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Monitoring Tumor Burden in Response to FOLFIRINOX Chemotherapy Via Profiling Circulating Cell-Free DNA in Pancreatic Cancer

Tao Wei[1,2], Qi Zhang[1,2], Xiang Li[1,2], Wei Su[1,2], Guogang Li[1,2], Tao Ma[1,2], Shunliang Gao[1,2], Jianying Lou[1,2], Risheng Que[1,2], Lei Zheng[3], Xueli Bai[1,2], and Tingbo Liang[1,2]

Abstract

We aimed to explore the application of circulating cell-free DNA (cfDNA) profiling in monitoring tumor burden in patients with pancreatic ductal adenocarcinoma (PDAC). Thirty-eight patients with advanced PDAC receiving first-line FOLFIRINOX chemotherapy were prospectively enrolled. Next-generation sequencing for a panel of 560 genes covering a wide range of cancer-related loci was performed to profile cfDNA. In total, 25 patients (65.8%) had at least one common driver gene alterations (*KRAS*, *TP53*, *SMAD4*, *CDKN2A*) detected within cfDNA. In contrast, no above tumor-related recurrent mutations were found in plasma from 13 healthy individuals. Concordant alterations in plasma cfDNA and tumor tissue DNA was confirmed in two of three patients with available tissues. Further analysis showed that mutant allele fraction (MAF) for altered loci in cfDNA correlated with tumor stage, metastatic burden, and overall survival. Serial blood samples were collected from 17 patients after chemotherapy. We found that allele fraction for specific altered loci declined in chemotherapy-responding subjects. For cases who were resistant to this therapeutic regimen, increased ctDNA MAF was observed at the time of disease progression. Meanwhile, the dynamics of total cfDNA concentration correlated with tumor burden following chemotherapy. Collectively, we provide evidence that pretreatment ctDNA level correlates with tumor burden in PDAC, and serial cfDNA analysis is a robust tool for monitoring cancer response to chemotherapy.

Introduction

Cancer arises through accumulation of genomic aberrations. Numerous evidence has revealed complicated mutational landscape in various types of cancer and genetic heterogeneity among patients (1, 2). These somatic alterations are almost exclusively present in tumor cells, and thereby serve as specific biomarkers for cancer patients. However, tumor tissue sampling is usually not convenient in clinic in particular for those who cannot undergo surgical resection. It has been shown that mutant DNA within tumor cells can be released into blood, and sequencing of circulating nucleic acids in plasma represents a way of liquid biopsy. Besides the ease of blood sample acquirement, the profiling of cell-free DNA (cfDNA) in plasma is more likely to recapitulate the whole genetic spectrum of tumor compared with tissue sequencing (3). Abundant evidence has confirmed that quantification of mutant circulating tumor DNA (ctDNA) is valuable in cancer diagnosis, prognositication, monitoring therapy response, and postoperative recurrence (4–6). Emerging studies are also reported its application in tracking clonal evolution and elucidating treatment-resistant mechanisms (7, 8).

Currently, ctDNA can be measured with distinct techniques and strategies. Digital PCR and other allele-specific PCR offer a high sensitivity for detecting individual loci like hotspot mutations (9, 10). Genome-wide analysis through next-generation sequencing (NGS) like whole-exome sequencing was also adopted, but the sensitivity is inadequate with detection limit of 5% to 10% of allele fraction (11, 12). In addition, targeted sequencing for specific DNA regions of interest constitutes a way to interrogate a broad range of loci. This strategy has the advantage to design multiplex patient-specific panels, and the sensitivity can be improved by increased sequencing depth and even ultra-deep sequencing (13).

The aim of this study was to explore the application of cfDNA profiling in monitoring tumor burden change following treatment. Instead of focusing on a few genes, we performed targeted NGS with a customized panel covering 560 cancer-related genes. To this end, a cohort of advanced pancreatic cancer patients receiving FOLFIRINOX (5-fluorouracil, Irinotecan, and Oxaliplatin) regimen was followed. The result showed a high degree of concordance between therapy response and dynamics of ctDNA allele fraction. Applying a broader panel provided additional value as infrequently mutated genes in pancreatic cancer can be detected in selected cases for tracking tumor burden, but this may limit to a small number of patients. Furthermore, we found that the total cfDNA concentration was also useful in monitoring tumor response for selected cases.

[1]Department of Hepatobiliary and Pancreatic Surgery, The Second Affiliated Hospital of Zhejiang University, School of Medicine, Hangzhou, China. [2]Zhejiang Provincial Key Laboratory of Pancreatic Disease, Hangzhou, China. [3]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

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T. Wei and Q. Zhang contributed equally to this article.

