A FISH assay efficiently screens for *BRAF* gene rearrangements in pancreatic acinar-type neoplasms

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Approximately 1–2% of pancreatic neoplasms are acinar cell carcinomas. Recently, *BRAF* gene rearrangements were identified in over 20% of acinar-type neoplasms, which included both pure acinar cell carcinomas and mixed carcinomas with acinar differentiation, using next-generation sequencing-based platforms, providing a potential therapeutic target for patients with these neoplasms. Thus, it is clinically important to develop a rapid, cost- and material-efficient assay to screen for *BRAF* gene fusions in pancreatic acinar-type neoplasms. We developed a dual color, break-apart FISH assay to detect *BRAF* gene rearrangements in these neoplasms and evaluated its performance in comparison to next-generation sequencing-based studies. A blinded *BRAF* rearrangement FISH investigation was performed on 31 acinar-type neoplasms that had been studied previously by next-generation sequencing-based analysis as well as on 18 additional acinar-type neoplasms that were accrued over the past 2 years. In total, *BRAF* fusions were identified in 12/49 (24%) acinar-type neoplasms by FISH. *BRAF* fusion partners were uncovered by using targeted next-generation sequencing studies in 11 FISH-positive cases that had sufficient material for next-generation sequencing studies. *SND1* was the most frequent fusion partner involved in *BRAF*-fusion acinar-type neoplasms (50%), followed by *HERPUD1* (18%). No *BRAF* fusions were identified by next-generation sequencing in any of the FISH-negative cases investigated. FISH analysis showed that *BRAF* rearrangements were diffusely present across tumor-rich areas in *BRAF*-fusion acinar-type neoplasms, which is consistent with an oncogenic driver alteration pattern. Thus, we demonstrated that, in comparison to targeted next-generation sequencing-based technologies, the FISH assay is highly sensitive and specific as well as time- and cost-efficient in the detection of *BRAF* fusions in acinar-type neoplasms. The FISH assay can be easily implemented in diagnostic settings to identify acinar-type neoplasms patients potentially suitable for targeted therapy to inhibit MAPK pathway activity.

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Pancreatic acinar cell carcinomas account for 1–2% of pancreatic cancers.¹ In addition to characteristic morphological features, the diagnostic hallmark of acinar cell carcinomas is the immunohistochemical expression of acinar-specific exocrine enzymes, such

as trypsin and chymotrypsin.² The common genetic aberrations of ductal adenocarcinomas, such as mutations in *KRAS*, *DPC4*, and *TP53* genes, are generally absent or uncommon in pancreatic acinar-type neoplasms. Alterations in the APC/ β -catenin pathway, either through activating mutations of *CTNNB1* gene or truncating mutations of *APC* gene, have been reported in a subset of pancreatic acinar-type neoplasms (20–50%).² As chemo and radiation therapies have limited efficacy against these tumors, exploring genetic aberrations in pancreatic acinar-type neoplasms to identify actionable molecular targets may trigger the development of novel therapies.

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In a recent study, our group performed comprehensive genomic profiling of 44 pancreatic acinar-type neoplasms (including mixed carcinomas with acinar differentiation) using a next-generation sequencing-based technical platform and identified *BRAF* gene rearrangements in over 20% of the neoplasms.³ The pilot next-generation sequencing study revealed eight different *BRAF* fusions, five of which were *SND1-BRAF* with minor differences in the fusion points. Despite a diversity of fusion partners and fusion points, all *BRAF* rearrangements involved relatively conserved *BRAF* break points that preserve the kinase domain. The pilot study also demonstrated that the most prevalent fusion in pancreatic acinar-type neoplasms, *SND1-BRAF*, was oncogenic through the activation of the MAPK pathway, which could be abrogated *in vitro* with MEK inhibition. The identification of *BRAF* fusions in a significant subset of pancreatic acinar-type neoplasms provides a potential therapeutic target for patients with this type of tumor. Thus, it is clinically important to develop a rapid, cost- and material-efficient assay to screen for *BRAF* gene fusions in acinar-type neoplasms.

Given that multiple fusion partners and variable fusion points challenge RT-PCR primer design, we developed a dual-color break-apart gene rearrangement FISH assay. In this study, we evaluated the FISH assay for the detection of *BRAF* fusions in acinar-type neoplasms with a comparison to next-generation sequencing-based studies.

