BRAF-MAD1L1 and BRAF-ZC3H7A: novel gene fusion in Rhabdomyosarcoma and Lung adenocarcinoma

Jiang Da
Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Hui Jin
Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Xinliang Zhou
Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Shaoshuang Fan
Hebei Medical University Fourth Affiliated Hospital and Hebei Provincial Tumor Hospital

Mian Xu
Origimed

Xiaoyan Chen (✉ xum@origimed.com)
The Hospital of Shunyi District Beijing

Case report

Keywords: novel gene fusion, BRAF-MAD1L1, BRAF-ZC3H7A, Rhabdomyosarcoma, Lung adenocarcinoma

DOI: https://doi.org/10.21203/rs.3.rs-368083/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background:** Rhabdomyosarcoma (RMS) and lung adenocarcinoma (LADC) epitomizes the success of cancer prevention by the development of conventional therapy, but huge challenges remain in the therapy of advanced diseases.

**Case presentation:** We reported two cases of novel *BRAF* gene fusion. The first case was a 34-year-old female with RMS harboring a *BRAF-MAD1L1* fusion. She suffered tumor resection, recurrence and rapid progression. The second case was a 72-year-old female with LADC harboring a *BRAF-ZC3H7A* fusion, and she gained rapid progression after receiving a first-line course of chemotherapy.

**Conclusions:** These two *BRAF* fusions retain the intact *BRAF* kinase domain (exon 11-18) and showed poor prognosis in RMS and LADC.

**Background**

Rhabdomyosarcoma (RMS) is an aggressive malignancy of soft-tissue in children and adolescents (1). There are 4.5 cases per 1 million in children per year and it accounts for 4–5% of all childhood malignancies (2). Despite most cases occur in children younger than 10 years, adult RMS still accounts for 41% of all RMS patients in the Surveillance, Epidemiology and End Results database between 1973 and 2005 (3). Pediatric patients with RMS are generally treated by standard protocols or within clinical studies. In the United State, most pediatric patients with RMS can be significantly improved with multimodality therapy (surgery, chemotherapy and radiotherapy) and the 5-year overall survival (OS) is 55% and 71% for IRS-I and IRS-IV, respectively (4–6). However, the treatment for adult RMS older than 19 years old is still non-standardized and depends on different physicians’ experience and personal choice. The treatment results of adult RMS have been shown to be inferior to that of pediatric RMS (3, 7). A recent study assessed the failure pattern and clinical outcome of adult patients with RMS who received multimodality treatment (surgery, chemotherapy and radiotherapy) in the first-line treatment of metastatic disease, observing approximately 21.7 months of median OS and 6.1 months of median OS especially for genitourinary (8). Lower 3-year DFS (0% versus 26.7%; p = 0.616) and OS (0% versus 53.5%; p = 0.12) of high-risk patients demonstrates the aggressive behavior of this disease.

Lung adenocarcinoma (LADC) is the most common type of lung cancer and occurs in both smokers and never smokers (9). Treatment strategy for LADC in advanced stages have changed obviously from the traditional platinum-based chemotherapy to a gene-based targeted therapy as a first-line treatment when the tumor carried targetable mutations (10). In primary LADC, screening for genomic alterations is becoming a clinical standard for guiding individual treatment options and identifying new targets (11). However, the majority of patients treated with targeted kinase inhibitors ultimately relapse, so new targets must be identified to improve the OS of patients with LADC.

Gene fusion is an important genomic alternation that has been observed in various cancers, including LADC (12) and RMS (13); such fusion is likely to contribute to cancer progression. Recent studies have
highlighted the important role of gene fusion in cancers, which is expected to provide new ideas for treatment and improving prognosis. It is reported that \textit{KIAA1549-BRAF} gene fusion is a prognostic factor in pilocytic astrocytomas and it associates with a better outcome (14). Recurrent \textit{BRAF} gene fusions with features reminiscent of infantile fibrosarcomas expand the spectrum of fusion-positive spindle cell sarcomas (15). A novel gene fusion, \textit{LMO7-BRAF}, was identified in papillary thyroid carcinoma and behaves as an oncogenic alteration (16). All the above studies indicate that BRAF gene fusion is involved in the development of cancer.