Corresponding Authors: Tingbo Liang, The Second Affiliated Hospital, Zhejiang University School of Medicine, 88 Jiefang Road, Hangzhou 310009, China. Phone: 86 571 87315006; Fax: 86 571 87315006; E-mail: liangtingbo@zju.edu.cn; and Xueli Bai, shirleybai@zju.edu.cn

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Materials and Methods

Study patients

Between October 2015 and April 2018, treatment-naïve patients with pancreatic ductal adenocarcinoma (PDAC) were enrolled from the Second Affiliated Hospital of Zhejiang University, School of Medicine, China. Informed consent was obtained from all patients for participation in the study. The study was approved by the institutional review board of the Second Affiliated Hospital of Zhejiang University, School of Medicine. Clinical information included age, gender, smoking status, tumor staging, tumor location, carbohydrate antigen 19-9 (CA19-9) level, number of FOLFIRINOX therapy cycles, and chemotherapy response. Diagnosis of pancreatic adenocarcinoma was confirmed by pathologic examination of either primary or liver metastasis biopsy for all enrolled subjects. The staging of PDAC was evaluated using American Joint Committee on Cancer (AJCC) criteria. Proximal location was defined as tumor in pancreatic head or neck, whereas distal tumor refers to location in body or tail. All included patients received FOLFIRINOX therapy as first-line treatment except one undergoing initial surgery. This regimen was modified by reducing Oxaliplatin and Irinotecan to 85% and 75% of their full dose, respectively, and omitting the Fluorouracil intravenous bolus (14). Assessment of treatment response was performed every four cycles of chemotherapy via abdominal enhanced CT and enhanced MRI of the liver. According to the Response Evaluation Criteria in Solid Tumors version 1.1 (15), the imaging measurement of tumor response was qualitatively classified into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). This work was conducted in accordance with the guideline of the Declaration of Helsinki.

Sample collection

Five to ten milliliters of peripheral blood was collected from each patient before treatment and at time when therapy response was assessed. Plasma was extracted within 3 hours of blood collection and was immediately frozen in −80°C freezer or liquid nitrogen. Peripheral blood from eight healthy donors was obtained for the same manipulation. Peripheral blood monocytes (PBMC) were obtained synchronously before chemotherapy. Fresh resected tumor tissues or biopsies were collected when possible.

Targeted sequencing of circulating mutant DNA

cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit. DNA from fresh frozen tissue and peripheral blood mononuclear cells (PBMC) were extracted using the DNeasy Blood & Tissue Kit. Purified DNA was quantified by Qubit DNA Assay Kit in Qubit 2.0 Flurometer. The size distribution of cfDNA was analyzed then (Supplementary Fig. S1A). Sequencing libraries were prepared using the Agilent SureSelect XT Custom Kit with an optimized manufacturer's protocol. Different libraries with unique indices were pooled together, and hybridized with SureSelect biotin labeled probes targeting 9,762 exons of 560 cancer-relevant genes (Supplementary Table S1). The target exons of genes were captured by streptavidin-coated magnetic beads. Captured libraries were enriched in a PCR reaction to add index tags. Library fragment size was confirmed then (Supplementary Fig. S1B). The clustering of the index-coded samples was performed on a cBot Cluster Generation System using Hiseq PE Cluster Kit. Then, the DNA libraries were sequenced on Illumina HiSeq4000 NGS platform. For cfDNA and tumor tissues, we intended to generate an average of 1,000 × sequencing depth. Reads from each sample were mapped to the reference sequence hg19. Somatic variant calls presenting at less than 1% mutant allelic frequency (MAF) in the paired PBMC, but with over 0.5% allelic frequency and at least four reads supporting variant alleles in cfDNA or tumor tissue samples, were retained. Raw sequence data have been deposited at the Sequence Read Archive (SRA) under accession number PRJNA491473.

Statistical analysis

Mann–Whitney *U* test was used for comparison of continuous parameters including total cfDNA concentration and MAF. Correlation analysis was performed using Pearson correlation method. Survival analysis was performed using Kaplan–Meier method and multivariate Cox regression analysis. All statistical calculations were performed using SPSS 22.0 (SPSS) and Prism 6.0 (Graphpad). Two-sized *P* value less than 0.05 was considered as significant.