Materials and methods

Study Population

This study was conducted with the approval of the Memorial Sloan Kettering Cancer Center Institutional Review Board. The study included both pure acinar cell carcinomas and mixed carcinomas with acinar differentiation, such as mixed acinar neuroendocrine carcinoma, mixed acinar ductal carcinoma, and mixed acinar neuroendocrine ductal carcinoma, all of which exhibit predominantly acinar differentiation and are known to be pathologically related to pure acinar cell carcinoma;⁴ for simplicity, all cases are collectively referred to as acinar-type neoplasms henceforth. We included 31 acinar-type neoplasms that were previously used in the pilot study (discovery cohort) for the discovery of *BRAF* fusions and an independent cohort (second cohort) of 18 acinar-type neoplasms cases that were accrued over the past 2 years. The entire study set of 49 acinar-type neoplasms included 45 pancreatic and 4 hepatic primaries, in 35 males and 13 females, with a median age of 59 (range of 15–80 years old). Demographics and specific histopathological diagnosis for each case are presented in Supplementary Table 1.

The discovery cohort included 17 pure acinar cell carcinomas, 7 mixed acinar neuroendocrine carcinomas, 4 mixed acinar ductal carcinomas, and 3 mixed acinar neuroendocrine ductal carcinomas. The discovery cohort, in which *BRAF* fusions were identified in six cases using a next-generation sequencing-based approach, was used to validate the FISH assay. The second cohort, used to further evaluate the prevalence of *BRAF* fusions in acinar-type neoplasms, included nine pure acinar cell carcinomas (three were hepatic primaries), six mixed acinar neuroendocrine carcinomas, one mixed acinar ductal carcinoma (also a hepatic primary), and two mixed acinar neuroendocrine ductal carcinomas. Representative hematoxylin and eosin images of acinar-type neoplasms in our series are presented in Figure 1.

BRAF Break-Apart FISH Assay

Formalin-fixed paraffin-embedded tissue samples from all 49 cases were tested for *BRAF* rearrangements by a break-apart FISH assay using a commercial *BRAF* break-apart FISH probe (Empire Genomics), which is a mixture of two clone contigs hybridizing to the chromosome band 7q34 flanking the *BRAF* gene locus. The green fluorochrome-labeled probe hybridizes telomeric (5') to *BRAF*; the orange fluorochrome-labeled probe hybridizes centromeric (3') to *BRAF*. Four-micron (4 µm) tissue sections generated from formalin-fixed paraffin-embedded blocks of tumor specimens were pretreated by deparaffinizing in xylene and dehydrating in ethanol. Dual-color FISH assays were conducted according to the protocol for formalin-fixed paraffin-embedded sections from Vysis/Abbott Molecular with minor adjustments of pepsin treatment as needed. FISH analysis and signal capture were conducted on a fluorescence microscope (Zeiss) coupled with the ISIS FISH Imaging System (Metasystems). We analyzed 100 interphase nuclei from tumor-rich areas in each specimen.

Similar to the FDA-approved *ALK* FISH assay, *BRAF* rearrangements may appear as either 'broken apart' signals (red and green signals separated by a gap) or a 'single orange (3')' signal (missing green signal), which indicates the loss of 5' *BRAF* but preservation of the 3' *BRAF* coding the kinase domain. For the 'broken apart' signal pattern, red and green signals separated by a gap larger than 2 signal diameters were considered diagnostically positive, which is identical to the diagnostic criteria for the *ALK*-rearrangement FISH assay.

DNA-Based Targeted Next-Generation Sequencing

For the 18 new cases recruited from June 2014 to May 2016, 14 patients receiving treatment at Memorial Sloan Kettering Cancer Center were consented

Figure 1 Representative images of acinar-type carcinomas in our series. (a, b) Pure acinar cell carcinomas revealed several different architectural patterns: (a) the solid pattern; (b) the acinar; (c) one of the four hepatic primary cases; (d) acinar differentiation was confirmed with trypsin immunohistochemical staining for each case; (e) the vast majority of mixed acinar neuroendocrine carcinomas were composed of a morphologically homogenous population of cells and the divergent differentiation could not be detected without immunohistochemical labeling. However, in some cases, there were two distinct populations of neoplastic cells, one with acinar features and the other having a neuroendocrine appearance as shown here; (f) in mixed acinar ductal carcinomas, glandular structures were also usually superimposed on the solid architecture of conventional acinar cell carcinomas.

