BRIEF REPORT

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AGAP3: A novel BRAF fusion partner in pediatric pancreatic-type acinar cell carcinoma

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Abstract

Most available molecular data on pancreatic acinar cell carcinoma (PACC) are provided by studies of adult cases. BRAF, RAF1, or RET rearrangements have been described in approximately 30% of cases. To the best of our knowledge, only seven cases with molecular data have been reported in pediatric PACC. We report here the comprehensive study of a pancreatic-type ACC from a 6-year-old patient. We detected an AGAP3::BRAF fusion. This result showing a BRAF rearrangement demonstrates a molecular link between adult and pediatric PACC. Moreover, it identifies AGAP3, a gene located at 7q36.1 that encodes a major component of the N-methyl-D-aspartate (NMDA) receptor signaling complex, as a partner gene of BRAF. The variability of BRAF partners is consistent with a driver role of BRAF alterations in PACC. The identification of such alterations is noteworthy for considering the use of MEK inhibitors in metastatic cases. We did not detect associated genomic instability. The better outcome of pediatric cases might be related to their stable genomic background.

KEYWORDS
acinar cell carcinoma, BRAF rearrangement, fusion gene, pediatric, RNA sequencing

1 | INTRODUCTION

Acinar cell carcinoma (ACC) is a rare and aggressive neoplasm that may originate from pancreas or from salivary glands. Pancreatic ACC (PACC) represents approximately 1%–2% of pancreatic tumors in adults, far behind pancreatic ductal adenocarcinoma.[1] In pediatric population, although extremely rare, PACC is slightly more frequent than in adults since it accounts up to 7%–15% of pancreatic tumors,

alongside solid pseudopapillary tumor, pancreatic neuroendocrine tumor (PanNET), and pancreatoblastoma.[1] Ductal adenocarcinoma is exceptional in children.[2] To the best of our knowledge, only 30 cases of pediatric pancreatic ACC (pPACC) have been reported in the literature. The average age of occurrence was 9.57 years (range 3–16 years).[2] A slight male predominance was noticed.[2] pPACC exhibits the same histological and immunohistochemical features as adult PACC.[2] Notably, a highly specific BCL10 expression is a distinctive hallmark.[3] While histopathological features of pPACC have been well documented, little is known about its molecular characteristics. Only a few molecular data extracted from large series of adult PACC including some pediatric cases are currently available (Table 1).[4–6]

In adults, the main molecular characteristic is the presence of recurrent rearrangements involving BRAF (7q34), described in 15%–24% of cases.[5,7,8] The genes RAF1 (3p25.2) and RET (10q11.21) were also shown as recurrently rearranged in approximately 2%–14% and 8% of PACC cases, respectively.[6,7,9] To date, the most prevalent fusion partner of BRAF in adult PACC is SND1 (7q31); the SND1::BRAF fusion results from a paracentric inversion of chromosome 7. Other partners involved in fusions with BRAF were HERPUD1 (16q13), ZSCAN30 (18q12.2), GATM (15q21.1), and GIPC2 (1p31.1) while fusion partners of RAF1 included HACL1, GOLGA4 (3p22.2), GATM, PDZNR3 (3p13), HERPUD1, and TRIM33 (1p13.2).[5–7] BRAF alterations also include BRAF mutations.[7,10] In a subset of PACC, other pathways have been reported to be altered such as Wnt pathway with CTNNB1 mutations or APC alterations, TP53 pathway with TP53 mutations and/or losses, as well as MYC amplification (formerly known as c-MYC) and/or chromosome 8 polysomy.[7,10–12] A high chromosomal instability has been reported, with loss of 1p and 18q and gain of 1q might be considered as early events.[7,10,11]

We report here the comprehensive study of a pediatric pancreatic-type ACC from a 6-year-old patient with a novel AGAP3::BRAF fusion that indicates a molecular link between adult and pediatric PACC.