Results

Patient characteristics

The demographics and clinical characteristics for included 38 patients are shown in Table 1. The majority of patients are male (71%). The median age is 62 years-old, and 11 patients are over 65 years of age. Twenty-eight cases were diagnosed with stage IV disease, of whom 26 had liver metastasis and 2 had peritoneal metastasis. For other 10 subjects, stage III disease was diagnosed due to unresectable tumor and absence of metastatic lesions. All cases were treatment-naïve at the time of diagnosis in our center, and FOLFIRINOX therapy was given as initial treatment except one patient. The last patient underwent curative resection first. However, liver metastasis was found 1 month postoperatively, and FOLFIRINOX regimen was given. A median of four cycles of

Table 1. Patients characteristics

Variables	All patients ($n = 38$)	ctDNA MAF <1.5% ($n = 17$)	ctDNA MAF >1.5% ($n = 21$)	*P* value
Age, median (range)	62 (42–74)	61	62	0.893
Gender, *n*				0.721
Male	27	13	14	
Female	11	4	7	
Smoking status, *n*				0.181
No	27	13	14	
Yes	11	4	7	
Tumor location, *n*				
Proximal	18	9	9	1.000
Distal	20	8	12	
Stage, *n*				
III	10	8	2	**0.023**
IV	28	9	19	
CA19–9, median (range)	1,964 (2–12,000)	879	4496	0.204
FOLFIRINOX cycles, median (range)	4 (2–13)	7	6	0.413
FOLFIRINOX response[a], *n*				0.542
PR	6	2	4	
SD	10	4	6	
PD	14	8	6	

[a]Eight patients did not finish four cycles of therapy and thus were not evaluated for response. The response showed here is the outcome after all cycles of therapy for each case.

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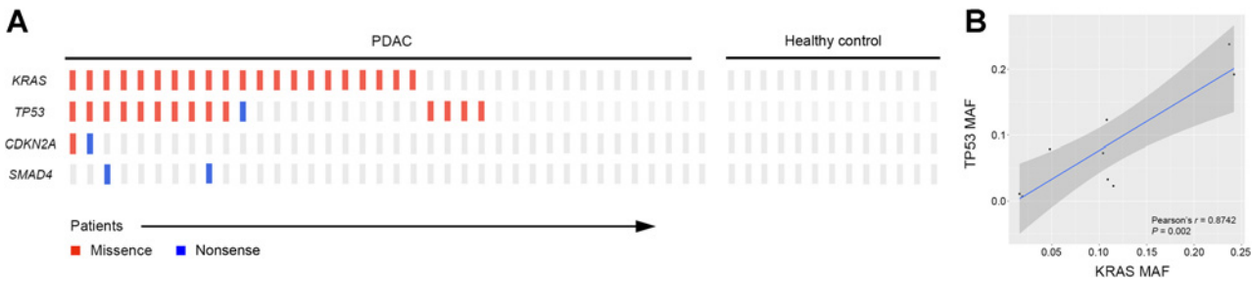

Figure 1.
cfDNA profiling in patients with advanced pancreatic cancer. The distribution of detected ctDNA in selected genes among included patients with PDAC and healthy individuals (**A**). The correlation between *KRAS* and *TP53* cfDNA MAF was analyzed (**B**).

chemotherapy were performed for all cases, and eight patients did not complete four cycles. For two subjects with stage III disease, remarkable shrinkage of primary tumor was observed after neoadjuvant treatment, and curative resection was performed thereafter.

cfDNA profiling

For cfDNA samples, a median of 987× sequencing depth was acquired after excluding duplication, and 95% of exons were at coverage >100× for all samples. We initially focused on the mutations in common driver genes for PDAC including *KRAS*, *TP53*, *CDKN2A*, and *SMAD4*. Twenty-five of 38 included subjects (65.8%) had at least one alteration for above genes in plasma (Fig. 1A). *KRAS* and *TP53* mutation were present in 84% and 60% of these cases, respectively. G12D ($n = 13$) was the most frequently observed alteration at *KRAS* followed by G12V ($n = 5$), G12R ($n = 3$), and G13D ($n = 1$). The MAF for altered *KRAS* varied between patients reflecting distinct tumor burden (median: 3.4%, range: 0.66–32.6%). One patient had simultaneous *KRAS* G12D and G13D mutation with comparable allele frequency (10.3% vs. 10.5%). For the remaining 13 cases without detection of the four driver gene alterations, we noted three patients harbored hotspot mutation in other loci including *GNAS* and *SF3B1*. The altered codon in all these genes have been reported in PDAC, and cause functional impact. In contrast, none aforementioned hotspot variants were detected in plasma from 13 healthy individuals (Fig. 1A). Noteworthy, 11 patients with PDAC had detectable amounts of *KRAS* and *TP53* mutation concomitantly, and they are presumably to be quantitative correlated as both of them are presented in early clone. Indeed, there was a significant correlation between MAF of them (Pearson's $r = 0.8742$; Fig. 1B). This provides yet another validation of the reliability of our assay and its quantitative nature.