for molecular testing with Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a capture-based next-generation sequencing assay that detects somatic single-nucleotide mutations, small indels, and copy number alterations in 410 cancer-related genes, and selected structural rearrangements of 14 recurrently rearranged genes, including *BRAF*, in solid tumors (MSK-IMPACT, detailed methods have been previously published).⁵ Briefly, 100–250 ng DNA extracted from formalin-fixed paraffin-embedded tissue with >10% tumor content and matched normal DNA from blood samples, if available, were obtained for testing with MSK-IMPACT.

Anchored Multiplex RT-PCR and Next-Generation Sequencing

Tumor-rich areas were macro-dissected from sections of formalin-fixed paraffin-embedded tissues, and subjected to RNA extraction using Mineral Oil-Qiagen RNeasy formalin-fixed paraffin-embedded kit (Qiagen, USA). Random priming was used to synthesize cDNAs from the RNA samples extracted. An anchored multiplex PCR assay based on the Rapid Amplification of cDNA Ends (RACE) strategy followed by next-generation sequencing (Archer FusionPlex, ArcherDX, USA) was employed to identify-specific partners involved in selected gene fusions.⁶ We used a custom fusion-screening panel focused on 35 genes, including *BRAF*, known to be involved in recurrent gene fusions in solid tumors. The list of genes in the panel is presented in the Supplementary Table 2. Within this panel, for *BRAF* fusions, 5' RACE was conducted using *BRAF*-specific 3' primers targeting exons 7–12, and the multiplex RACE products were sequenced on MiSeq (Illumina), and sequencing data were analyzed by Archer software (version 3.3.0).

Immunohistochemical Staining

Immunolabeling for phospho-ERK was performed on tissue sections of each tumor using a rabbit monoclonal antibody (Cell Signaling Technology), phospho-p44/42MAPK (202Y284). The primary antibody was applied at a dilution of 1:1000 with an incubation time of 60 min. The secondary biotinylated anti-rabbit antibody was applied at a dilution of 1:200 for 60 min, and diaminobenzidine was used as the chromagen. Staining was performed using DISCOVERY DAB Map Detection Kit (Ventana) and the Ventana Discovery XT automated immunohistochemical staining platform. The immunolabeled slides were scored as previously described.³ In brief, labeling of $\geq 25\%$ of nuclei and cytoplasm was scored as 'positive'; labeling of $< 25\%$ of nuclei and cytoplasm was interpreted as 'focal', and no staining was considered 'negative'.

Statistical Analysis

All statistical tests were performed using the IBM-SPSS version 20.0, and the threshold for statistical significance was set at $P < 0.05$. Data were analyzed using a Chi-square and Fisher exact tests to compare the *BRAF* fusion status with age, gender, tumor size, lymphovascular invasion, tumor stage (pT), and lymph node stage (pN). Survival data were analyzed by using the Kaplan–Meier method and were assessed by log-rank test to compare differences in survival between the patients whose tumors revealed *BRAF* fusions and those whose tumors did not reveal *BRAF* fusions.

Results

Development of *BRAF* FISH and New Patient Screening

To validate the dual-color break-apart *BRAF* rearrangement FISH assay, a blinded FISH investigation was performed on 31 acinar-type neoplasms from the discovery cohort, among which, *BRAF* fusions were previously identified in six cases by next-generation sequencing-based methods. FISH identified *BRAF* gene rearrangements in seven acinar-type neoplasms (23%) in the discovery cohort, including all six cases positive for *BRAF* fusions in the prior next-generation sequencing-based studies and one additional case that had not been identified by next-generation sequencing (case #1_10, Table 1 and Supplementary Table 1). FISH analysis was repeated in this sample, and in both assays bona fide *BRAF* gene rearrangement was observed in a high percentage of cells (~90%). Considering the limited technical sensitivity of targeted DNA-seq in the detection of gene fusions, we performed targeted RNA-seq (Archer FusionPlex) to further investigate this case; however, RNA-seq failed due to suboptimal sample quality. The validated *BRAF* rearrangement FISH assay was then employed to screen for *BRAF* fusions in 18 newly accrued acinar-type neoplasms (second cohort), and 5 additional *BRAF*-rearranged cases were identified (28%). Representative FISH images are presented in Figure 2.

