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CLINICAL REPORT

A novel fusion between *CDC42BPB* and *ALK* in a patient with quadruple wild-type gastrointestinal stromal tumor

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Abstract

Background: Gastrointestinal stromal tumors (GISTs) are the most common type of mesenchymal tumor in gastrointestinal tract, with striking features of morphology and immunohistochemistry. But GISTs in pregnancy could seldom be found. Pathogenic activating mutations of the proto-oncogene *KIT* and *PDGFRA* are detected in majority GISTs, and adjuvant imatinib therapy targeting *KIT* and *PDGFRA* mutations is recommended for patients with high-risk GIST. However, some rare subgroups with distinct molecular features remain uncovered and more therapeutic targets need to be revealed.

Methods: The DNA/RNA samples were detected using the NGS-based YuanSu450 gene panel. After identifying the *CDC42BPB-ALK* fusion by NGS, this novel fusion was further confirmed by Sanger sequencing. Subsequently, FISH analysis was performed using the Vysis ALK Break Apart FISH Probe kit to testify the *ALK* status. ALK protein expression was confirmed by IHC (D5F3 and 5A4).

Results: Herein, we reported the first case of quadruple wild-type (WT) GIST with *ALK-CDC42BPB* fusion and ALK (D5F3) overexpression. In this study, we described a 33-year-old pregnant patient in lactation who had a massive space occupying lesion (with the maximum diameter of 22 cm) in the stomach and was eventually diagnosed as quadruple WT GIST (KIT^{WT}/$PDGFRA^{WT}$/SDH^{WT}/$RAS\text{-}P^{WT}$).

Conclusion: We unexpectedly found that this GIST patient showed ALK (D5F3) overexpression and harbored a novel fusion *CDC42BPB* exon 24-*ALK* in exon 20.

KEYWORDS

ALK (D5F3) expression, *ALK* rearrangement, gastrointestinal stromal tumors, quadruple WT GIST, receptor tyrosine kinase

Wen Huang and Wei Yuan contributed equally to this work.

1 | BACKGROUND

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors occurring within the gastrointestinal tract (Mayr et al., 2019). Approximately 85% GISTs harbor *KIT* or *PDGFRA* mutations (Vankova et al., 2020), and GISTs that do not harbor any mutation of the *KIT* and *PDGFRA* genes are defined as wild type (WT) GISTs (Huss et al., 2013). In addition, a small subset of all GISTs (5%) that lack *KIT/PDGFRA/SDH/RAS-P* (RAS pathways, RAS-P) mutations can be regarded as a specific molecular event and referred as quadruple WT GIST (Pantaleo et al., 2015). Up to now, some kinds of genetic alternations have been identified in this GIST subgroup, including *ETV6-NTRK3* fusion, *FGFR1* or *FGF4*, *TP53*, *MEN1*, and *MAX* mutations (Astolfi et al., 2020).

The tyrosine kinase inhibitors (TKIs), especially imatinib, have been used as a standard first-line treatment for patients with localized and advanced GIST (Casali et al., 2018). What's more, there are increasing literatures illustrating the differential responses to TKIs (or imatinib) in GIST patients with different mutations (Corless et al., 2005). For example, the majority of GIST patients are imatinib-sensitive, especially with *KIT* exon 11 mutations; while GISTs harboring a mutation in *KIT* exon 17 or *PDGFRA* exon 18 (p.D842V) are confirmed imatinib-resistant (Cassier et al., 2012). Additionally, TKIs are not that effective for WT GIST patients, especially the *SDH*-deficient cases (Boikos & Stratakis, 2014). Therefore, identifying gene mutations in different patients is critical to guide the therapy and improve the prognosis by matching targeted drugs.

To our knowledge, most GISTs are ALK-negative, which helps to differentiate GISTs from other mesenchymal neoplasms, such as inflammatory myofibroblastic tumors (IMTs) (Kataoka et al., 2014). ALK is a transmembrane receptor tyrosine kinase and its overexpression can be caused by gene fusion, mutation, and amplification (Fan et al., 2020). Previous researches demonstrated that *ALK* rearrangement led to a new driver oncogene and served as a biomarker in human cancers (Choi et al., 2008; Perner et al., 2008). Until now, *ALK* rearrangement has been reported in various neoplasms, including pseudosarcomatous myofibroblastic proliferation (Albores-Saavedra et al., 1990), secretory carcinoma (Sasaki et al., 2020), and non-small cell lung cancer (NSCLC) in China (Fei et al., 2019). However, only one *ALK-PPP1R21* fusion in GIST has been reported yet (Zhao et al., 2020).