Concordance of alterations in plasma and tumor tissue could be evaluated in three patients. The time interval of acquisition between tumor biopsy or tissue and synchronous blood sample was within 3 days for all cases. In the first patient, liver metastatic biopsy was obtained for analysis, and all four somatic nonsynonyms mutations at *KRAS*, *TP53*, *SMAD4*, and *C8orf34* presented in biopsy was also found in the plasma cfDNA (Table 2). $KRAS^{G12V}$ alteration detected in this patients' tumor was validated by Sanger sequencing (Supplementary Fig. S2). Other two patients underwent curative resection after neoadjuvant chemotherapy, and thus primary tumors were available. In one case, same hotspot variant at *TP53* was detected in tissue and plasma (Table 2). For another patient, $SF3B1^{R625C}$ was detected in plasma, whereas there were no alterations found in tumor tissue possibly due to extremely low viable tumor cell fraction after FOLFIRINOX therapy.

Correlation of ctDNA level and tumor burden

The median total cfDNA concentration for patients with PDAC was significantly higher than those of healthy donors (28.4 ng/mL vs. 10.3 ng/mL plasma, $P < 0.001$; Fig. 2A). Moreover, stage IV patients tended to have increased cfDNA concentration compared with stage III cases, although the difference was not significant (Fig. 2B). Then we determined the relationship between ctDNA allele fraction and tumor staging. For cases with multiple alterations detected, the one with highest allele fraction was used to represent the ctDNA MAF. Elevated MAF was observed in stage IV metastatic patients compared with stage III cases (Fig. 2C, $P < 0.01$). We further divided 21 stage IV patients with liver metastasis into two groups with either limited metastatic lesion (≤2 lesions) or disseminated lesions (>3 lesions). Patients with multiple isolated liver metastasis had markedly higher ctDNA MAF compared with those having fewer lesions (Fig. 2D, $P < 0.01$). These results indicated that ctDNA MAF was correlated with disease burden, implying its potential application for tracking tumor response to therapy.

We further divided patients into two groups with either low or high ctDNA MAF using threshold of 1.5%, which is the median value for all subjects. The comparison of clinical parameters for this two groups showed that only stage was significantly different (Table 1). Besides, the overall survival analysis demonstrated that patients with ctDNA MAF ≥1.5% had worse prognosis compared with those have lower than 1.5%. But the difference was not significant in multivariate cox regression analysis possibly due to small number of cases included (Supplementary Fig. S3).

Monitoring tumor burden following FOLFIRINOX therapy via cfDNA

The role of ctDNA in monitoring treatment response was studied in 17 patients with PDAC receiving FOLFIRINOX regimen

Table 2. Concordance of altered loci in tumor tissue and plasma.

Patient	Concordant mutations in tumor tissue and plasma
Case 1	*KRAS*, p.G12V *TP53*, p.E153K *SMAD4*, p.E161X *C8orf34*, p.S262Y
Case 2	*TP53*, p.C153R

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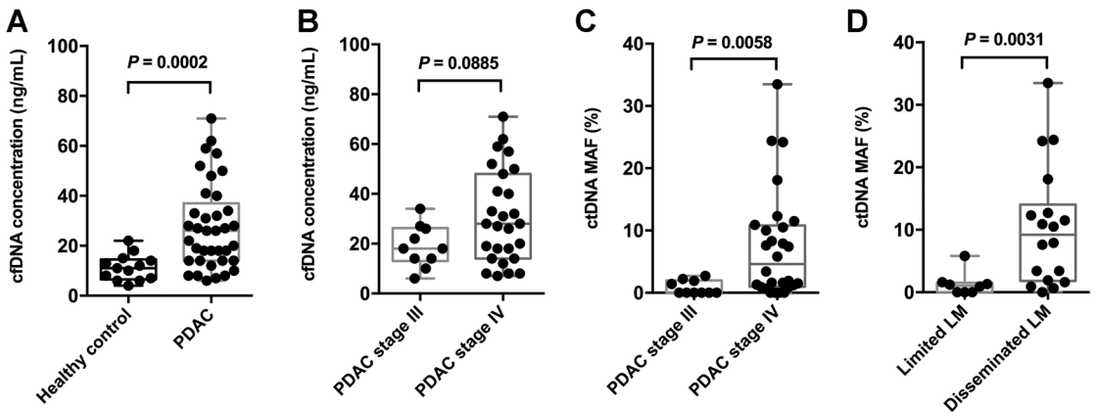

Figure 2.

