Gastroenterology 2016;151:267–270

BRIEF REPORT

Detection of Hot-Spot Mutations in Circulating Cell-Free DNA From Patients With Intraductal Papillary Mucinous Neoplasms of the Pancreas

Andreas W. Berger,[1,*] **Daniel Schwerdel**,[1,*] Ivan G. Costa,[2] Thilo Hackert,[3] Oliver Strobel,[3] Sandra Lam,[1] Thomas F. Barth,[4] Bernd Schröppel,[1] Alexander Meining,[1] Markus W. Büchler,[3] Martin Zenke,[5] Patrick C. Hermann,[1] Thomas Seufferlein,[1,§] and Alexander Kleger[1,§]

[1]*Department of Internal Medicine I, Ulm University, Ulm, Germany;* [2]*IZKF Computational Biology Research Group, RWTH Aachen University Medical School, Aachen, Germany;* [3]*Department of General Surgery, University Hospital Heidelberg, Heidelberg, Germany;* [4]*Institute of Pathology, Ulm University, Ulm, Germany; and* [5]*Department of Cell Biology, Institute for Biomedical Engineering, RWTH Aachen University Medical School, Aachen, Germany*

Intraductal papillary mucinous neoplasms (IPMNs) are the most frequent cystic pancreatic tumors. Little is known about their molecular alterations, but mutations in *GNAS* have been reported to promote IPMN formation. A tumor-derived fraction of circulating cell-free DNA (cfDNA), isolated from blood samples, contains many of the same mutations as the primary tumor, and could be a tool for noninvasive disease monitoring. We found that the total amount of cfDNA can discriminate between individuals without pancreatic lesions (controls) and patients with Fukuoka-negative branch-duct IPMN or pancreatic cancer. Furthermore, we detected *GNAS* mutations in cfDNA from patients with IPMN, but not in patients with serous cystadenoma or controls. Analyses of cfDNA might therefore be used in the diagnosis of patients with IPMN or in monitoring disease progression.

Keywords: Genetic; Biomarker; Prognostic Factor; Diagnostic; IPMN; PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer type of the pancreas. The 3 PDAC precursor lesions are pancreatic intraepithelial neoplasia, mucinous cystic neoplasm, and intraductal papillary mucinous neoplasm (IPMN). In contrast, serous cystadenomas (SCAs) are strictly benign cystic neoplastic lesions and rarely require surgery.[1] Frequently, differential diagnosis of neoplastic cysts remains cumbersome. Therefore, noninvasive diagnostic stratification would be welcome. Such a test should allow discrimination of IPMN from strictly benign pancreatic cysts and also low- from high-grade IPMNs.

Somatic mutations in the guanine nucleotide-binding protein (G-protein)-stimulating α-subunit (*GNAS*) gene are found in up to 100% of IPMN cases. Also *KRAS* is frequently mutated.[1–3] Preclinical studies suggest that, in contrast to other precursor lesions, pancreatic intraepithelial neoplasias (PanIN) can shed cells into the bloodstream[4] as putative metastatic precursors. Similarly, circulating pancreatic epithelial cells were found in IPMN patients' blood.[5] Tumors shed measurable amounts of a tumor-derived fraction of cell-free DNA (cfDNA) into the blood, which allow noninvasive tumor-specific genotyping in malignancies.[6] No data are available in the premalignant/benign setting for pancreatic lesions. We hypothesized that cfDNA analysis complemented with targeted genotyping in IPMN can fulfill these requirements for a noninvasive diagnostic test.

Results

Study Population

In this retrospective study, the following 5 cohorts were analyzed (Supplementary Tables 1–4 and 6): 21 IPMN patients (blood; IPMN surveillance); 38 healthy controls (blood; control); 24 patients with metastatic PDAC (blood; PDAC); 26 patients with resected SCA (blood; SCA); and 16 patients with borderline IPMN (blood/tissue; IPMN resected).

Cell-Free DNA Levels

IPMN patients showed a mean cfDNA value of 0.2887 ± 0.0319 ng/μL, controls had significantly less cfDNA with 0.1360 ± 0.0203 ng/μL ($P < .001$) (Figure 1*A*, Supplementary Tables 1 and 2). Total cfDNA was independent of age or sex (Figure 1*B* and *C*). The amount of cfDNA was significantly higher in PDAC compared with IPMN and controls (4.220 ± 2.501 ng/μL; $P = .0005$ vs IPMN; $P < .0001$ vs control, Figure 1*A*, Supplementary Table 3).