Herein, we describe the first case of RMS harboring a \textit{BRAF-MAD1L1} fusion and one case of LADC harboring a \textit{BRAF-ZC3H7A} fusion revealed by next-generation sequencing. These fusions result in BRAF kinase domain activation. However, both patients were not treated with BRAF inhibitors, resulting in a poor prognosis clinically.

**Case Presentation**

**Case 1**

A 34-year-old female attended a gynecologist in November 2017 with significant intermittent dull pain in the left lumbar region. The ultrasonic wave inspection revealed a 23.2*17.4cm solid-cystic lesion in the left pelvic cavity. Exploratory laparotomy was performed on November 30, 2017, and found that the goitre was difficult to remove. Combined with morphology and immunohistochemistry (IHC), the pathological biopsy revealed the mass as RMS. The IHC results of pathological sections were: Des (+), Myogenin (+), S-100 (-), SMA (-), CD34 (-), HMB45 (-), Ki-67 (+, 60–70%), Dog1 (-), CD117 (-), MyoD1 (-) (Fig. 1). For further diagnosis and treatment, she was admitted to the Fourth Hospital of Hebei Medical University. In December 2017, she underwent intestinal adhesion lysis, left abdomen massive tumor resection, left nephrectomy, left hemicolecotomy, transverse colon rectal anastomosis, partial ileal resection and ileal end anastomosis. During the operation, it was observed that the tumor was located in the left abdomen with 50*45*40cm in size, of which part was in the left colon with the surface of the intestinal wall showing 25*20*9cm mass and part was in left kidney. IHC results for tumor cells were: CD34 (+), CD117 (+), Dog1 (-), Ki67 (60%), Desmin (+), Calponin (-), S100 (-), SMA (+/-), MDM2 (-), MyoD1 (-), Myo (-), Masson (+), PTAH (+). After the operation, the patient recovered well.

In March 2018, PET-computed tomography (CT) scans revealed the recurrence and metastasis of RMS with multiple enlarged lymph nodes near the abdominal aorta and multiple nodules around the spleen (Fig. 2B). The patient was treated with ifosfamide and doxorubicin hydrochloride liposomes for 3 cycles and received a partial response (PR) (Fig. 2C). In August 2018, after 6 cycles of chemotherapy, CT scans showed multiple low-density nodules in thyroid and multiple masses around the spleen and retroperitoneum, with increased size and PD (Fig. 2D). After 3 cycles of second-line treatment with albumin paclitaxel, the size of the mass significantly increased with PD (Fig. 2E). In October 2018, the patient received anlotinib hydrochloride (12 mg 1/day, d1-d14 for 3 weeks). In December 2018, pelvic tumors increased slightly, and new liver metastases were observed with PD.
Peripheral blood samples were collected for next-generation sequencing to identify additional therapeutic options. Genomic testing revealed a $BRAF$-$MAD1L1$ fusion (Fig. 3A), $FGFR1$ gene amplification, $TP53$ R248W mutation, and $KMT2C/VWC2$ rearrangement as well as high bTMB (14.05 Muts/Mb). First-generation sequencing (Sanger) confirmed $BRAF$-$MAD1L1$ fusion in the RMS patient (Fig. 3B-C). On December 28, 2019, the patient’s blood was drawn for ctDNA test, during which the disease progressed rapidly, and the patient died in March 2019.

**Case 2**

A female patient at age 72 was diagnosed with LADC in November 2016 (Fig. 4). Pemetrix combined with cisplatin was applied as a first-line chemotherapy regimen for five cycles with SD in February 2017. In November 2016, next-generation sequencing of the original lung tumor tissue was pursued to identify additional therapeutic options and revealed the following: $BRAF$-$ZC3H7A$ fusion (Figure. 4) and $TP53$ V172F mutation. First-generation sequencing (Sanger) confirmed the $BRAF$-$ZC3H7A$ gene fusion in the LADC patient (Figure.5). In March 2017, CT scans showed rapid disease progression and the patient died with failing to use sorafenib, which was recommended as the second-line therapy.;113-114104