The correlation analysis of cfDNA concentration and ctDNA MAF with tumor burden. The comparison of total cfDNA concentration was done in PDAC versus healthy individuals (**A**), and AJCC stage III versus stage IV (**B**). ctDNA MAF between patients with PDAC stage III and stage IV was compared (**C**). ctDNA MAF was compared for patients with limited and disseminated liver metastasis (LM; **D**).

who had longitudinal blood samples collected after one course of chemotherapy (Table 3). For 12 subjects responding to chemotherapy with six PR and six SD, the cfDNA MAF reduced concomitantly in 11 patients. Among them, alterations in multiple loci can be detected in four subjects, and all had consistent trend in MAF with tumor burden. One patient exhibited SD, and ctDNA of altered *KRAS* was detectable with MAF of 0.7% after chemotherapy which was not present at baseline. Other five cases showed resistance to chemotherapy, and increased ctDNA MAF was observed at the time of disease progression. In contrast, CA19-9 was not reliable to track disease burden for five cases. Among them, CA19-9 was within normal range throughout the treatment course in three subjects. For other two cases, the change of CA19-9 level was not in consistent with the clinical scenario (Table 3).

We further highlighted six cases. In case 1, remarkable shrinkage of primary tumor and almost disappearance of multiple liver metastasis were observed after four cycles of FOLFIRINOX therapy. But disease progressed with markedly enlarged lesions in liver when the second course was completed (Fig. 3A). Both cfDNA concentration and MAF for driver gene *KRAS*, *TP53*, and *CDKN2A* were in line with clinical course. The second patient experienced continued response representing as extensive tumor necrosis on imaging, and then curative resection was performed (Fig. 3B). The microscopic examination of resected tumor

Table 3. Dynamics of the ctDNA MAF following FOLFIRINOX therapy

		MAF (%)		CA19–9 (U/mL)		
Patient no.	**Altered loci in cfDNA**	**Before therapy**	**After therapy[a]**	**Before therapy**	**After therapy[a]**	**Therapy response**
1	*KRAS* (G12D)	10.8	0.82	2	2	PR
	TP53 (R172H)	12.3	0.78			
	CDKN2A (H83Y)	8.7	0			
2	*KRAS* (G12D)	10.3	2.8	51.7	36.7	PR
	KRAS (G13D)	10.5	2.8			
	U2AF1 (S34F)	4.6	0.76			
3	*KRAS* (G12V)	1.9	0	12000	3921	SD
	TP53 (E153K)	0.73	0			
	SMAD4 (E163X)	0.74	0			
4	*TP53* (R150W)	0	1.6	162.7	681.5	PD
	SMAD4 (R361H)	0	0.75			
5	*GNAS* (R201S)	3.4	2.2	512	235.7	SD
6	*SF3B1* (K700E)	1.6	0	1261.2	374.2	SD
7	*KRAS* (G12D)	0.66	0	12000	12000	PR
8	*TP53* (C153R)	1.2	0	43	10.9	PR
9	*KRAS* (G12D)	1.2	0	2	2	SD
10	*SF3B1* (R625C)	2.9	0.63	121.8	61.9	PR
11	*TP53* (R175H)	0	1.3	4018.9	3569.1	PD
12	*KRAS* (G12R)	4.6	59.6	134.1	12000	PD
	TP53 (I195T)	1.0	37.0			
13	*KRAS* (G12D)	32.6	0	12000	3746.7	PR
	TP53 (Q165X)	33.5	12.8			
14	*KRAS* (G12C)	0	0.7003	1594.5	1161.1	SD
15	*TP53* (G295V)	0	5.9	587.3	1287.1	PD
16	*KRAS* (G12D)	3.4	11.2	108.5	884.2	PD
17	*TP53* (R175H)	5.8	5.1	2	2	SD

[a]Three to four cycles of chemotherapy.

