OS and PFS benefit in advanced non-squamous NSCLC patients [6,7]. Moreover, several second-line trails of pemetrexed has showed positive results regarding OS and PFS [8-10].

Although elevated mRNA expression of TS in cultured cancer cell lines was reversely correlated with sensitivity to pemetrexed [11-14], it remained controversial that TS protein expression levels

can predict the clinical efficacy of pemetrexed-based chemotherapy [15-19]. SNP rs11545077 (c.91G>T p. Ala31Thr) in the GGH gene was demonstrated to be significantly associated with the therapeutic effects of pemetrexed in NSCLC patients [20]. A predictive peptide model, using MS serum peptidome profiling, can predict effectiveness of pemetrexed-based regimen in patients with advanced lung adenocarcinoma [21]. The plasma miR-25 expression level, which was measured by microarray technique, can predict the insensitivity of pemetrexed and platinum-based chemotherapy with high degree of accuracy [22]. The polymorphisms in TS and MTHFR genes seem to be molecular predictor factors for pemetrexed-based front-line chemotherapy in non-squamous NSCLC patients [23]. In one latest research, which investigated the relationship between promoter methylation status of RAS association domain family (RASSF1A) in BAL (bronchoalveolar lavage) and response to pemetrexed-based chemotherapy, unmethylated RASSF1A is regarded as a favorable prognostic indicator for patients receiving pemetrexed doublets [24]. However, in spite of a few of researches that were devoted to identify predictive biomarkers of pemetrexed-based chemotherapy, none of these biomarkers was approved to be used as a routine test.

We hypothesized that genetic variation was the key mechanism underlying the sensitivity to pemetrexed-based chemotherapy in NSCLC. To test this hypothesis, using next generation sequencing, we sequenced and compared the genome sequences of tumor tissues from the good response group and the no response group.

Patients and Methods

Patient eligibility

We retrospectively reviewed the metastatic lung adenocarcinoma patients who received pemetrexed-based chemotherapy at thoracic surgery department of PLA general hospital between February 2013 and December 2018. The inclusion criteria are listed below: Patients aged 18 years or older; pathologically verified lung adenocarcinoma; stage III or IV; at least two cycles of pemetrexed plus cisplatin/carboplatin chemotherapy were administrated as first-line treatment; evaluable lung tumor on CT scanning; the eastern cooperative oncology group performance status was 0-2; archived tumor tissue is enough for DNA extraction and subsequent next-generation sequencing. Tumor tissues were obtained by CT-guided lung biopsy, bronchoscopy or Endobronchial Ultrasound Guided-Transbronchial Needle Aspiration (EBUS-TBNA).

After every two cycles of chemotherapy, the treatment response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The screened patients were sorted to the good response group, which included the patients who achieved complete or partial response, and the no response group consisting of patients with stable or progression disease. The institutional review board approved this study, and all the patients involved provided informed consent.

DNA extraction and next-generation sequencing

The genomic DNA was extracted from archived FFPE samples using Pure Link Genomic DNA Mini Kit according to the manufacturer's instructions. Qubit® 4.0 Fluorometer and Agarose gel electrophoresis system were used to measure the quantity of the extracted DNA.

The DNA library was prepared using a paired-end DNA sample prep kit (Illumina, Inc., San Diego, CA, USA) and following the manufacturer's instructions. An Agilent Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA, USA) was used to detect the fragment size and yield, and the results of the library revealed that it contained the expected size and yield. Following quality control, the library generated was used in the cBot system for cluster generation and the samples were then sequenced using the Illumina sequencing system (Nova Seq 6000 platform; Illumina, Inc.), which is based on sequencing by synthesis technology.

Bioinformatic and statistical analysis

Sequencing quality of the whole genome sequencing reads from all samples was checked using Fast QCv0.11.8 [25]. The Burrows-Wheeler Aligner (BWA) v0.7.15 was utilized to align sequencing reads to the human reference genome GRCh37 (hg19) [26], and the Genome Analysis Toolkit (GATK) v3.8 was used for SNP calling [27]. Subsequently, the detected variants were filtered by the GATK filter expression "QD<2.0 || FS>60.0 || MQ<40.0 || Haplotype Score >13.0 || Mapping Quality Rank Sum < -12.5|| Read Pos Rank Sum <-8.0". The filtered SNPs were annotated with the ANNOVAR tool (version 20191024) [28], and only non-synonymous SNPs were considered for further analysis. To identify non-synonymous SNPs with a significance difference between the sensitive and control groups, Fisher's exact test was performed by the statistical software "R" v3.6.0 [29]. It was considered statistical significance while p value is greater than 0.01.