**Discussion And Conclusion**

$BARF$, located on human chromosome 7, encodes the serine/threonine protein kinases of RAF family that play an important role in cell division, differentiation and secretion by regulating the MAPK/ERK signaling pathway (17), which is critical for the normal development of cells. However, aberrant signaling of this pathway can occur through activating mutations of $BRAF$ and result in uncontrolled growth and tumorigenesis. Most mutations in the BRAF cluster happen in two regions: the glycine-rich P loop of the N lobe and the activation segment and flanking regions. Another abnormality of the BRAF gene is fusion. Although present data and the published literature demonstrate $BRAF$ fusions are enrichment in many cancers, including pilocytic astrocytoma, spitzoid melanoma, pancreatic acinar carcinoma and papillary thyroid cancer (18–21), it has not previously been described in RMS. In this report, we first reported a novel $BRAF$-$MAD1L1$ fusion and $BRAF$-$ZC3H7A$ fusion resulted from a genomic rearrangement in the RMS patient and LADC patient, respectively.

To our knowledge, $MAD1L1$, the mitosis checkpoint gene, and other gene fusion partner, contains a conserved an N-terminal coil domain and C-terminal domain (595–718 amino acids) that plays a role in the kinetochore targeting (22). Depletion of $MAD1L1$ genes in mammal cells severely affects the spindle checkpoint function, leading to aneuploidy and tumorigenesis (23), and abnormal expression of MAD1L1 is associated with poor prognosis (24). A patient with melanoma harboring a $MAD1L1$-$BRAF$ fusion (intron 16 of $MAD1L1$ and intron 7 of $BRAF$), which is a similar translocation with case 1, demonstrated a partial response to treatment with certain types of $RAF$ inhibitors, such as sorafenib (25). So, $BRAF$-$MAD1L1$ fusion keeps yet the intact in-frame $BRAF$ kinase domain observed in case1 is likely to be effective in the treatment of certain RAF inhibitors. More evidence needs further research.
ZC3H7A (zinc finger CCCH domain-containing protein 7A) is a member of the CCCH zinc finger family and was highly enriched in macrophage-related organs, suggesting that they may play a role in the regulation of innate immunity and inflammatory response. A study had confirmed that ZC3H7A, together with TP53 and KRAS, were clustered into a single network that is strongly related to cancer development (26). Here, we also detected ZC3H7A and TP53 mutations in case 2. These preclinical results suggest the role of BRAF-MAD1L1 and BRAF-ZC3H7A fusion as oncogenic-drivers in a variety of tumors, deserving targeted inhibition by small molecule kinase inhibitors.

As the first BRAF inhibitor, sorafenib is used in the first-line treatment for advanced liver cancer and kidney cancer. Another class of BRAF inhibitors, vemurafenib, which have high inhibitory activity against BRAF, especially BRAF V600E, is mainly used to treat melanoma. However, results of ongoing preclinical studies could not provide a signal of the efficacy of BRAF inhibitors on these diseases. The ARMC10-BRAF fusion patient-derived xenograft PDX models showed the highest growth rate in melanomas and significant response to downstream MAPK inhibition with trametinib, a MEK inhibitor, but no response to vemurafenib; a metastatic spitzoid melanoma featured with a ZKSCAN1-BRAF fusion responded to treatment with the MEK inhibitor trametinib (27). Sorafenib monotherapy for current or progressive low-grade astrocytomas harboring KIAA1549-BRAF fusion showed significant progression upon treatment (19). Here, both patients did not use trametinib or sorafenib as a therapeutic drug, and the cancers developed rapidly and led to the patients’ death, suggesting that the combination of trimetidine or sorafenib may have a better therapeutic effect on patients with BRAF-fused cancers.

In this study, we reported two novel BRAF fusions, BRAF-MAD1L1 and BRAF-ZC3H7A fusion, which has resulted in a poor prognosis in RMS and LADC. With regard to the treatment strategy of these novel BRAF fusions, our cases suggest that the trametinib or sorafenib may benefit patients. A single drug or multi-drug combination could be selected according to the patient’s physical status. And With the rapid development of NGS techniques, newly identified gene fusions and concomitant molecular alterations in oncology practice may provide novel therapeutic targets and bring significant clinical improvement for RMS and LADC patients.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the ethics committee of Fourth Hospital of Hebei Medical University. Written informed consent for the study was obtained from the patients.

**Consent for publication**

Written informed consent for publication has be obtained from the patients.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.
Competing interests

No potential conflicts of interest were disclosed.

