

# World Journal of *Clinical Cases*

*World J Clin Cases* 2021 April 26; 9(12): 2696-2950



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**ABOUT COVER**

Editorial Board Member of *World Journal of Clinical Cases*, Jing Liu, MD, PhD, Chief Doctor, Professor, Department of Neonatology and NICU, Beijing Chaoyang District Maternal and Child Healthcare Hospital, Beijing 100021, China. liujingbj@live.cn

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**RESPONSIBLE EDITORS FOR THIS ISSUE**

Production Editor: Ji-Hong Lin; Production Department Director: Xiang Li; Editorial Office Director: Jin-Lai Wang.

**NAME OF JOURNAL**

*World Journal of Clinical Cases*

**ISSN**

ISSN 2307-8960 (online)

**LAUNCH DATE**

April 16, 2013

**FREQUENCY**

Thrice Monthly

**EDITORS-IN-CHIEF**

Dennis A Bloomfield, Sandro Vento, Bao-Gan Peng

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/2307-8960/editorialboard.htm>

**PUBLICATION DATE**

April 26, 2021

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<https://www.wjgnet.com/bpg/GerInfo/288>

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<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

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## Detection of *EGFR-SEPT14* fusion in cell-free DNA of a patient with advanced gastric cancer: A case report

Boyeon Kim, Yoonjung Kim, Inho Park, Jae Yong Cho, Kyung-A Lee

**ORCID number:** Boyeon Kim 0000-0003-1867-8648; Yoonjung Kim 0000-0002-4370-4265; Inho Park 0000-0003-2250-6507; Jae Yong Cho 0000-0002-0926-1819; Kyung-A Lee 0000-0001-5320-6705.

**Author contributions:** Kim B reviewed the literature and contributed to manuscript drafting; Cho JY provided clinical information regarding the patient; Kim Y and Park I contributed to panel assay design and gene curation; Kim B, Kim Y, and Lee KA performed analysis of genetic data; Lee KA and Cho JY were responsible for revision of the manuscript for important intellectual content; All authors issued final approval for the version to be submitted.

**Informed consent statement:** Informed written consent was obtained for specimen collection and genetic analysis.

**Conflict-of-interest statement:** The authors declare that they have no conflict of interest.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**Open-Access:** This article is an

**Boyeon Kim**, Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul 03722, South Korea

**Boyeon Kim, Yoonjung Kim, Kyung-A Lee**, Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, South Korea

**Inho Park**, Center for Precision Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, South Korea

**Jae Yong Cho**, Division of Medical Oncology, Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul 06273, South Korea

**Corresponding author:** Kyung-A Lee, MD, PhD, Full Professor, Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-Ro, Gangnam-Gu, Seoul 06273, South Korea. [kal1119@yuhs.ac](mailto:kal1119@yuhs.ac)

### Abstract

#### BACKGROUND

Gastric cancer is the fifth most diagnosed cancer worldwide and the third most common cause of cancer-related death. In recent decades, increasing application of next-generation sequencing has enabled detection of molecular aberrations, including fusions. In cases where tissue is difficult to obtain, cell-free DNA (cfDNA) is used for detecting mutations to identify the molecular profile of cancer. Here, we report a rare case of *EGFR-SEPT14* fusion detected from cfDNA analysis in a patient with gastric cancer.

#### CASE SUMMARY

A 49-year-old female diagnosed with advanced gastric cancer in July 2019 received capecitabine and then combination chemotherapy of ramucirumab and paclitaxel, but ascites was detected. The therapy was switched to nivolumab, but disease progression was observed on a positron emission tomography/computed tomography scan in May 2020. Therapy was discontinued, and cfDNA next-generation sequencing was immediately evaluated. All genomic variants, including fusions, were analyzed from cfDNA. The following somatic alterations were detected from the patient's cfDNA: an *APC* frameshift mutation (NM\_000038.5:c.6579del, p.V2194fs) with variant allele frequency of 0.5%, an *EGFR* amplification with a copy number of 17.3, and an *EGFR-SEPT14* fusion with variant allele frequency of 45.3%. The site of the fusion was exon 24 of *EGFR* fused to exon 10 of *SEPT14*. The fusion was in-frame and considered to be proto-