Diagnostic Power of Cell-Free DNA

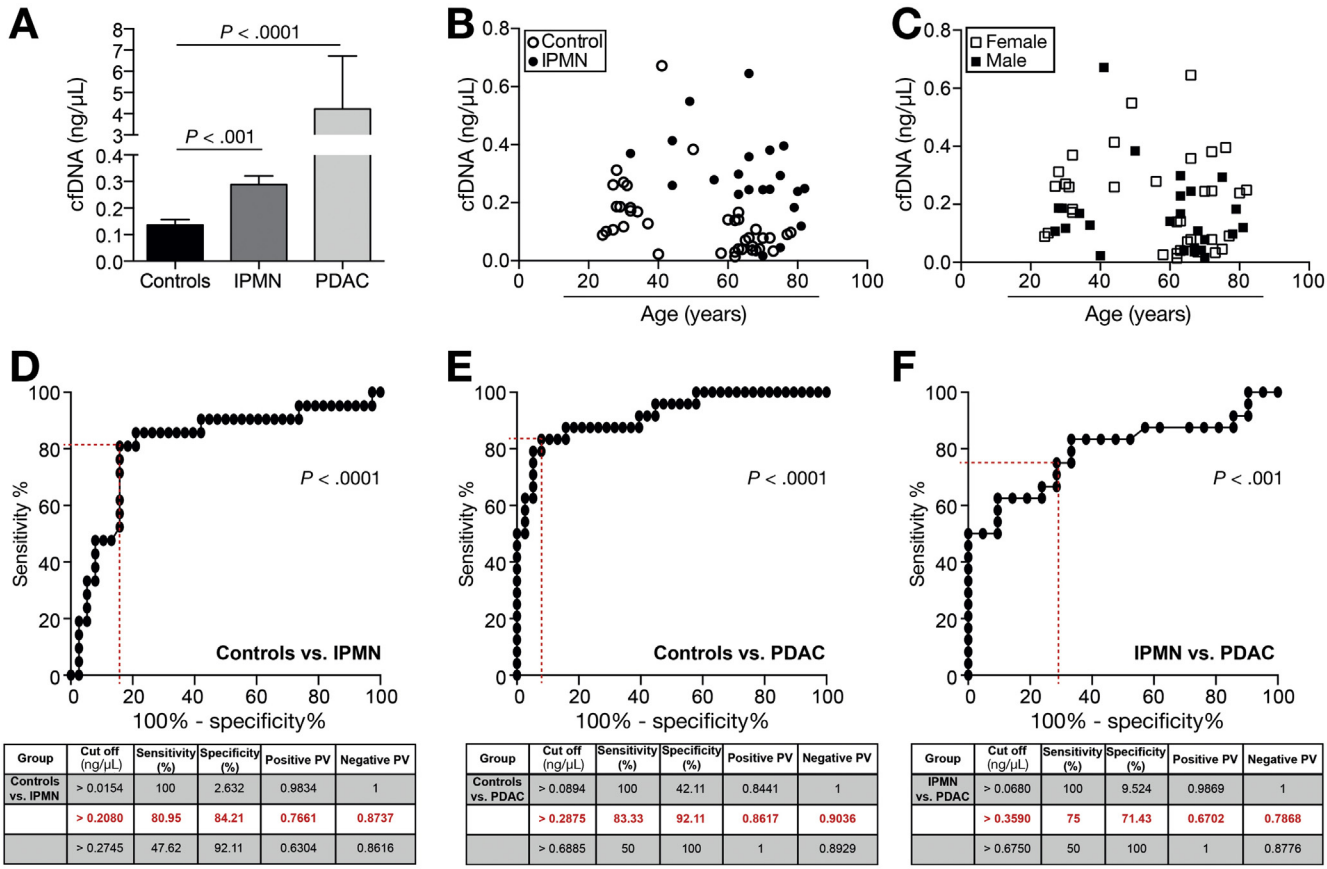
We tested the diagnostic power of cfDNA quantity using receiver operating characteristics analysis. The calculated receiver operating characteristic area under the curve was 0.81 (95% CI, 0.65–0.98), suggestive for a high discrimination power ($P < .0001$). Accuracy was highest using a

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviation used in this paper: cfDNA, circulating cell-free DNA; IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma; SCA, serous cystadenoma.