In total, we identified *BRAF* fusions in 12 of 49 acinar-type neoplasms (24%). The frequency of *BRAF* rearrangement identified in this expanded study is similar to that in the prior study. Table 1 summarizes the percentage of positive cells and the dominant FISH signal pattern for each of the 12 *BRAF*-rearranged samples. The specific histologic subtypes (pure acinar cell carcinoma, mixed acinar neuroendocrine, mixed acinar ductal, or mixed acinar neuroendocrine ductal carcinomas), *BRAF* fusion partners and phosphorylated ERK (pERK) immunohistochemical staining results are shown in Table 1 as well. In all except one (11/12) *BRAF*-rearranged case, positive signals were observed in 60–100% of the cells analyzed (Avg = 84%) in tumor-rich areas. The one 'outlier' case (#1_25, Table 1 and

Table 1 Twelve acinar-type carcinomas with *BRAF* fusions

Case ID	Primary tumor site	Histologic diagnosis	FISH % of positivity	FISH signal pattern	<i>BRAF</i> fusion (5' partner exon- <i>BRAF</i> exon)	Predicted chromosomal rearrangement	pERK IHC
1_4	Pancreas	Pure ACC	99%	F+single 3' <i>BRAF</i>	<i>GATM1-BRAF</i> (e2-e11)	t(7;15)(q34;q21)	Positive
1_8	Pancreas	Pure ACC	97%	F+single 3' <i>BRAF</i>	<i>HERPUD1-BRAF</i> (e4-e7)	t(7;16)(q34;q13)	Positive
1_10	Pancreas	Pure ACC	89%	Broken-apart ± F	n/a	n/a	Positive
2_2	Pancreas	Pure ACC	90%	F+single 3' <i>BRAF</i>	<i>SND1-BRAF</i> (e10-e9)	inv(7)(q31q34)	Positive
2_3	Pancreas	Pure ACC	100%	F+ broken-apart	<i>HERPUD1-BRAF</i> (e6-e7)	t(7;16)(q34;q13)	Positive
1_18	Pancreas	Mixed ACC/NE	95%	F+single 3' <i>BRAF</i>	<i>ZSCAN30-BRAF</i> (e3-e10)	t(7;19)(q34;q12)	Positive
2_13	Pancreas	Mixed ACC/NE	95%	F+single 3' <i>BRAF</i>	<i>GIPC2-BRAF</i> (e3-e10)	t(1;7)(p31q34)	Positive
1_25	Pancreas	Mixed ACC/D	38%	F+ broken-apart	<i>SND1-BRAF</i> (e14-e11)	inv(7)(q31q34)	Positive
1_26	Pancreas	Mixed ACC/D	73%	F+single 3' <i>BRAF</i>	<i>SND1-BRAF</i> (e10-e11)	inv(7)(q31q34)	Positive
1_28	Pancreas	Mixed ACC/D	68%	F+ broken-apart	<i>SND1-BRAF</i> (e9-e9)	inv(7)(q31q34)	Focal
2_16	Liver	Mixed ACC/D	60%	Broken-apart, no F	<i>SND1-BRAF</i> (e14-e9)	inv(7)(q31q34)	Positive
2_17	Pancreas	Mixed ACC/NE/D	83%	Broken-apart ± F	<i>SND1-BRAF</i> (e9-e9)	inv(7)(q31q34)	Positive

Abbreviations: ACC, acinar cell carcinoma; ACC/NE, acinar cell/neuroendocrine carcinoma; ACC/D, acinar cell/ductal carcinoma; F, fusion signals; IHC, immunohistochemistry.

Supplementary Table 1), which harbored an *SND1-BRAF* fusion, showed positivity in 38% of the cells, according to our stringent FISH signal scoring criteria. However, many cells that were not considered diagnostically positive by the scoring criteria had 'broken apart' red and green signals separated by 1.5–2 signal diameters, which fell below the threshold for positivity. *SND1* and *BRAF* are ~13 Mb apart on genomic DNA, respectively mapping to 7q32 and 7q34, and running in opposite directions on chromosome 7. We suspect a complex chromosomal rearrangement occurred at 7q in this sample.