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**Manuscript source:** Unsolicited manuscript

**Specialty type:** Medicine, research and experimental

**Country/Territory of origin:** South Korea

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0  
Grade B (Very good): B  
Grade C (Good): 0  
Grade D (Fair): 0  
Grade E (Poor): 0

**Received:** December 4, 2020

**Peer-review started:** December 4, 2020

**First decision:** January 24, 2021

**Revised:** February 2, 2021

**Accepted:** March 3, 2021

**Article in press:** March 3, 2021

**Published online:** April 26, 2021

**P-Reviewer:** Jin X

**S-Editor:** Fan JR

**L-Editor:** Filipodia

**P-Editor:** Yuan YY



oncogenic. Although the patient refused to continue therapy, we suggest that EGFR-targeted therapies be tried in such future cases.

## CONCLUSION

The expanded applications of the cfDNA assay may open a new horizon in treatment of patients with advanced gastric cancer.

**Key Words:** Gene fusion; Cell-free DNA; Liquid biopsy; Gastric cancer; EGFR tyrosine kinase inhibitor; Case report

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**Core Tip:** In recent decades, increasing application of next-generation sequencing has enabled detection of molecular aberrations, including fusions. In cases where tissue is not easily obtainable, cell-free DNA is used for detecting mutations to determine the molecular profile of cancer. In this study, we report the first case of EGFR-SEPT14 fusion detected from next-generation sequencing analysis of cell-free DNA from a patient with advanced gastric cancer. We suggest expanded applications of the cell-free DNA assay regardless of cancer type, which may open a new horizon in treatment of patients with advanced gastric cancer.

**Citation:** Kim B, Kim Y, Park I, Cho JY, Lee KA. Detection of EGFR-SEPT14 fusion in cell-free DNA of a patient with advanced gastric cancer: A case report. *World J Clin Cases* 2021; 9(12): 2884-2889

**URL:** <https://www.wjgnet.com/2307-8960/full/v9/i12/2884.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v9.i12.2884>

## INTRODUCTION

Gastric cancer is the fifth most diagnosed cancer worldwide with a particularly high incidence in East Asia and the third most common cause of cancer-related death<sup>[1]</sup>. Curative surgery is the primary treatment of choice, but systemic chemotherapies are used for patients with metastatic or unresectable advanced or recurrent gastric cancer. Because systemic chemotherapies are nonspecific and can cause serious adverse effects, development of molecular targeted drugs has been attempted to improve outcomes in patients with gastric cancer.

In recent decades, increasing application of next-generation sequencing (NGS) has enabled detection of molecular aberrations such as copy number gains or losses, somatic mutations, and gene fusions. For cases where tissue is not easily obtainable, cell-free DNA (cfDNA) is used for detecting mutations to determine the molecular profile of cancer<sup>[2]</sup>. Successful identification of oncogenic gene fusions can aid in diagnosis and molecular treatment of patients<sup>[3]</sup>. Here, we report a rare case of EGFR-SEPT14 fusion detected from cfDNA analysis in a patient with gastric cancer.

## CASE PRESENTATION

### Chief complaints

A 49-year-old female patient had been treated for advanced gastric cancer (AGC) with chemotherapy. After therapy, she expressed whole body pain, especially on the left side of the pelvis.