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2016.04.034



Group	Cut off (ng/μL)	Sensitivity (%)	Specificity (%)	Positive PV	Negative PV
Controls vs. IPMN	> 0.0154	100	2.632	0.9834	1
	> 0.2080	**80.95**	**84.21**	**0.7661**	**0.8737**
	> 0.2745	47.62	92.11	0.6304	0.8616

Group	Cut off (ng/μL)	Sensitivity (%)	Specificity (%)	Positive PV	Negative PV
Controls vs. PDAC	> 0.0894	100	42.11	0.8441	1
	> 0.2875	**83.33**	**92.11**	**0.8617**	**0.9036**
	> 0.6885	50	100	1	0.8929

Group	Cut off (ng/μL)	Sensitivity (%)	Specificity (%)	Positive PV	Negative PV
IPMN vs. PDAC	> 0.0680	100	9.524	0.9869	1
	> 0.3590	**75**	**71.43**	**0.6702**	**0.7868**
	> 0.6750	50	100	1	0.8776

Figure 1. Total amount of cfDNA (*A*) in IPMN (n = 21), controls (n = 38), and PDAC (n = 24), correlation with age (*B*) and sex (*C*). Receiver operating characteristics (ROC): control vs IPMN (*D*), control vs PDAC (*E*), IPMN vs PDAC (*F*). *Red lettering* indicates the optimal cutoff value for cfDNA (*D–F*).

threshold of 0.208 ng/μL, establishing high sensitivity (81.0%) and specificity (84.2%) (Figure 1*D*). Similar discrimination power was found for controls and PDAC (Figure 1*E*), as well as for IPMN and PDAC (Figure 1*F*).

Quantification of Circulating GNAS *and* KRAS *Mutations in cfDNA*

We used droplet digital polymermase chain reaction (ddPCR) to detect circulating *GNAS* codon 201 mutations (namely R201C/H)[7] in cfDNA. In controls and SCA, no *GNAS* codon 201 mutations were observed, whereas 15 of 21 (71.4%) patients in the IPMN-surveillance cohort harbored *GNAS* mutations (Figure 2*A*, Supplementary Tables 1, 2, and 4): 10 of 21 (47.6%) with *GNAS* R201C or R201H mutations, respectively. Five of 21 samples (23.9%) showed both *GNAS* codon 201 mutations. In PDAC, 6 of 24 patients (25.0%) carried *GNAS* codon 201 mutations (Figure 2*A*, Supplementary Table 3). Differences in *GNAS* codon 201 mutation rates were significantly different across all cohorts (Supplementary Table 5).

KRAS codon 12 mutations (namely G12D and G12V[3]) in cfDNA were not detectable in controls, SCA, and IPMN. Ten of 24 patients (41.7%) of the PDAC cohort had *KRAS* codon 12 mutations (Figure 2*A*, Supplementary Tables 1–4).

Additionally, we assessed the concordance of *GNAS* codon 201 mutations in cfDNA and tissue DNA from historically stored material in 16 patients with resected borderline IPMN (Supplementary Table 6), leading to an overall concordance rate of 56.3% (Figure 2*B*).

Finally, the comparison of our cfDNA-based measurements of *GNAS* mutations with previous reports in resected IPMN tissue,[3] pancreatic juice,[8] and cyst fluid[1] found similar ranges (Figure 2*C*).

Discussion

Our main findings are that cfDNA discriminates IPMN patients from controls, but also from metastatic PDAC; cfDNA allows targeted genotyping for known driver mutations, even in benign pancreatic lesions; and the detection of *GNAS* and *KRAS* mutations allow discriminating IPMN patients from those with per se harmless pancreatic tumors, such as SCA.

Recent studies reported on the diagnostic potential of cfDNA quantification in benign and malignant pulmonary[9,10] and colorectal diseases.[11] In the pancreas, recent data in mice and humans found the detection of circulating pancreatic epithelial cells already at the pancreatic intraepithelial neoplasia stage and in IPMN,[4,5] a biological