Acknowledgments

We owe thanks to the patients and their families. We thank the staff at The Hospital of Shunyi District Beijing and Fourth Hospital of Hebei Medical University. We thank OrigiMed for NGS technical support and scientific comments.

Funding

There was no supported funding for this study.

Authors’ contributions

Conception/Design: Da Jiang, Xiaoyan Chen

Provision of study material or patients: Hui Jin and Xinliang Zhou

Collection and/or assembly of data: Shaoshuang Fan, Junying Li

Data analysis and interpretation: Junying Li

Manuscript writing: Da Jiang, Xiaoyan Chen, Mian Xu

Final approval of manuscript: Da Jiang, Xiaoyan Chen

All authors have read and approved the submitted version of the manuscript.

Abbreviations

RMS, Rhabdomyosarcoma; LADC, lung adenocarcinoma; OS, overall survival

References

1. Skapek SX, Ferrari A, Gupta AA, Lupo PJ, Butler E, Shipley J, et al. Rhabdomyosarcoma Nat Rev Dis Primers. 2019;5(1):1.

2. Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975–2005. Cancer. 2009;115(18):4218–26.

3. Parham DM, Barr FG. Classification of rhabdomyosarcoma and its molecular basis. Adv Anat Pathol. 2013;20(6):387–97.

4. Stewart E, Federico SM, Chen X, Shelat AA, Bradley C, Gordon B, et al. Orthotopic patient-derived xenografts of paediatric solid tumours. Nature. 2017;549(7670):96–100.
5. Italiano A, Di Mauro I, Rapp J, Pierron G, Auger N, Alberti L, et al. Clinical effect of molecular methods in sarcoma diagnosis (GENSARC): a prospective, multicentre, observational study. Lancet Oncol. 2016;17(4):532–8.

6. Drager J, Simon-Keller K, Pukrop T, Klemm F, Wilting J, Sticht C, et al. LEF1 reduces tumor progression and induces myodifferentiation in a subset of rhabdomyosarcoma. Oncotarget. 2017;8(2):3259–73.

7. Breneman JC, Lyden E, Pappo AS, Link MP, Anderson JR, Parham DM, et al. Prognostic factors and clinical outcomes in children and adolescents with metastatic rhabdomyosarcoma—a report from the Intergroup Rhabdomyosarcoma Study IV. J Clin Oncol. 2003;21(1):78–84.

8. Xu N, Hua Z, Ba G, Zhang S, Liu Z, Thiele CJ, et al. The anti-tumor growth effect of a novel agent DMAMCL in rhabdomyosarcoma in vitro and in vivo. J Exp Clin Cancer Res. 2019;38(1):118.

9. Tanner NT, Thomas NA, Ward R, Rojewski A, Gebregziabher M, Toll B, et al. Association of Cigarette Type With Lung Cancer Incidence and Mortality: Secondary Analysis of the National Lung Screening Trial. JAMA Intern Med. 2019.

10. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol. 2011;12(2):175–80.

11. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR Jr, Tsao A, et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov. 2011;1(1):44–53.

12. Chen HF, Wang WX, Xu CW, Huang LC, Li XF, Lan G, et al. A novel SOS1-ALK fusion variant in a patient with metastatic lung adenocarcinoma and a remarkable response to crizotinib. Lung Cancer. 2020;142:59–62.

13. Helm BR, Zhan X, Pandya PH, Murray ME, Pollok KE, Renbarger JL, et al. Gene Co-Expression Networks Restructured Gene Fusion in Rhabdomyosarcoma Cancers. Genes (Basel). 2019;10(9).

14. Becker AP, Scapulatempo-Neto C, Carloni AC, Paulino A, Sheren J, Aisner DL, et al. KIAA1549: BRAF Gene Fusion and FGFR1 Hotspot Mutations Are Prognostic Factors in Pilocytic Astrocytomas. J Neuropathol Exp Neurol. 2015;74(7):743–54.

15. Kao YC, Fletcher CDM, Alaggio R, Wexler L, Zhang L, Sung YS, et al. Recurrent BRAF Gene Fusions in a Subset of Pediatric Spindle Cell Sarcomas: Expanding the Genetic Spectrum of Tumors With Overlapping Features With Infantile Fibrosarcoma. Am J Surg Pathol. 2018;42(1):28–38.