### History of present illness

This patient had been diagnosed with AGC in July 2019. The pathological diagnosis indicated signet ring cell carcinoma. While receiving her first round of chemotherapy with capecitabine, the patient developed acute pyelonephritis and hydronephrosis in both kidneys, leading to a suspicion of periureteral metastases. Therefore, the patient started a new regimen of combination chemotherapy with ramucirumab and

paclitaxel. However, ascites was observed after two cycles of chemotherapy. The treatment was switched to nivolumab. After five cycles, an abdominopelvic computed tomography scan was performed in April 2020 that showed improvement in peritoneal carcinomatosis compared to an image from February 2020. She received seven cycles of nivolumab, but progressive disease was observed by the positron emission tomography/computed tomography scan, and other therapeutic options were needed to be discussed.

### **History of past illness**

The patient did not have any other medical history beyond AGC.

### **Personal and family history**

The patient reported a family history of gastric cancer in her grandfather.

### **Physical examination**

Physical examination revealed pain on the left side of the pelvis.

### **Laboratory examinations**

Blood analysis revealed mild leukocytosis ( $14 \times 10^9/L$ ) with low hemoglobin (10.3 g/dL). Platelet count was in the normal range. Serum C-reactive protein was increased at 181 mg/L (normal range, 0.1-6.0 mg/L).

### **Imaging examinations**

A positron emission tomography/computed tomography scan obtained in May 2020 revealed bone, multiple nodal, and right lateral abdominal wall soft tissue metastases after the patient had received seven cycles of nivolumab. The therapy was discontinued, and cfDNA NGS was performed immediately.

### **Further genetic diagnostic work-up**

For genetic testing, the patient provided informed written consent for specimen collection and genetic analysis. This study was approved with a waiver of informed consent by the Institutional Review Board of Gangnam Severance Hospital, Seoul, Korea (IRB No. 3-2020-0268).