2 | MATERIALS AND METHODS

2.1 | RNA sequencing

Total RNA was extracted from formalin-fixed paraffin-embedded tissue. We used the Archer FusionPlex Comprehensive Sarcoma Panel (ArcherDX Inc., Boulder, CO) targeting recurrent fusion genes in sarcomas using 407 gene sequence primers corresponding to 52 genes classically involved in translocation-related sarcomas. The experimental procedures were performed according to the manufacturer's recommendations (ArcherDX). Emulsion PCR and sequencing were performed as targeted DNA NGS experiments (Thermo Fisher Scientific). Base calling, barcode sorting and trimming, alignment to the human reference genome, and variant calling were achieved using Torrent Suite v5.6. Annotations were based on the human reference hg19 (Genome Reference Consortium Human Build 37 [GRCh37]) and performed using Archer Analysis software (version 6.0; ArcherDX).

TABLE 1 Molecular features of pediatric cases of acinar cell carcinoma (pACC)

Gender/age (years)	Fusion or rearrangement (method)	Point mutations (method)	CNA/LOH (method)	References
M/6	AGAP3-BRAF fusion (targeted RNA sequencing)	None (targeted DNA NGS)	No (aCGH/SNP)	Present case
ND/7	PPP1CC-BRAF fusion (Comprehensive genomic profiling[a])	None (Comprehensive genomic profiling[a])	MYC amplification (Comprehensive genomic profiling[a])	Rankin et al. (2021)
F/15		BRAF V600E mutation, VAF 54% MLL3 R2463C mutation, VAF 17% (Comprehensive genomic profiling[a])	None (Comprehensive genomic profiling[a])	Rankin et al. (2021) Cramer et al. (2020)
M/17	No BRAF/RAF1/RET rearrangement (FISH)	ND	ND	Prall et al. (2020)
F/15	ND	APC wild-type CTNNB1 wild-type (Sanger sequencing)	No LOH 11q (PCR)	Abraham et al. (2002)
F/2	ND	APC wild-type CTNNB1 wild-type (Sanger sequencing)	LOH 11q (PCR)	Abraham et al. (2002)
M/16	No BRAF rearrangement (FISH/DNA targeted NGSe)	ND	ND	Wang et al. (2018)
F/15	No BRAF rearrangement (FISH/DNA targeted NGS)	ND	ND	Wang et al. (2018)

Abbreviations: aCGH/SNP, Comparative Genomic Hybridization/Single-Nucleotide Polymorphism on array; CNA/LOH, copy number alteration/loss of heterozygosity; F, female; FISH, Fluorescence in situ hybridization; M, male; ND, not done; NGS, next-generation sequencing; PCR, polymerase chain reaction; VAF, variant allele frequency.
[a]Targeted hybrid capture panel.

2.2 | Comparative genomic hybridization/single-nucleotide polymorphism on array

Copy number alteration (CNA) and loss of heterozygosity were assessed using the Affymetrix OncoScan CNV FFPE Assay (Affymetrix, Santa Clara, CA). Experimental procedures were performed according to the manufacturer's recommendations. Raw data have been submitted to Gene Expression Omnibus (GEO) database with the accession number GSE203175.

2.3 | Targeted DNA next-generation sequencing

We used the Ion AmpliSeq Cancer Hotspot Panel v2 designed to amplify and sequence 207 amplicons covering 2800 COSMIC point mutations from 50 oncogenes and tumor suppressor genes (Thermo Fisher Scientific, Waltham, MA) and the BRCA Tumor MASTR PlusDx

Panel (Multiplicom, Agilent, Santa Clara, CA). The experimental procedures were performed according to the manufacturer's recommendations, using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific). Emulsion polymerase chain reaction (PCR) was performed on the Ion Chef System (Thermo Fisher Scientific) and sequencing on the Ion GeneStudio System S5 using semiconductor-based technology (Thermo Fisher Scientific).