Comparison of FISH to Next-generation Sequencing-Based Studies in the Detection of *BRAF* Fusion

BRAF fusion was also explored by the targeted DNA-seq (MSK-IMPACT) assay in 14 of 18 acinar-type neoplasms in the second cohort, including all FISH-positive cases (five cases) and nine FISH-negative cases. MSK-IMPACT identified *BRAF* fusions in four cases, and the remaining FISH-positive case was further analyzed by the targeted RNA-seq (Archer), and an *HERPUD1-BRAF* fusion was identified. It is worth noting that, besides the strong evidence from MSK-IMPACT or Archer showing bona fide *BRAF* fusions, no other gene fusion was identified in any of the five FISH-positive samples. Targeted RNA-seq (Archer) was also used to analyze two of four FISH-negative cases that could not be analyzed by MSK-IMPACT, and no *BRAF* fusions were identified. The remaining two *BRAF* FISH-negative cases had insufficient tumor tissue for targeted DNA-seq or RNA-seq studies. As comprehensive mutation profiling of acinar-type neoplasms has been presented previously by our group and others,^{3,7} a complete genetic aberration analysis was not performed on the next-generation sequencing data obtained from the second cohort cases in the present study; instead, we focused on *BRAF*-related genetic alterations only. No mutation in *BRAF* or *RAS* was identified in any of the samples in the second cohort.

In our expanded study, *SND1* was still the most frequent fusion partner involved in *BRAF*-fusion acinar-type neoplasms (50%), followed by *HERPUD1* (2/11) (Table 1). All *BRAF* fusion partners in acinar-type neoplasms, except *SND1*, are located on chromosomes other than 7. A summary of findings in all 49 acinar-type neoplasms by FISH and next-generation sequencing-based studies is available in Supplementary Table 1. In brief, using next-generation sequencing-based studies (targeted DNA-seq or RNA-seq), in-frame *BRAF* fusions were identified in all 11 FISH-positive cases in which next-generation sequencing studies could be successfully performed, whereas no *BRAF* fusions were identified in any of the FISH-negative cases investigated.

We also looked into FISH signal patterns in each positive case and the possible correlation with different

Figure 2 Interphase FISH detection of *BRAF* rearrangement in acinar-type carcinomas. Representative images for each FISH signal patterns. (a) FISH negative for *BRAF* rearrangement, showing fusion signals of 5' and 3' *BRAF*; (b) FISH positive for *BRAF* rearrangement—'broken apart' signal pattern (red and green signals separated by a gap); (c) FISH positive for *BRAF* rearrangement—'single orange' (3' *BRAF*) signal pattern, which indicates the loss of 5' *BRAF* but preservation of the 3' *BRAF* coding the kinase domain.

fusion partners. Interestingly, all six cases except one with the fusion partner other than *SND1* showed the 'single 3' *BRAF*' FISH signal pattern; in contrast, the 'broken apart' signal was slightly dominant (4/6) in *SND1*–*BRAF* fusions. FISH signal pattern and *BRAF* fusion partner are listed case by case in Table 1.

Immunohistochemical Staining for pERK

Immunohistochemical staining for pERK, a readout of MAPK pathway activation, was performed on all samples in the discovery cohort and on 11 of 18 cases in the second cohort for which sufficient material was available (Supplementary Table 1). Representative immunohistochemical staining for pERK is shown in Figure 3. Of the fusion-positive cases ($n=12$), 11 had sufficient material for immunohistochemical analyses; 10 stained strongly positive for pERK and the remaining case showed focal positivity.

Of the 31 fusion-negative samples with material available for immunohistochemistry, 3 were positive (all in the discovery cohort), 4 were focally positive (1 in the discovery and 3 in the second cohort), and 24 were negative for pERK staining. Cases from the discovery cohort were discussed, regarding the mechanisms for positive pERK staining, in our previous publication.³ In terms of the second cohort, amplification of *BRAF* and *FGFR1* were likely responsible for the activation in the MAPK pathway and pERK focal staining in two samples, respectively. The mechanism for focal pERK staining in the remaining one sample could not be elucidated as next-generation sequencing mutation profiling was not performed due to the insufficiency of tumor material available for the study.