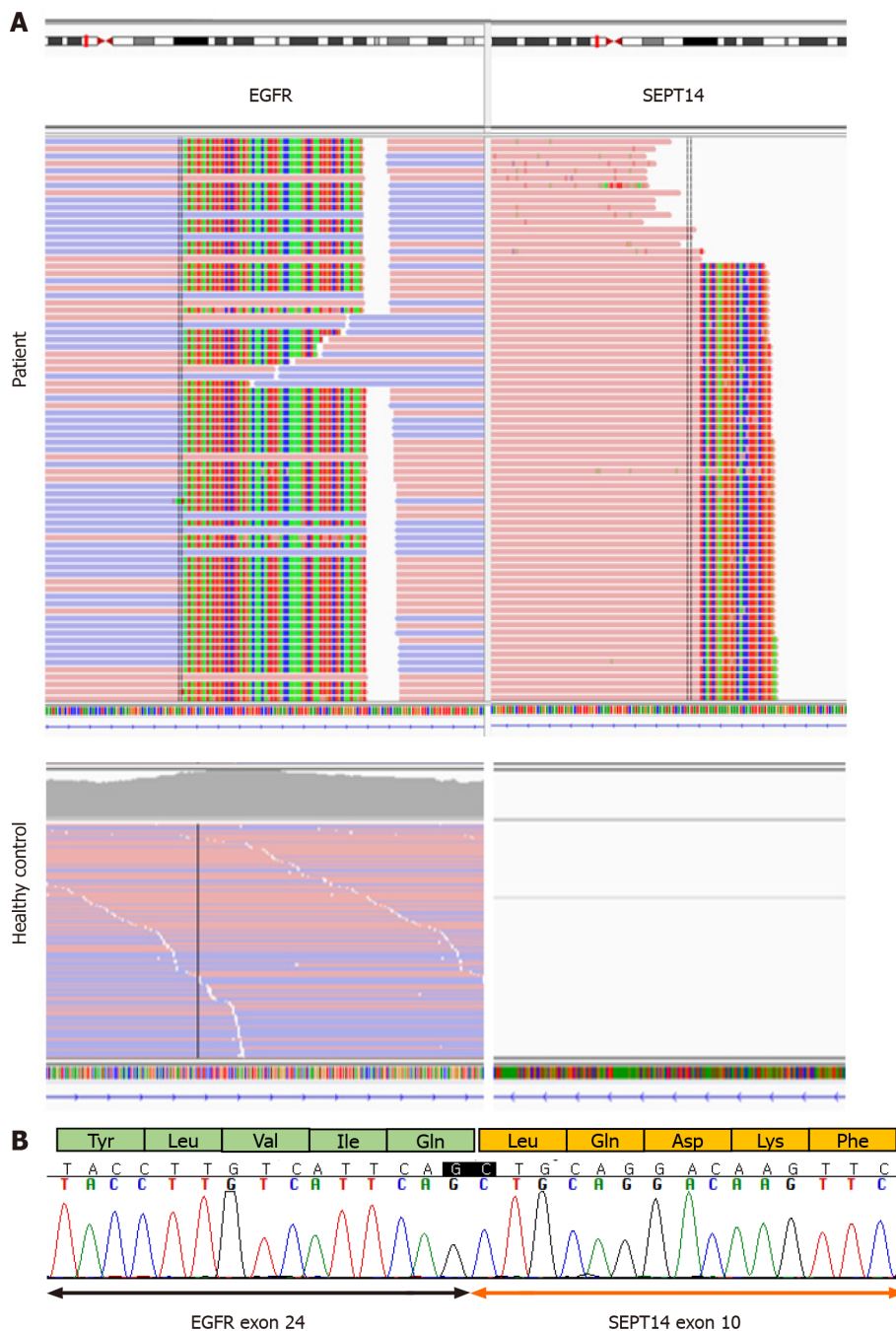
cfDNA was extracted using the MagMAX Cell-Free Total Nucleic Acid Kit (Thermo Fisher Scientific, Waltham, MA, United States). A DNA library was constructed with the AlphaLiquid<sup>®</sup>100 kit (IMBDx Inc., Seoul, Korea), which was designed to include intronic regions of target genes. Hybrid-capture-selected libraries were sequenced to a mean coverage of 14237x (cfDNA) and 735x (DNA) on an Illumina NextSeq-550 (Illumina, San Diego, CA, United States). GeneFuse was used to detect fusions<sup>[4]</sup>, and a Genome Reference Consortium Human Build 38 was used for variant interpretation. All genomic variants, including fusions, were analyzed from cfDNA. Because of the patient's family history, the presence of germline mutation was tested in parallel for the following genes: *APC*, *ATM*, *BRCA1*, *BRCA2*, *CDH1*, *CDK4*, *CDKN2A*, and *MLH1*. No germline mutations were detected from the genomic DNA. Somatic alterations detected from the cfDNA were an *APC* frameshift mutation (NM\_000038.5:c.6579del, p.V2194fs) with variant allele frequency of 0.5%, an *EGFR* amplification with a copy number of 17.3, and an *EGFR-SEPT14* fusion with variant allele frequency of 45.3% (Figure 1A). Because the *EGFR* and *SEPT14* genes are closely located on chromosome 7, we tested 50 normal healthy controls with the same panel and confirmed that the fusion detected in the patient was a true positive. We also confirmed *EGFR-SEPT14* fusion by complementary DNA sequencing, which was processed using the patient's cell-free RNA extracted by MagMAX Cell-Free Total Nucleic Acid Kit. The site of fusion was exon 24 of *EGFR* fused to exon 10 of *SEPT14* (Figure 1B). The fusion was in-frame and considered to be proto-oncogenic.

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## **FINAL DIAGNOSIS**

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The final diagnosis of the present case was *EGFR-SEPT14* fusion in AGC.



**Figure 1** *EGFR-SEPT14* fusion. A: Genomic fusion of *EGFR* exon 24 with exon 10 of *SEPT14*; B: RNA sequencing analysis of the *EGFR-SEPT14* fusion.

## TREATMENT

The patient refused further treatment.