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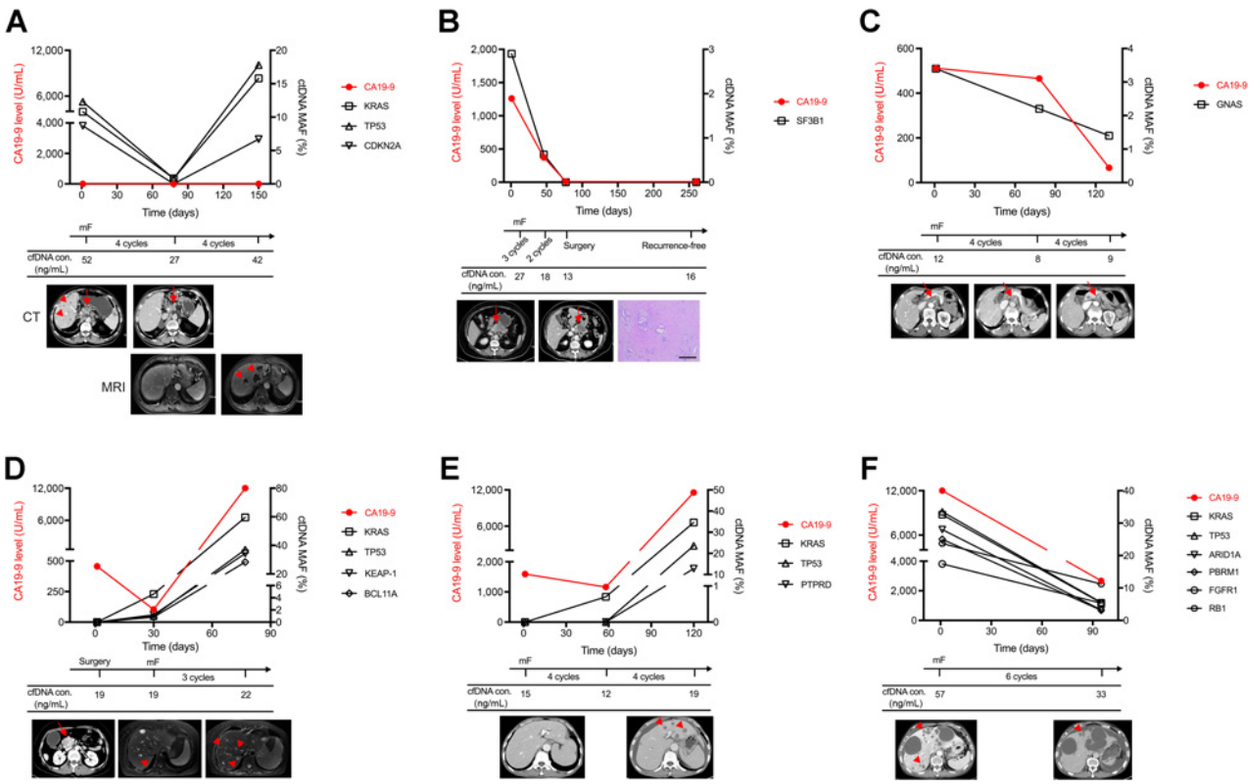

Figure 3.

Representative cases of ctDNA dynamics and correlation with treatment response (**A–F**). Arrows represent primary pancreatic tumors. Arrowheads represent liver metastatic tumors. Low tumor fraction and necrosis was shown by H&E staining, and there were typical cholesterol-like crystals with multinuclear giant cell reaction after chemotherapy (**B**). cfDNA con. denotes cfDNA concentration. The scale bar is 200 μm.

specimen showed typical post-chemotherapy structure and massive necrosis (Fig. 3B). *SF3B1*^{R625C} alteration was detected before chemotherapy, and in parallel, its MAF gradually decreased along with treatment course. The kinetics of cfDNA concentration was also consistent with tumor burden change. In case 3 with localized tumor, moderate response was noted, which correlated with decline of *GNAS*^{R201S} MAF and stable level of cfDNA concentration after the first and second course of therapy (Fig. 3C). In the fourth subject, surgical resection was first performed. Emerging liver metastatic lesions were found one month postoperatively, and ctDNA of *KRAS* and *TP53* was detectable concomitantly. Metastasis progressed despite multiple times of FOLFIRINOX treatment, along with the dramatic increase of ctDNA MAF (Fig. 3D). Interestingly, we observed that two other somatic alterations at *KEAP-1*^{R483H} and *BCL11A*^{R141H} exhibited consistent dynamics as *KRAS* and *TP53*. These two mutations have not been reported in pancreatic cancer and the functional impact remains to be defined. In case 5, disease continuously progressed with multiple emerging liver metastasis following chemotherapy (Fig. 3E). In line with the clinical findings, MAF of ctDNA at multiple loci increased with *KRAS* being up to 10% after two course of therapy. At the same time, a new variant of *PTPRD*^{I972V} was detected. The last patient suffered from disseminated liver metastasis and had high tumor burden, and high MAF for multiple cfDNA loci was noted before treatment (Fig. 3F). This patient exhibited favorable response to the FOLFIRINOX regimen with substantial reduction of metastatic burden and even disappearance for some lesions. Multiple altered loci in cfDNA were detected at baseline including *KRAS*^{G12D}, *PBRM1*^{T377S}, *RB1*^{Y498C}, *TP53*^{Q165X}, *ARID1A*^{Q564X}, and *FGFR1*^{M390I}. In consistent with clinical scenario, all these detected ctDNA showed decline of MAF. The total cfDNA concentration also decreased concomitantly.