In this study, we presented a case of a 33-year-old woman diagnosed with quadruple WT GIST, and ALK (D5F3) was overexpressed. Surprisingly, *CDC42BPB-ALK* fusion was identified by next-generation sequencing (NGS) and confirmed by Sanger sequencing, enriching the molecular profiling of GIST and highlighting the importance of personalized cancer therapy.

2 | CASE PRESENTATION

A 33-year-old pregnant woman was initially admitted to the hospital for routine antenatal testing and a massive space-occupying lesion (with an estimated diameter of 20.5 cm) located in the right upper abdomen was discovered incidentally on MRI. Subsequently, this patient was transferred to another hospital for further examination. The computed tomography (CT) revealed a mass adhering to the stomach, and adjacent to the liver and pancreas (Figure 1a). The tumor and part of the stomach were surgically removed in July 2020 (Figure 1b). No lymph node metastases were observed. Immunohistochemically, the spindle cells were positive for CD117, DOG-1, CD34, SDHA, and SDHB (Figure 1d–h), but negative for a-SMA, desmin, and S100. Ki-67 was estimated to be 5%. As a result, the diagnosis of NIH high-risk GIST was rendered.

Subsequently, the mutation status of the patient was detected by Sanger sequencing, and no mutations were observed in *KIT* (exon 9, 11, 13, 17), *PDGFRA* (exon 12, 18), *KRAS* (exon 2, 3, 4), *BRAF* (exon 15), *NRAS* (exon 2, 3, 4), and *PIK3CA* (exon 20). *SDH* deficiency could be excluded based on its IHC status of SDHA and SDHB expression (Figure 1g,h). For further verification, the DNA/RNA samples were detected by using the NGS-based by using the NGS-based YuanSu450 gene panel (OrigiMed, Shanghai, China), which covers all the coding exon of 450 tumor-related genes that are frequently rearranged in solid tumors. The result confirmed that this GIST lacked mutations in *KIT/PDGFRA/SDH/RAS-P (NF-1, BRAF, RAS)*. Therefore, this GIST was quadruple WT GIST (KIT^{WT}/$PDGFRA^{WT}$/SDH^{WT}/$RAS\text{-}P^{WT}$). Interestingly, a novel rearrangement involving CDC42BPB exon 24 (chr14:103417527) and ALK exon 19 (chr20:29446465), which encode an in-frame fusion protein containing the ALK kinase domain (Shimizu et al., 2019). After identifying the CDC42BPB-ALK fusion by NGS-based DNA (Figure 2a) and RNA (Figure 2b) targeted sequencing, we further confirmed the novel fusion by Sanger sequencing (Figure 3a).

In order to testify the *ALK* status, FISH analysis was performed using the Vysis ALK Break Apart FISH Probe kit (Abbott Molecular, Des Plaines, IL, USA), and split signals for *ALK* were detected in 90% of tumor cells, indicating the *ALK* gene breaking (Figure 3b). Additionally, ALK protein expression was confirmed by IHC (D5F3 and 5A4). However, ALK was strongly stained only by the antibody of D5F3 (Figure 3c,d). Generally speaking, these results mentioned above suggested that this case was proven to be quadruple WT GIST with a novel *CDC42BPB-ALK* fusion,