## OUTCOME AND FOLLOW-UP

The patient could have tried *EGFR* targeted therapy such as erlotinib, which has been used in other types of carcinomas with *EGFR-SEPT14* fusion<sup>[5]</sup>, but she refused further treatment and passed away about 1 month after discontinuation of nivolumab.

## DISCUSSION

*EGFR1* (*EGFR*; *ErbB1*; *HER1*) is one of four transmembrane growth factor receptor



proteins that constitute the *EGFR* family of receptor tyrosine kinases<sup>[6]</sup>. Activation of *EGFR* leads to cell proliferation, differentiation, motility, and metastasis<sup>[7]</sup>. *SEPT14* is a member of a highly conserved septin family of guanosine 5'-triphosphate-binding cytoskeletal proteins with multiple cellular functions, such as membrane transport, apoptosis, cell polarity, cell cycle regulation, cytokinesis, and oncogenesis<sup>[8]</sup>. Among all septins, *SEPT14* shows the highest mutation frequency in skin cancer followed by *SEPT9* exhibiting high mutation frequency in stomach cancer<sup>[9]</sup>.

The *EGFR-SEPT14* fusion was first reported in glioblastoma in which the site of fusion was the tyrosine kinase domain of *EGFR* and the coiled-coil domain of *SEPT14*. The *EGFR-SEPT14* fusion is the most frequent functional gene fusion in human glioblastoma<sup>[10]</sup>. The *EGFR-SEPT14* fusion was also identified in tissue from salivary gland secretory carcinoma using fluorescence in situ hybridization. That previous case indicated that a tumor harboring this fusion would be sensitive to *EGFR* inhibitors<sup>[11]</sup>. Recently, the *EGFR-SEPT14* fusion was reported in colorectal adenocarcinoma by using a comprehensive NGS assay on tumor samples<sup>[5]</sup>.

In the present study, the tissue biopsy of the patient was difficult. Therefore, we used a comprehensive NGS assay with a sample of cfDNA from the patient. We identified an *EGFR-SEPT14* fusion in AGC. To our knowledge, this is the first case of *EGFR-SEPT14* fusion identified in a cfDNA sample from an AGC patient. The patient went through unusually rapid disease progression, and this progression might have been caused by the fusion mutation. Unfortunately, because the patient refused to continue therapy, we could not determine whether the *EGFR-SEPT14* fusion responded to *EGFR* targeted therapies, such as tyrosine kinase inhibitors. However, the use of such therapies might have been effective in AGC with an *EGFR-SEPT14* fusion because there was a report of a patient with colorectal cancer with an *EGFR-SEPT14* fusion treated with erlotinib therapy. The fusion site reported in that study is the same as that in the present study, and the patient was administered erlotinib therapy to which the *EGFR-SEPT14* fusion is known to be sensitive<sup>[10]</sup>. However, soon after treatment, an *EGFR* variant III was detected and can result in resistance to erlotinib<sup>[5]</sup>. To confirm the treatment effect and disease progression in AGC, further studies are needed.

Nevertheless, detection of genomic fusion by the well-established cfDNA NGS assay confirmed that cfDNA can serve as an alternate source for detecting gene aberrations, including fusions. Furthermore, *EGFR-SEPT14* fusion has been reported in various types of cancer. Therefore, expanded applications of cfDNA assays should be considered regardless of cancer type. We also suggest that genomic variants including fusions can be therapeutic targets in AGC, which may open a new horizon in treatment.

## CONCLUSION

To the best of our knowledge, this is the first case of an *EGFR-SEPT14* fusion identified in a cfDNA sample from a patient with AGC. Detection of genomic fusion by the well-established cfDNA NGS assay confirmed that cfDNA can serve as an alternate source for detecting gene aberrations, including fusions. Successful identification of genomic variants, including fusions, from cfDNA can aid in diagnosis and molecular treatment of patients with AGC.

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