Comparison of *BRAF* Fusion Status to Clinicopathologic Features

Tumors with *BRAF* fusion found to be significantly smaller than those without *BRAF* fusion (median

tumor size 3.5 vs 5.5 cm, $P=0.001$). However, there was no significant difference in other clinicopathologic features, including age, gender, lymphovascular invasion, tumor stage (pT), and lymph node stage (pN). Similarly, *BRAF* fusions did not bear any prognostic significance (3- and 5-year disease-free survival of the patients whose tumors revealed *BRAF* fusions were 28 and 14 vs those of the patients whose tumors did not reveal *BRAF* fusions were 54% and 40%, respectively; $P=0.24$) (Supplementary Table 3).

Discussion

The discovery of recurrent *BRAF* fusions in acinar-type neoplasms, including pure acinar cell carcinomas and mixed acinar carcinomas, indicates a new potential therapeutic target for patients whose tumors harbor these changes. In this study, we evaluated the utility of a *BRAF*-rearrangement FISH assay for the detection of *BRAF* fusions in acinar-type neoplasms and demonstrated 100% analytical sensitivity and specificity. In *BRAF*-rearranged cases, the fusion signal being observed in over 60% of the cells analyzed (Avg=84%). Our FISH study also indicated that *BRAF* gene rearrangements are diffusely present in *BRAF*-fusion acinar-type neoplasm samples, which is consistent with an oncogenic driver alteration pattern. The relative proximity of *SND1* (the most frequent fusion partner involved in *BRAF*-fusion acinar-type neoplasms) and *BRAF* at 7q was not an issue for the detection of *SND1*–*BRAF* fusions using this test. The 'single 3' *BRAF*' signal pattern (loss of 5' *BRAF*) was commonly observed in *BRAF*-rearranged cases (50%), which is compatible with functional studies of previously identified *BRAF* fusions showing fusions with *BRAF* truncations of the N terminus function as constitutive kinases through RAF dimerization.^{3,8–14}

Compared to the FISH assay, targeted next-generation sequencing-based DNA-seq studies are

capable of identifying fusions and also the specific fusion partners and fusion points, provided that the fusions occur within exons or introns included in the assay.⁵ However, the sensitivity of *BRAF* fusion detection varies due to the nature of the genomic locus around the fusion point as well as the technical limitations of the methodology. For instance, *HERPUD1-BRAF* fusions occurred at *BRAF* exon 7 in cases #1_8 and #2_3 were not detected by MSK-IMPACT because our design does not include capture baits targeting intron 6 of *BRAF*. Given that multiple *BRAF* fusion partners occur in acinar-type neoplasms, it is not feasible to design individual RT-PCR tests for diagnostic use. Complementarily, RNA-seq is an ideal method for the detection of fusion transcripts. However, the quality of RNA extracted from formalin-fixed paraffin-embedded tissues may limit the utility of this technical platform. In fact, a few distinct features of FISH, such as fast turn-around time, extremely low sample consumption and tumor content requirement, cost-efficiency, as well as high sensitivity and specificity, make FISH superior to targeted DNA-seq or RNA-seq when a test for a specific fusion is needed in the diagnostic setting.

Prior to the discovery of *BRAF* fusions in pancreatic acinar-type neoplasms, *BRAF* fusions had been identified in several other cancer types,^{8–11,13–15} with an enrichment in pilocytic astrocytomas.^{9,14} *KIAA1549-BRAF* is the most frequent *BRAF* fusion in pilocytic astrocytomas, followed by *FAM131B-BRAF*. Interestingly, both fusions are derived from subtle unbalanced genomic alterations at chromosome locus 7q34, a ~2 Mb tandem duplication and a ~2.5 Mb interstitial deletion, respectively. In contrast, the *BRAF* fusion partners identified in acinar carcinomas are physically distant from *BRAF*, with *SND1*, which is ~13 Mb away from *BRAF* on chromosome 7, being the closest one. Our study in acinar-type neoplasms demonstrated that the *BRAF* rearrangement FISH test presented here is fully

capable of detecting *SND1-BRAF* fusions. However, this FISH assay may not be optimal for the detection of *KIAA1549-BRAF* fusions in pilocytic astrocytomas where *BRAF* fusions resulted from subtle genomic alterations. In fact, we have developed another *BRAF* FISH assay that is optimized to screening for *KIAA1549-BRAF* in pilocytic astrocytomas (data not shown).