3 | RESULTS

3.1 | Patient presentation

A 6-year-old boy was admitted to intensive care unit in the context of multisystemic inflammatory system in children (MIS-C) related to Sars-Cov2 disease. An intra-abdominal mass measuring $40 \times 42 \times 60$ mm and located anteriorly to left kidney (Figure 1) was incidentally
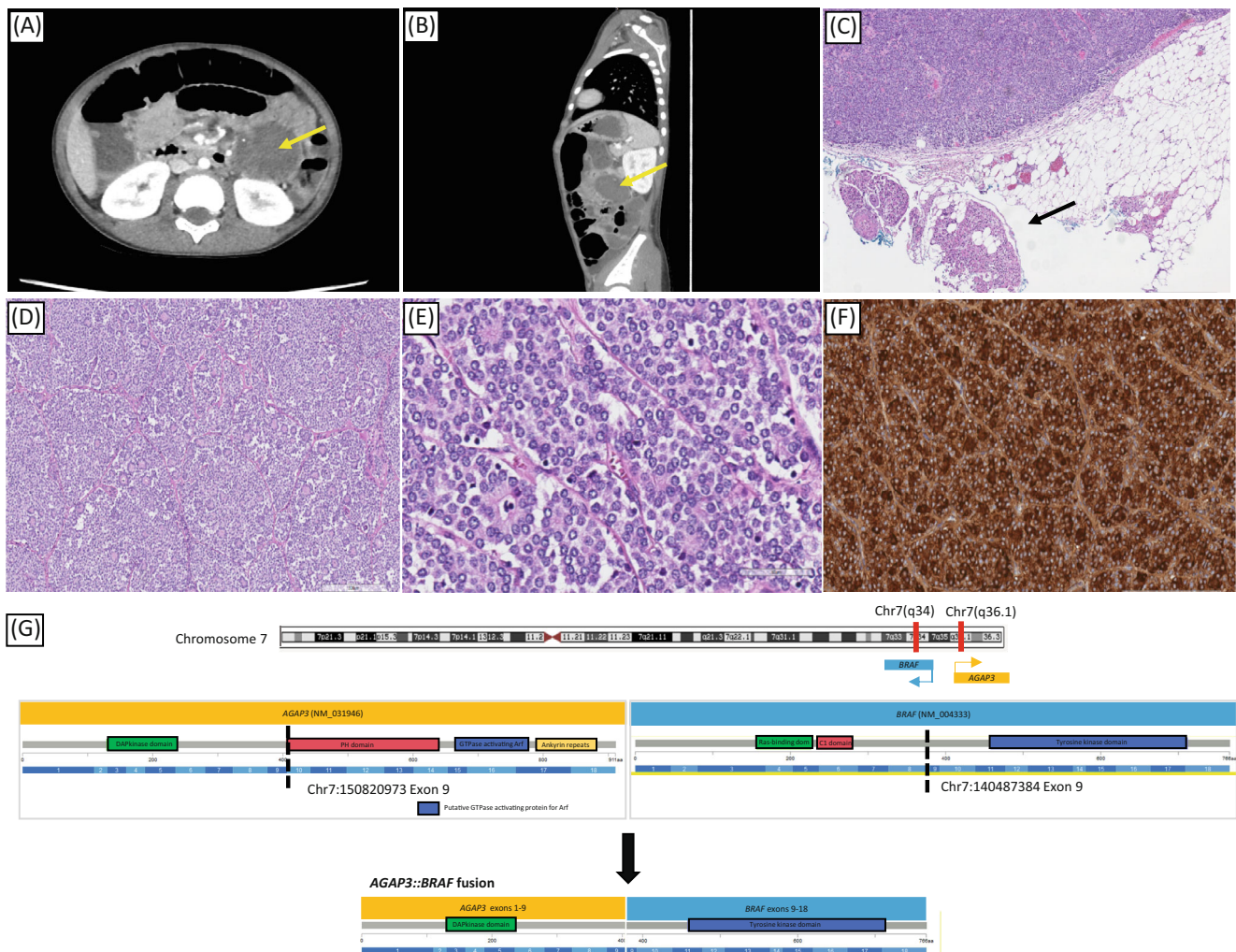