16. He H, Li W, Yan P, Bundschuh R, Killian JA, Labanowska J, et al. Identification of a Recurrent LMO7-BRAF Fusion in Papillary Thyroid Carcinoma. Thyroid. 2018;28(6):748–54.

17. Flaherty KT, McArthur G. BRAF, a target in melanoma: implications for solid tumor drug development. Cancer. 2010;116(21):4902–13.

18. El-Osta H, Falchook G, Tsimberidou A, Hong D, Naing A, Kim K, et al. BRAF mutations in advanced cancers: clinical characteristics and outcomes. PLoS One. 2011;6(10):e25806.

19. Karajannis MA, Legault G, Fisher MJ, Milla SS, Cohen KJ, Wisoff JH, et al. Phase II study of sorafenib in children with recurrent or progressive low-grade astrocytomas. Neuro Oncol. 2014;16(10):1408–16.
20. Wiesner T, He J, Yelensky R, Esteve-Puig R, Botton T, Yeh I, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun. 2014;5:3116.

21. Chmielecki J, Hutchinson KE, Frampton GM, Chalmers ZR, Johnson A, Shi C, et al. Comprehensive genomic profiling of pancreatic acinar cell carcinomas identifies recurrent RAF fusions and frequent inactivation of DNA repair genes. Cancer Discov. 2014;4(12):1398–405.

22. Maldonado M, Kapoor TM. Constitutive Mad1 targeting to kinetochores uncouples checkpoint signalling from chromosome biorientation. Nat Cell Biol. 2011;13(4):475–82.

23. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer. 2005;5(10):773–85.

24. Ryan SD, Britigan EM, Zasadil LM, Witte K, Audhya A, Roopra A, et al. Up-regulation of the mitotic checkpoint component Mad1 causes chromosomal instability and resistance to microtubule poisons. Proc Natl Acad Sci U S A. 2012;109(33):E2205-14.

25. Botton T, Yeh I, Nelson T, Vemula SS, Sparatta A, Garrido MC, et al. Recurrent BRAF kinase fusions in melanocytic tumors offer an opportunity for targeted therapy. Pigment Cell Melanoma Res. 2013;26(6):845–51.

26. Zhou B, Irwanto A, Guo YM, Bei JX, Wu Q, Chen G, et al. Exome sequencing and digital PCR analyses reveal novel mutated genes related to the metastasis of pancreatic ductal adenocarcinoma. Cancer Biol Ther. 2012;13(10):871–9.

27. Ross JS, Wang K, Chmielecki J, Gay L, Johnson A, Chudnovsky J, et al. The distribution of BRAF gene fusions in solid tumors and response to targeted therapy. Int J Cancer. 2016;138(4):881–90.

**Figures**
Figure 1

Clinical pathologic characteristics of the RMS case. Tumor cells showed nuclear immune positivity for (A) Des; (B) Myogenin; (C) Ki-67 (60-70%) (Bar=100μm). (D) Tumor cells with nuclear hyperchromasia and the presence of frequent mitotic figure (H&E, Bar=100μm).
Figure 2

CT scans of the RMS patient. (A) No metastases were found around the spleen or near the abdominal aorta before the surgery. (B) Metastases of RMS with multiple enlarged lymph nodes are shown around the spleen and near the abdominal aorta. (C) The lesions around the spleen and near the abdominal aorta decreased significantly. (D) The lesions around the spleen increased notably. (E) The lesions around the spleen increased notably.
Figure 3

The BRAF-MAD1L fusion. (A) NGS data indicated a somatic genomic rearrangement between BRAF and MAD1L as demonstrated by Integrative Genomics Viewer. (B) First-generation sequencing (Sanger) confirmation of BRAF-MAD1L1 gene fusion in the RMS patient presented.
Figure 4

CT scans of the RMS patient. (A) Left pulmonary lesion with atelectasis. (B) The left bronchus was narrowed and partially blocked; and soft tissue protruded into the trachea cavity, leading to a high probability of tumor.
The BRAF-ZC3H7A fusion. (A) NGS data indicated a somatic genomic rearrangement between BRAF and ZC3H7A as demonstrated by Integrative Genomics Viewer. (B) First-generation sequencing (Sanger) confirmation of BRAF-ZC3H7A gene fusion in the LADC patient presented.