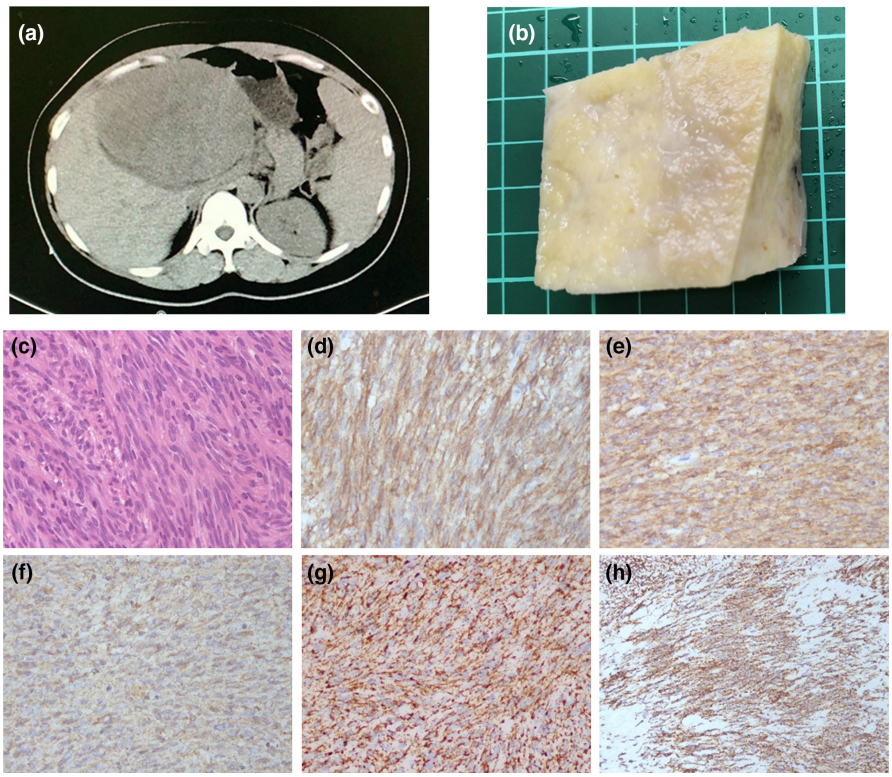

FIGURE 1 Abdominal CT scan showed a massive space-occupying lesion which was related to gastric wall (a). The gross image showed that the cut surface of this tumor has yellowish-white appearance (b). Hematoxylin and eosin (H&E) staining (c, 400×) and immunohistochemistry staining for CD117 (d, 400×), Dog1 (e, 400×), CD34 (f, 400×), SDHA (g, 400×), and SDHB (h, 400×)

which was likely to function as the oncogenic driver in this tumor.

According to the previous study (Gao et al., 2012), TKIs could offer a treatment option with relativly good responses for wild-type patients (36.4% imatinib response rate). Besides, curative effects are currently undetermined in *ALK*-rearranged GIST. Thus, postoperative adjuvant imatinib therapy or *ALK* inhibitors were recommended for this patient. This patient was still in lactation and declined medical treatment. Alternatively, close follow-up was suggested. Three months after resection (from July 2020), the patient started to take imatinib (400 mg/day) and feels well. No sign of recurrence is observed till now.

3 | DISCUSSION

Here, we described a quadruple WT GIST in pregnancy with a novel *CDC42BPB-ALK* fusion. According to the relevant researches, the relation between *ALK* rearrangement and the ALK protein expression is uncertain. In 2020, Fan et al. (2020) reported a case of GIST with *PDGFRA* p. D842V mutation, which showed ALK overexpression (both of D5F3 and 5A4 clones) but lacked *ALK* rearrangement. Zhao et al. (2020) presented a GIST patient with a *PPP1R21-ALK* rearrangement, and ALK protein expression was confirmed by IHC. According to the standard methods advocated by the US Food and Drug Administration (FDA) and the China Food and Drug Administration (CFDA), FISH based on break-apart FISH probes and IHC using D5F3 antibody were standard methods for *ALK* arrangement detection in NSCLC (Liu et al., 2020). In our study, the FISH analysis showed that a significant proportion of *ALK* gene were split, and ALK D5F3 overexpression was confirmed by IHC, indicating that the *CDC42BPB-ALK* fusion result in the expression of a CDC42BPB-ALK fusion protein.

CDC42BPB encodes a member of the serine/threonine-protein kinase family and is a key mediator of cell growth, proliferation, and apoptosis (Manning & Cantley, 2007). Shkolyar et al. (2021) discovered that *CDC42BPB* was as a cancer-associated gene for risk stratification in bladder cancer, and would serve as potential drug candidates to prevent tumor growth (Nagaraj & Reverter, 2011). *ALK* fusion after exon 20 on the ALK side, which includes the complete *ALK* kinase domain, has been reported to activate a carcinogenic kinase in various *ALK*-rearranged tumors including NSCLC (Shimizu et al., 2019), IMT (Lawrence et al., 2000), peritoneal mesothelioma, and various other carcinomas (Huang, 2018). We supposed that the *CDC42BPB-ALK* fusion is that it may cause the neo- or over-expression of *ALK* kinase in tissues where it would be silent under physiological condition but this mechanism in this *ALK*-rearranged GIST still needs further study.