As shown in Table 1, *BRAF* fusions were observed in 11 primary pancreatic samples and one primary hepatic sample, which indicates that hepatic acinar carcinomas can share the same genetic changes as their pancreatic counterparts. The occurrence of acinar-type neoplasm as a primary liver neoplasm has been reported in a few cases.^{16–18} In this study, we included four primary liver carcinomas that showed acinar cell differentiation without any evidence of hepatocellular differentiation. For each case, a pancreatic primary was excluded on preoperative imaging procedures, during surgery, and based on follow-up after surgery, thus justifying their classification as primary acinar-type neoplasms of the liver.

In terms of histopathologic subtypes, *BRAF* gene rearrangements were observed in all variants of acinar-type neoplasms: five pure acinar cell carcinomas, four mixed acinar ductal carcinomas, two mixed acinar neuroendocrine carcinomas, and one acinar neuroendocrine ductal carcinoma. Table 2 illustrates the prevalence of *BRAF* fusions in each histologic subtype. Although the numbers of cases are relatively small, it is worth noting that 4/5 (80%) mixed acinar ductal carcinomas in our study had *BRAF* fusions, and all were *SND1-BRAF* fusions. In contrast, *BRAF* fusions were much less prevalent in other histologic subtypes, ie, roughly 20% of each of the other subtypes had *BRAF* fusions. In addition, fusion partners other than *SND1* seemed to be predominate in other histologic subtypes, and both *HERPUD1-BRAF* cases were pure acinar cell carcinomas. Why *BRAF* fusions, in particular

Table 2 The prevalence of *BRAF* fusions in each acinar carcinoma histologic subtype

Histologic subtype	Number of cases	Number of <i>BRAF</i> fusions (%)	Number of <i>SND1-BRAF</i> fusions
Pure acinar cell carcinoma	26	5 (19%)	1
Mixed acinar cell/neuroendocrine carcinoma	13	2 (20%)	0
Mixed acinar cell/ductal carcinoma	5	4 (80%)	4
Mixed acinar cell/neuroendocrine/ductal carcinoma	5	1 (20%)	1

Figure 3 Representative immunohistochemical staining for pERK and corresponding morphology feature and *BRAF* rearrangement FISH analysis finding. (a) A pure acinar cell carcinoma case with solid growth pattern, expressing nuclear and cytoplasmic pERK labeling. *BRAF* rearrangement was detected by the FISH assay showing multiple 'single orange (3'*BRAF*)' signals; (b) a mixed acinar ductal carcinoma case, positive for pERK staining. *BRAF* rearrangement was detected by the FISH assay showing 'broken apart' signal pattern; (c) a pure acinar cell carcinoma case with glandular growth pattern is focally positive for pERK by immunohistochemistry. *BRAF* rearrangement was not detected by the FISH assay, but the FISH assay revealed *BRAF* gain/amplification.

SND1-BRAF, are enriched in mixed acinar ductal carcinomas is unclear; the prevalence of *BRAF* fusion in pancreatic ductal adenocarcinomas is extremely rare ($< 0.1\%$),¹⁵ so it seems unlikely that the components with a ductal lineage contributed to the observed increased frequency. Whether or not 5' partners in *BRAF* fusions impact clinical outcomes or sensitivity to targeted therapy will be addressed in future studies.

In summary, we developed a *BRAF*-rearrangement FISH assay to screen for *BRAF* fusions in pancreatic acinar-type neoplasms. In our expanded study cohort, we demonstrated *BRAF* fusions in 24% (12/49) of acinar-type neoplasms, a rate similar to that reported in our previous study. In comparison to other sequencing-based technologies, we demonstrated that the FISH assay is highly sensitive and specific as well as time- and cost-efficient in the detection of *BRAF* fusions in acinar-type neoplasms. The FISH assay can be easily implemented in diagnostic settings to identify acinar-type neoplasm patients suitable for targeted therapy to inhibit *MAPK* pathway activity.

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Disclosure/conflict of interest

Juliann Chmielecki, Jeffrey Ross, and Philip J Stephens are employees and stockholders in Foundation Medicine.

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Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)