Discussion

In this study, we described the cfDNA analysis as a way to monitor tumor burden in response to treatment. Our cfDNA profiling was based on targeted NGS strategy. Compared with many other studies (16, 17), we designed a larger multigene panel constituted of 560 genes including both high- and low-frequently altered loci reported in PDAC (18). Hybrid capture technique was used in order to cover broad genomic regions of our customized panel. Sensitivity might be compromised given the wide genomic region to be detected. Nevertheless, for well-known driver genes in PDAC including *KRAS*, *TP53*, *SMAD4*, and *CDKN2A*, the results showed that detection rate for at least one of above loci among included patients is 65.6%. Although the sensitivity is lower than a few PCR-based assays, this result is superior to other NGS profiling for ctDNA in pancreatic cancer. By using PCR-amplicon and AmpliSeq platform, Pietrasz and colleagues found 48.1% of patients with unresectable tumor had detectable ctDNA with detection limit of 0.1% (19). Another study reported that

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57.7% of patients with advanced pancreatobiliary carcinomas had detectable *KRAS* mutation in cfDNA. Notably, the coverage depth for a 54-gene panel averaged approximately 10,000× in their study (20). However, it has been recognized that the risk of false positive increases with the size of panel owing to multiple hypothesis testing (4). In addition, the mutant cfDNA could be originated from hematopoietic clone and thus lead to false positive detection. However, allele fractions of these nontumor-derived cfDNA are generally low and a threshold of 0.5% MAF defined in the present analysis should avoid these contaminations. Indeed, MAF of ctDNA for most cases was higher than 1% in our analysis. To determine the specificity, plasmas from 13 healthy donors with comparable age to enrolled patients were analyzed with same pipeline, and there were no somatic variants found in PDAC driver genes. It should be noted that only a small population of healthy individuals were included in the control group. Furthermore, the accuracy of ctDNA detection was evaluated in three cases given the available corresponding tumor tissues. The alterations found in tumor tissues were favorably matched to those in cfDNA for two subjects. Finally, as altered *KRAS* and *TP53* are well-characterized drivers for PDAC at an early stage, their MAF among cfDNA is supposed to be correlated quantitatively. This was corroborated in nine patients with concomitant mutation at both genes, which is consistent with other report using digital PCR analysis (21). In one case, mutant codon 12 and 13 of *KRAS* was found with almost same MAF. These results overwhelmingly strengthened the robustness of our sequencing strategy.

One potential clinical utility of ctDNA is to monitor disease longitudinally in response to therapy given the ease of repeated blood drawing (22, 23). This is rationalized by the relationship between disease burden and circulating mutant DNA level. Here we included a highly-selected group of patients with advanced PDAC with uniform first-line treatment of FOLFIRINOX chemotherapy. FOLFIRINOX regimen has been recommended as a first-line treatment choice for advanced PDAC (24). We confirmed that ctDNA level could mirror tumor burden at baseline as higher cfDNA MAF was associated with liver metastasis. The serial blood analysis showed that ctDNA dynamics in allele frequency was consistent with therapy response in all 12 patients evaluated. In comparison, as the standard clinical biomarker for PDAC, CA19-9 remained undetectable in three cases and thus was futile for tumor monitoring. Indeed, certain percentage of patients are negative for Lewis antigen (25). Therefore, ctDNA could be a useful compensate for conventional serum biomarker in tracking therapy response. Although we noted a generally consistency between change of CA19-9 level and tumor burden, other studies found superiority of ctDNA over standard biomarker. In colorectal cancer, researchers revealed that ctDNA outperformed carcino-embryonic antigen in predicting recurrence and tracking disease (26).