Results

Participant characteristics

After screening 210 metastatic lung adenocarcinoma patients, there are only 19 patients who fulfill the criteria of this study. Among the 19 patients, 14 patients showed PR and were sorted to the good response group, and the remaining 5 patients experienced SD or PD and were regarded as having no response. The median age of patients in the good response group and in the no response group was 51.0 (range, 28 to 68 years) and 54.0 (range, 42 to 64 years) respectively. There are 9 (64.3%) male patients in the good response group and 4 (80.0%) male patients in the no response group. In the good response group, 3 (21.4%) patients were classified into the clinical stage III and the rest into stage IV. All the patients in the no response group were classified into the clinical stage IV.

Next generation sequencing and genotypes related to treatment sensitivity

The average depth of next generation sequencing is 30, and 99.89% of reads were mapped to the reference genomic sequence. Averagely, 4×10^6 SNPs were detected in one sample. Eleven SNPs in nine genes are significantly associated with good response to pemetrexed-based chemotherapy, while twenty-two SNPs in fifteen genes with no response. The relationships between good response and genotypes are presented in Table 1, and Table 2 displays the relationships between no response and genotypes.

Discussion

In the era of personalized medicine, introduction of predictive biomarkers into clinical practice made it possible to identify the patients who may response to the targeted therapy of lung cancer, to improve the effectiveness and to avoid unnecessary treatment associated toxicities. Unfortunately, discovery and validation of biomarker is a strenuous and prohibitive task, for according to previous experience, only 3% to 5% investigated biomarkers entered

Table 1: The relationships between good response and genotypes.

Gene	SNP	Good Response	No Response	*p* value
KATNAL2	rs7233515	12/13(92.3%)	1/5(20.0%)	0.0077
KIR3DL1	rs45556431	12/13(92.3%)	1/5(20.0%)	0.0077
	rs1049150	12/13(92.3%)	1/5(20.0%)	0.0077
NEK11	rs16836266	11/13(84.6%)	0	0.0025
NLRP5	rs10409555	11/13(84.6%)	0	0.0025
OR2F2	rs2240359	12/13(92.3%)	1/5(20.0%)	0.0077
OR8G5	rs2512168	12/13(92.3%)	1/5(20.0%)	0.0077
	rs2512167	12/13(92.3%)	1/5(20.0%)	0.0077
PDZD2	rs157496	12/13(92.3%)	1/5(20.0%)	0.0077
RAET1G	rs9397449	12/13(92.3%)	1/5(20.0%)	0.0077
SLC22A4	rs272893	11/13(84.6%)	0	0.0025

Table 2: The relationships between no response and genotypes.

Gene	SNP	No Response	Good Response	*p* value
ADSSL1	rs80097179	4/5(80.0%)	0	0.0016
AQP10	rs6668968	5/5(100.0%)	3/13(23.1%)	0.0065
	rs6685323	5/5(100.0%)	3/13(23.1%)	0.0065
CETN1	rs61734344	4/5(80.0%)	1/13(7.7%)	0.0077
DBF4	rs2041049	4/5(80.0%)	0	0.0016
ISG20L2	rs3795737	5/5(100.0%)	2/13(15.4%	0.0025
KLK4	rs1654551	4/5(80.0%)	1/13(7.7%)	0.0077
MYO5C	rs62623565	4/5(80.0%)	0	0.0016
	rs55712142	4/5(80.0%)	0	0.0016
PRSS3	rs201773718	4/5(80.0%)	0	0.0016
SCNN1D	rs6690013	4/5(80.0%)	0	0.0016
SVEP1	rs3818764	4/5(80.0%)	1/13(7.7%)	0.0077
TARP	rs1053760	4/5(80.0%)	1/13(7.7%)	0.0077
TYMP	rs11479	4/5(80.0%)	1/13(7.7%)	0.0077
UGT1A5	rs3755323	4/5(80.0%)	1/13(7.7%)	0.0077
	rs3755322	4/5(80.0%)	1/13(7.7%)	0.0077
	rs3755321	4/5(80.0%)	1/13(7.7%)	0.0077
	rs17862867	4/5(80.0%)	1/13(7.7%)	0.0077
	rs2012736	4/5(80.0%)	1/13(7.7%)	0.0077
	rs17862868	4/5(80.0%)	1/13(7.7%)	0.0077
	rs3892170	4/5(80.0%)	1/13(7.7%)	0.0077
ULK4	rs3774372	5/5(100.0%)	3/13(23.1%)	0.0065

the clinic eventually [30-32]. Furthermore, most research aimed to identify biomarkers focused on the targeted therapy of lung cancer, while little attention was paid to biomarker of chemotherapy. So, until now, there is no approved biomarker of chemotherapy of lung cancer, including pemetrexed-based chemotherapy. To our knowledge, this paper reported the first research in which the next generation sequencing was used to identify the SNPs related to response to pemetrexed-based chemotherapy.