FIGURE 1 (A, B) CT-scan showing an intra-abdominal mass, located anteriorly to left kidney (arrows). (C–E) Histological features of the tumor showing (C, H&E ×2) a well-circumscribed proliferation closed to an ectopic pancreatic parenchyma (arrow), (D, H&E x5) arranged in a predominant acinar pattern (E, H&E ×10) with minute lumina. (F) Immunohistochemical analysis with BCL10 antibody showing a diffuse and strong expression (×10). (G) Schematic of the *AGAP3::BRAF* fusion transcript, depicting chromosomal locations, genomic and exonic breakpoints as well as protein domains.

detected on CT-scan. An enucleation of this extra-pancreatic tumor located 20 mm behind the pancreatic tail was done. During surgery, no pancreatic anatomical abnormality was observed. Pathological and molecular analyses led to the diagnosis of pancreatic-type ACC. Resection was microscopically incomplete. There was no nodal involvement and no distant metastasis. The national multidisciplinary team board including oncologists and pathologists expert in pediatric and adult pancreatic tumors decided to treat the patient on the basis on guidelines defined for adult patients of PACC. This young patient received adjuvant chemotherapy with 12 cycles of FOLFIRINOX modified regimen (5-Fluoro-uracil 1200 mg/m^2, Oxaliplatin 85 mg/m^2, and Irinotecan 150 mg/m^2 at D1 of each cycle). The treatment was well tolerated. After a follow-up of 12 months post chemotherapy, the patient was in first complete remission. Disease monitoring is clinically and radiologically evaluated every 3 months. Referred to an oncogenetic consultation, no predisposition syndrome was detected.

3.2 | Histological and immunohistochemical features

On gross examination, we observed a well-circumscribed, tan to red, soft and fleshy mass of $7 \times 4 \times 3$ cm. Hemorrhage and necrosis were extensive, representing up to 50% of tumor volume.

Microscopically, the tumor was partially surrounded by a thin fibrous pseudocapsule and was highly cellular with a multinodular architecture. Tumor cells were predominantly arranged in acinar structures with minute lumina and basally located nuclei (Figure 1). Focally, glandular architecture was observed (Figure 1). The resection margins were evaluated as R1. Pancreatic parenchyma was focally identified on one slide (Figure 1). No vascular or perineural invasion was observed. Tumor cells were diffusely positive for cytokeratin (AE1-AE3) and for BCL10 (Figure 1) and negative for neuroendocrine markers. Phospho-ERK was heterogeneously expressed by tumor cells. Expression of DNA MisMatch Repair proteins (MLH1, PMS2, MSH2, and MSH6) was retained.

3.3 | Molecular features

Using RNA sequencing, we observed an in-frame fusion of the exon 9 of AGAP3 (ArfGAP with GTPase domain, ankyrin repeat and PH domain 3) with the exon 9 of BRAF (Figure 1). The breakpoints on chromosome 7 were located at chr7:150820973 and chr7:140487384, respectively. We did not detect any other alterations. Notably, we did not observe chromosomal imbalance or loss of heterozygosity using aCGH/SNP and no point mutation using NGS.

4 | DISCUSSION

Most available molecular data on PACC have been provided by studies of adult cases. Several molecular driver alterations have been