PDAC has remarkable genetic heterogeneity with a large number of low-frequently mutated loci, and importantly, a fraction of patients does not harbor mutations in *KRAS* and *TP53* (27). For three patients, we detected alterations at *GNAS* and *SF3B1* in plasma but not at those high-frequently altered driver genes. *GNAS* has been characterized as an oncogene in pancreatic cancer, and the hotspot mutation can be found in about 5% to 10% of cases (28). *SF3B1* encoded the protein involving in RNA splicing, and both R625C and K700E variant detected here resulted in functional change. The frequency for *SF3B1* mutation in PDAC was reported to be around 5% (29). In addition, a few detected somatic mutations in cfDNA have not been reported in pancreatic cancer before including *ARID1A*^{Q564X}, *PBRM1*^{T377S}, and *KEAP-1*^{R483H}. These data supported the additional value of application of large gene panel in ctDNA analysis.

Besides the ctDNA level, total cfDNA concentration was also clinically relevant in selected patients. A significantly higher cfDNA concentration was present in PDAC patients in contrast to healthy donors. Meanwhile, stage IV patients tended to have elevated concentration. Indeed, the correlation between tumor staging and cfDNA concentration has been well characterized. It is thus presumably that tumor would give rise to a considerable percentage of cfDNA in plasma. Goodall and colleagues reported that decline of total cfDNA concentration was able to predict the progression-free and overall survival in prostate cancer patients treated with PARP inhibitor. Moreover, the change of cfDNA concentration was consistent with that of allele frequency of somatic mutation in plasma (30). Another recent study also demonstrated that total cfDNA concentration was a surrogate marker for tumor burden and is prognostic for overall survival (31). Compared with mutation profiling, measurement of cfDNA concentration has the distinct advantages being faster, easier, and cheaper. Its clinical application warrants further investigation.

In this cohort, a few patients exhibited initial response to FOLFIRINOX therapy, but secondary resistance occurred after the next course of treatment. It would be clinically significant to interrogate resistant mechanisms though analysis of genomic evolution via cfDNA (32). In fact, several piece of emerging evidence indicated that serial cfDNA analysis informed the underlying mechanisms for acquired therapy resistance. Two recent studies uncovered that acquired *BRCA2* or *PALB2* reversion mutations conferred insensitivity to PARP inhibitor olaparib or talazoparib in prostate cancer (30, 33). Another study reported polyclonal secondary *FGFR2* mutations drove resistance to *FGFR* inhibitor in patients with cholangiocarcinoma who initially responded due to *FGFR2* fusion-positive (34). FOLFIRINOX regimen is a combination of three cytotoxic drugs. The genomic alterations associated with therapy sensitivity are lacking except that DNA repair genes status predicts response to platinum-based drugs like oxaliplatin. Nevertheless, there were no detected pathogenic variants in genes regulating DNA damage response like *BRCA1*, *BRCA2*, *ATM*, and *PALB2*. It is worthwhile for further investigation as a significant proportion of patients develop acquired drug tolerance (14).

Clinically, the widely-used circulating protein biomarker for pancreatic cancer is CA19-9, and CA125 and CEA are useful in a small fraction of patients. It is proposed that ctDNA has greater dynamic range and shorter half-life than protein biomarkers (4). This should enable ctDNA to be more sensitive in monitoring tumor burden following treatment. In particular, the current method like ultra-deep sequencing allows extremely low abundance ctDNA to be detected. Meanwhile, ctDNA-based markers might be more specific than proteins. Increased CA19-9 has been seen in other nontumor contexts like pancreatitis and cholestasis. However, we believe that ctDNA suffers from some drawbacks similar as protein-based blood diagnosis. For instance, for early-stage cancer and those with minimum residual disease after resection, the detection sensitivity remains low as ctDNA level tightly correlate with tumor burden.

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Conclusions

In summary, we provide evidence that ctDNA level constitute as an indicator for tumor burden. Serial cfDNA analysis is a noninvasive and robust tool for tumor monitoring in patients with pancreatic cancer receiving FOLFIRINOX chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Wei, Q. Zhang, L. Zheng, X. Bai, T. Liang
Development of methodology: T. Wei, Q. Zhang, X. Li, T. Liang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Wei, Q. Zhang, X. Li, W. Su, G. Li, T. Ma, S. Gao, J. Lou, R. Que
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Wei, Q. Zhang, W. Su, G. Li
Writing, review, and/or revision of the manuscript: T. Wei, Q. Zhang, X. Bai, T. Liang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Liang
Study supervision: Q. Zhang, T. Liang

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