Wild-type GISTs have been proved to respond poorly to TKI-therapy owing to the lack of target oncogenic alteration (Park et al., 2020). However, one 2012 study of Chinese advanced GIST patients found that the

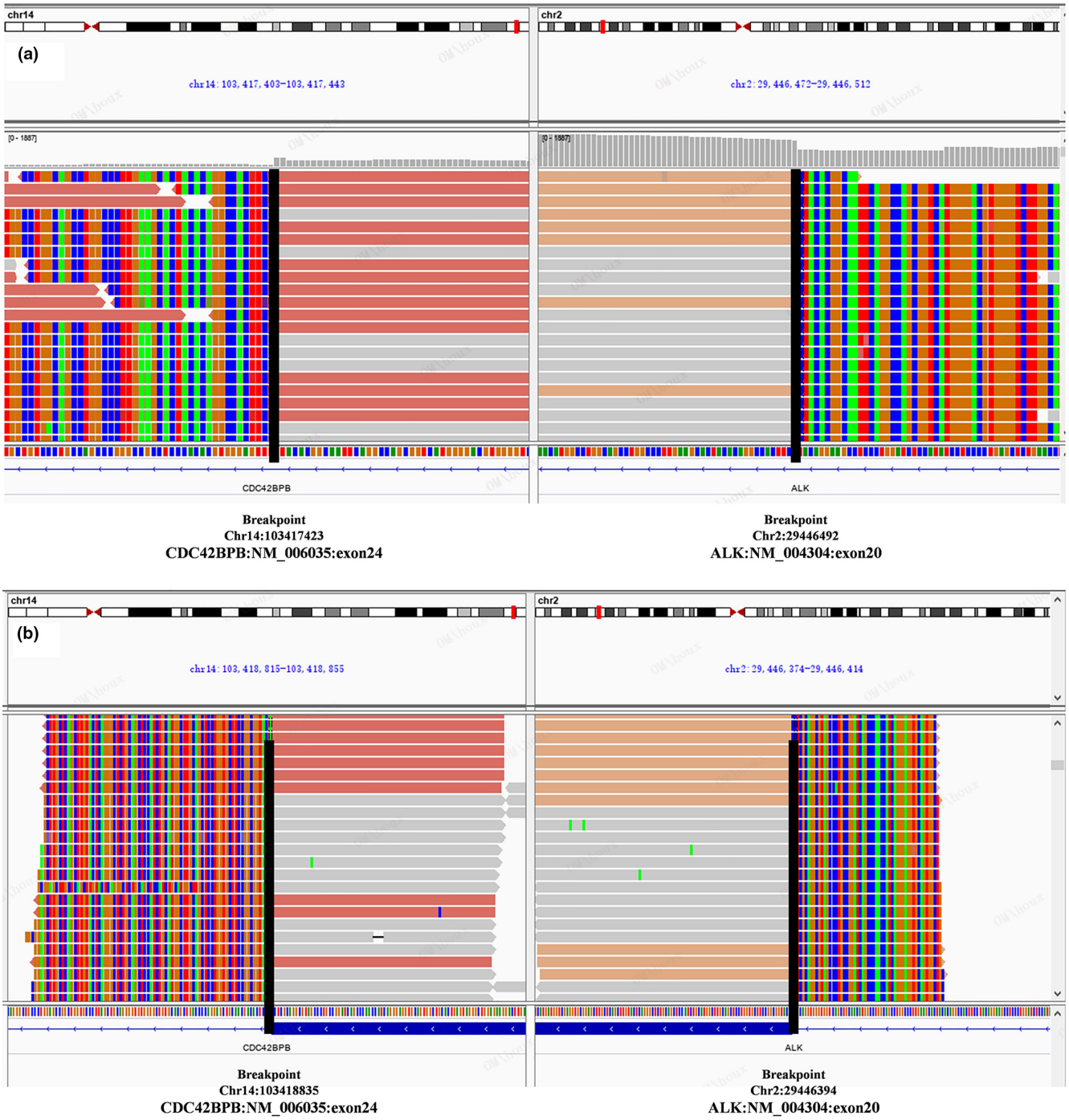

FIGURE 2 The IGV images of the *CDC42BPB-ALK* fusion identified DNA sequencing (a) and RNA sequencing (b)

imatinib response rate was 36.4% for wild-type patients (Gao et al., 2012). Although *ALK* inhibitor has been reported as a possible therapeutic option in some *ALK* fusion cancers (Fei et al., 2019; Sasaki et al., 2020), the efficacy and safety of *ALK* inhibitors were still unknown in *ALK*-rearranged GIST. Considering the two factors above, postoperative adjuvant imatinib therapy or *ALK* inhibitors were recommended for this patient. To avoid the adverse effect of breast milk secreted-imatinib on infant, the patient tended to choose close follow-up (without medicine treatment) for 2.5-month after resection, and subsequently, imatinib therapy (400 mg/day) was carried out after stopping breastfeeding from September 2020. It would be reasonable to carry out targeted therapy with *ALK* tyrosine kinase inhibitors when the patient developed tumor progression. Now 10 months after resection (from July 2020), this patient is alive with no evidence of recurrence.