All the identified SNPs in this study are related to response to pemetrexed-based chemotherapy for the first time. Interestingly, seven SNPs in gene UGT1A5 (UDP glucuronosyltransferase family 1 member A5) were demonstrated to associated with no response simultaneously. The gene UGT1A5 encodes a UDP-glucuronosyltransferase, which is an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. Previous studies also revealed that the gene UGT1A5 related to lung and stomach cancer [32,33]. As exampled by EGFR TKI scenario, the sensitive mutations can lead to response to targeted therapy of lung cancer. In this study, we have shown that mutations may be a potential mechanism conferring resistance to pemetrexed-based chemotherapy, while other patients without this mutation remain sensitive to the treatment.

Definitely, this study has several shortcomings: Firstly, it is a retrospective research, so it has inherent weakness, and perspective clinical trials are warranted in the future; secondly, because the patient numbers in the two groups are imbalanced, this imbalance will incur severe statistical bias. Adding more patients to group with no response to pemetrexed-based chemotherapy was considered as a priority of future research; thirdly, although all the patients in the good response group achieved PR according to the RESICT, they responded to the treatment with different degree, indicating the good response group a heterogeneous patient population. Finally, the agents combined with pemetrexed in this study was cisplatin or carboplatin, therefore this combination therapy can obscure the association between pemetrexed and the biomarkers screened.

In the future research, the biospecimens should be handled according to the best-practice guideline for biospecimen resources [16], which provides detailed instructions to tissue acquisition, processing and banking. Furthermore, the intratumoural heterogeneity, characterized by diversity of cell types in solid tumors, must be taken into consideration in the research of biomarkers; Laser Capture Micro-dissection (LCM) technology can be utilized to select tumor cells to next generation sequencing. More attention and resources should be paid to the development of predictive biomarkers of chemotherapy, thereby ensuring the patients best treatment options and reducing the health costs.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-32.
2. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small-cell lung cancer. J Clin Oncol. 2008;26(21):3543-51.
3. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, et al. Maintenance Pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: A randomised, double-blind, phase 3 study. Lancet. 2009;374:1432-40.
4. Paz-Ares L, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): A double-blind, phase 3, randomised controlled trial. Lancet Oncol. 2012;13(3):247-55.
5. Paz-Ares L, De Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. PARAMOUNT: Final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. J Clin Oncol. 2013;31(23):2895-902.
6. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik

A, Powell SF, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamousnon-small-cell lung cancer: A randomised, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol. 2016;17(11):1497-508.

7. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, Angelis FD, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl JMed. 2018;378(22):2078-92.

8. Smit EF, Mattson K, von Pawel J, Manegold C, Clarke S, Postmus PE. ALIMTA (pemetrexed disodium) as second-line treatment of non-small-cell lung cancer: A phase II study. Ann Oncol. 2003;14(3):455-60.

9. Russo F, Bearz A, Pampaloni G, The Investigators of The Italian Pemetrexed monotherapy of NSCLC group. Pemetrexed single agent chemotherapy in previously treated patients with locally advanced or metastatic non-small cell lung cancer. BMC Cancer. 2008;8:216.

10. Hanna N, Sheperd FA, Fossella FV, Pereira JR, Marinis FD, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol. 2004;22(9):1589-97.

11. Hanauske AR, Eismann U, Oberschmidt O, Pospisil H, Hoffmann S, Hanauske-Abel H, et al. *In vitro* chemosensitivity of freshly explanted tumor cells to pemetrexed is correlated with target gene expression. Investig New Drugs. 2007;25(5):417-23.

12. Sigmond J, Backus HHJ, Wouters D, Temmink OH, Jansen G, Peters GJ. Induction of resistance to the multitargeted antifolate Pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. Biochem Pharmacol. 2003;66(3):431-8.