described, including alterations of the Wnt pathway, the TP53 pathway, the DNA repair pathways (with BRCA1/2 and MMR alterations), and the RAF/MAPK pathway.[7,10,11] This latter pathway is frequently altered with BRAF and RAF1 rearrangements, occurring in approximately 30% of PACC cases.[5–8] Only a few pPACC have been subject to molecular analyses (Table 1). The most significant results were extracted from a study of a large series of 3633 pediatric tumors screened for BRAF alterations: the sole two pPACC included in this series showed a PPP1CC::BRAF fusion in one case and BRAF mutation in the other case (Table 1).[13] Interestingly, the case with BRAF fusion also presented MYC amplification (formerly known as c-MYC). Cramer et al. had also described a case of pPACC that harbored a BRAF (V600E) mutation associated with a MLL3 (R2463C) mutation (Table 1).[14] The other studies that presented pediatric cases of PACC were less informative. The series from Abraham et al. was published before the identification of BRAF/RAF1/RET rearrangements and was only focused on APC/β-Catenin pathway (Table 1).[4] The study of Wang et al. was focused on the detection of BRAF fusions but was not strictly pediatric since the youngest patients among 49 cases were two adolescents (Table 1).[5] No BRAF rearrangement or fusion was found in those two cases. In the study of Prall et al., the youngest patient was a young adult. No BRAF/RAF1/RET rearrangements were found by FISH analyses (Table 1).[6] In the pediatric case presented here, we observed a fusion gene involving BRAF. Accounting this new result, BRAF fusion has been observed in 33% of the six cases of pPACC studied at the molecular level, which is close to the proportion of BRAF alterations in adult PACC. To the best of our knowledge, the fusion partner, AGAP3 (7q36.1), is new on the PACC scene. AGAP3 encodes a major component of the N-methyl-D-aspartate (NMDA) receptor-signaling complex, which mediates long-term potentiation in synapses.[15] The encoded protein contains an N-terminal GTPase-like domain, a pleckstrin homology domain, an ArfGAP domain, and several C-terminal ankryn repeat domains.[15] The protein is mainly physiologically expressed in brain cortex, while overexpression has been reported in colorectal carcinoma and adenoma.[16] The AGAP3::BRAF fusion has previously been reported in a few cases from various tumor origins: colorectal carcinoma, lung adenocarcinoma, ovarian serous carcinoma, melanoma, and gastrointestinal stromal tumor.[17–19] The detection of this AGAP3::BRAF fusion seems important for the choice of a targeted treatment since it has been described to confer resistance to EGFR-targeted and BRAF-targeted therapies, in colorectal carcinoma and melanoma, respectively.[20,21] Fusion genes involving BRAF, RAF1, and RET are mutually exclusive in PACC. They are known to activate the mitogen-activated protein kinase (MAPK) signaling pathway and might be targetable by MEK inhibitors.[17,22,23] Whether the BRAF-fusion gene partner has an impact on sensitivity or resistance to those targeted drugs is not known yet.[24] The use of BRAF and/or MEK inhibitors has been reported in two patients with metastatic BRAF V600E-mutated PACC, showing at least a transient complete response.[14,25] Moreover, pre-clinical studies demonstrated sensitivity to MEK inhibitors in SND1::BRAF-transformed cells.[7]

Several methods are currently available to detect gene rearrangements or fusion genes. In PACC, the preferred method should be

targeted RNA sequencing, ideally with an anchored multiplex PCR technique and adequate panel including *BRAF*, *RAF1*, and *RET*. FISH analyses are not an optimal option for detecting *BRAF*, *RAF1*, and *RET* rearrangements: it implies to perform sequential analyses with different break-apart probes and in case of rearrangement, the identification of the fusion partner will not be possible. Moreover, some complex rearrangements or chromosomal inversion may remain cryptic.

We have demonstrated that adult and pediatric PACC shares some molecular features such as *BRAF* fusions. It will be further interesting to evaluate the prognostic impact of all these reported molecular alterations. Indeed, although aggressive, pPACC seems to have a better prognosis than adult PACC.[2] Most adult PACC harbor a high level of chromosomal instability while our pPACC case showed no chromosomal imbalance associated with the fusion gene.[10,11] If this absence of genomic instability were representative of pediatric PACC, it could be hypothesized that this better outcome is related to the differences of genomic background.

In conclusion, we described a novel fusion gene *AGAP3::BRAF* in a pediatric pancreatic-type ACC. No associated genomic instability was found. The identification of *AGAP3* as a new fusion partner gene in the PACC scene confirms the variability of partners and therefore the main pathogenic role of *BRAF* in PACC. These results indicate that pediatric cases of PACC share with adult cases a deregulation of *BRAF* that is probably a founder event. The better outcome of pediatric cases might be related to their stable genomic background. The detection of *BRAF* fusion gene is important to be done in pediatric cases since they might be targetable by MEK inhibitors.

CONFLICT OF INTEREST
The authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in Gene Expression Omnibus at https://www.ncbi.nlm.nih.gov/geo/info/update.html, reference number GSE203175.

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