In conclusion, we identified a quadruple WT GIST in pregnancy with a novel *CDC42BPB-ALK* fusion. Our results expanded the molecular spectrum of this tumor beyond the

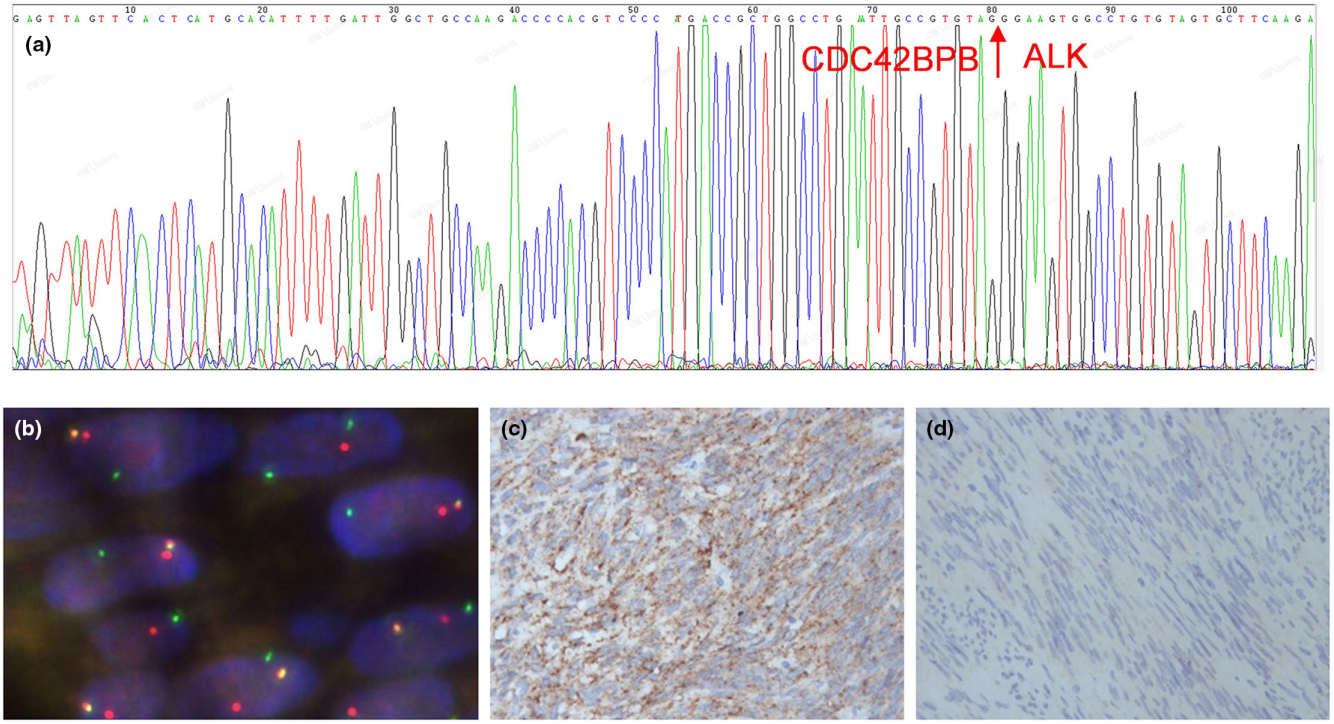

FIGURE 3 Sanger analysis further confirmed the breakpoint of *CDC42BPB-ALK* fusion (a). FISH analysis using the FISH break-apart probes shows split signals for *ALK* (red, 3′; green, 5′) (b). IHC staining showed that ALK protein expression was strongly positive for antibody D5F3, but negative for antibody 5A4 (c,d, 400×)

well-known GIST driver genes. These oncogenic events may have implications for therapeutic targets in patients with *ALK*-rearranged GIST, which deserves further investigation.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Wen Huang and Mian Xu reviewed the literature and contributed to manuscript drafting; Wen Huang, Wei Yuan and Yingyong Hou conceptualized and designed the study; Lei Ren, Chen Xu, Rongkui Luo, Yuhong Zhou, Weiqi Lu and Qing Hao collected the data; Wen Huang and Yingyong Hou critically reviewed the manuscript for important intellectual content. All authors issued final approval for the version to be submitted.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board (IRB) of Zhongshan Hospital Fudan University. Informed consent was obtained from the patient.

DATA AVAILBILITY STATEMENT

All data are included in the manuscript and no additional data need to be disclosed.

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