13. Takezawa K, Okamoto I, Okamoto W, Takeda M, Sakai K, Tsukioka S, et al. Thymidylate synthase as a determinant of pemetrexed sensitivity in non-small-cell lung cancer. Br J Cancer. 2011;104(10):1594-601.

14. Ozasa H, Oguri T, Uemura T, Miyazaki M, Maeno K, Sato S, et al. Significance of thymidylate synthase for resistance to pemetrexed in lung cancer. Cancer Sci. 2010;101(1):161-6.

15. Christoph DC, Asuncion BR, Hassan B, Tran C, Maltzman JD, O'Shannessy DJ, et al. Significance of folate receptor alpha and thymidylate synthase protein expression in patients with non-small-cell lung cancer treated with pemetrexed. J Thorac Oncol. 2013;8(1):19-30.

16. Wynes MW, Konopa K, Singh S, Reyna-Asuncion B, Ranger-Moore J, Sternau A, et al. Thymidylate synthase protein expression by IHC and gene copy number by SISH correlate and show great variability in non-small cell lung Cancer. J Thorac Oncol. 2012;7(6):982-92.

17. Sun JM, Han J, Ahn JS, Park K, Ahn MJ. Significance of thymidylate synthase and thyroid transcription factor 1 expression in patients with nonsquamous non-small cell lung cancer treated with pemetrexed-based chemotherapy. J Thorac Oncol. 2011;6(8):1392-9.

18. Nicolson MC, Fennell DA, Ferry D, O'Byrne K, Shah R, Potter V, et al. Thymidylate synthase expression and outcome of patients receiving pemetrexed for advanced nonsquamous non-small-cell lung cancer in a prospective blinded assessment phase II clinical trial. J Thorac Oncol. 2013;8(7):930-9.

19. Shimizu T, Nakanishi Y, Nakagawa Y, Tsujino I, Takahashi N, Nemoto N, et al. Association between expression of thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase and efficacy of pemetrexed in advanced non-small cell lung cancer. Anticancer Res. 2012;32(10):4589-96.

20. Zhang X, Zhang D, Huang L, Li G, Chen L, Ma J, et al. Discovery of novel biomarkers of therapeutic responses in Han Chinese pemetrexed-based treated advanced NSCLC patients. Front Pharmacol. 2019;10:944.

21. Wang L, Tang C, Xu B, Yang L, Qu L, Li L, et al. Mass spectrometry-based serum peptidome profiling accurately and reliably predicts outcomes of pemetrexed plus platinum chemotherapy in patients with advanced lung adenocarcinoma. PLoS One. 2017;12(6):e0179000.

22. Zhu J, Qi Y, Wu J, Shi M, Feng J, Chen L. Evaluation of plasma microRNA levels to predict insensitivity of patients with advanced lung adenocarcinomas to pemetrexed and platinum. Oncol Lett. 2016;12(6):4829-37.

23. Krawczyk P, Kucharczyk T, Kowalski DM, Powrózek T, Ramlau R, Kalinka-Warzocha E, et al. Polymorphisms in TS, MTHFR and ERCC1 genes as predictive markers in first-line platinum and pemetrexed therapy in NSCLC patients. J Cancer Res Clin Oncol. 2014;140(12):2047-57.

24. Deng Q, Su B, Xiu X, Fang Q, Zhou S, Zhou C. Predictive value of unmethylated RASSF1A on disease progression in non-small cell lung cancer patients receiving pemetrexed-based chemotherapy. Cancer Biomark. 2020;27(3):313-23.

25. Babraham Bioinformatics.

26. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM; 2013.

27. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297-303.

28. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.

29. DiMasi JA, Feldman L, Seckler A, Wilson A. Trends in risks associated with new drug development: Success rates for investigational drugs. Clin. Pharmacol Ther. 2010;87(3):272-7.

30. Arrowsmith J. Trial watch: Phase II failures: 2008-2010. Nat Rev Drug Discov. 2011;10(5):328-9.

31. Arrowsmith J. Trial watch: Phase III and submission failures: 2007-2010. Nat Rev Drug Discov. 2011;10(2):87.

32. Bao L, Zhang Y, Wang J, Wang H, Dong N, Su X, et al. Variations of chromosome 2 gene expressions among patients with lung cancer or non-cancer. Cell Biol Toxicol. 2016;32(5):419-35.

33. Cengiz B, Yumrutas O, Bozgeyik E, Borazan E, Igci YZ, Bozgeyik I, et al. Differential expression of the UGT1A family of genes in stomach cancer tissues. Tumour Biol. 2015;36(8):5831-7.