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Detection of Circulating Pancreas Epithelial Cells in Patients With Pancreatic Cystic Lesions

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Hematogenous dissemination is thought to be a late event in cancer progression. We recently showed in a genetic model of pancreatic ductal adenocarcinoma that pancreas cells can be detected in the bloodstream before tumor formation. To confirm these findings in humans, we used microfluidic geometrically enhanced differential immunocapture to detect circulating pancreas epithelial cells in patient blood samples. We captured more than 3 circulating pancreas epithelial cells/mL in 7 of 21 (33%) patients with cystic lesions and no clinical diagnosis of cancer (Sendai criteria negative), 8 of 11 (73%) with pancreatic ductal adenocarcinoma, and in 0 of 19 patients without cysts or cancer (controls). These findings indicate that cancer cells are present in the circulation of patients before tumors are detected, which might be used in risk assessment.

Keywords: Early Detection; IPMN; Circulating Tumor Cells; Pancreatic Cancer.

A widely accepted paradigm in cancer biology is that epithelial cancers progress in a linear manner whereby cancer-defining properties are acquired sequentially.[1] In this model, cancer cells acquire metastatic potential after large primary tumors are established. However, in pancreatic ductal adenocarcinoma (PDAC), the linear progression model cannot be reconciled with clinical observations. A number of patients undergoing pancreatectomy for chronic pancreatitis will develop disseminated PDAC, although only precancerous pancreatic intraepithelial neoplasias, but no tumors, are found on histologic analysis.[2] In addition, in patients with small primary tumors (<2 cm) who have no clinical evidence of metastatic disease, 5-year survival after pancreatectomy is less than 18% owing to recurrent metastatic disease.[3] These data suggest that metastatic seeding may occur before the formation of large primary tumors. Moreover, we recently showed that hematogenous dissemination occurs before tumor formation, in a lineage-labeled genetic model of PDAC,[4] at which time the pancreas contained only pancreatic intraepithelial neoplasias. Based on the clinical characteristics of PDAC and our findings within a recapitulative mouse model, we hypothesized that bloodstream seeding of pancreas-derived epithelial cells can occur in patients with clinical evidence of only precancerous lesions of the pancreas and no detectable invasive carcinoma.

To test our hypothesis, we performed a blinded prospective pilot study of 3 cohorts, as follows: (1) patients with no history of cancer presenting for average-risk, age-appropriate colonoscopy screening and no adenomas detected; (2) patients with precancerous cystic lesions (intraductal papillary mucinous neoplasm [IPMN] or mucinous cystic neoplasms) of the pancreas with no evidence of tumor or metastasis on computerized tomography or magnetic resonance imaging, who did not qualify for surgery using the Sendai criteria[5] (including no evidence of dysplasia or cancer on fine-needle aspiration, if performed); and (3) patients with cytology-confirmed PDAC. Peripheral blood was obtained from patients who consented before the procedure. We analyzed blood samples using geometrically enhanced differential immunocapture (GEDI), a microfluidic platform that has been shown to detect circulating tumor cells from patients with prostate cancers with high sensitivity.[6,7] Here, we functionalized the GEDI device using antibodies to epithelial cell adhesion molecule to capture circulating epithelial cells (CECs). Captured cells then were stained with 4′,6-diamidino-2-

Abbreviations used in this paper: **CEC, circulating epithelial cell; DAPI, 4′,6-diamidino-2-phenylindole; GEDI, geometrically enhanced differential immunocapture; IPMN, intraductal papillary mucinous neoplasm; PDAC, pancreatic ductal adenocarcinoma; Pdx-1, pancreatic and duodenal homeobox protein-1; CK, cytokeratin 19.**

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Table 1. Patient Characteristics

	Age, y	Race	Sex	FHx	BMI	Smoking	EtOH, avg/wk	CA19-9 serum	CEA serum	CEC	Size of cyst/tumor, mm	Cyst type/ cancer stage
Cancer-free controls (n = 19)	53	Cauc	M		35.7	Never	0			0		
	40	Cauc	M		26.3	Never	1			0		
	64	Cauc	F		28.3	Never	0			0		
	61	Cauc	M		31.0	Never	1			0		
	48	Cauc	F		21.0	Never	0			0		
	62	AAM	F		35.4	Never	0			0		
	74	Cauc	F		19.8	Never	0			0		
	56	AAM	F		24.7	Never	0.5			0		
	70	Cauc	M		26.4	Previous	0			0 (CK)		
	51	AAM	F		56.6	Never	0			0 (CK)		
	60	Cauc	M		29.5	Never	0			0 (CK)		
	66	Cauc	M		21.9	Never	1.5			0 (CK)		
	84	AAM	F		29.3	Never	0			0 (CK)		
	53	AAM	F		46.1	Never	0			0 (CK)		
	59	AAM	F		26.6	Never	0			1 (CK)		
	50	AAM	F		36.6	Never	1.5			2 (CK)		
	58	AAM	F		30.2	Never	0			3 (CK)		
	73	AAM	F		26.6	Never	0			0 (CK)		
	50	Cauc	F		24.0	Never	0			0 (CK)		
Mean	**59.6**				**30.3**					**0.3 ± 0.8**		
Cystic lesion (n = 21)	67	Cauc	F		31.6	Never	0			0	9	Side-branch IPMN, multiple
	62	Cauc	F	Y	20.2	Never	3		0	0	8	Side-branch IPMN
	64	Cauc	M		34.9	Never	5		86.7	0	16	MCN
	75	Cauc	M		24.3	Never	0			0	15	Side-branch IPMN
	65	Cauc	F	Y	22.4	Never	0			6	9.5	Side-branch IPMN
	60	Cauc	M		28.3	Never	0	1.9	22	16	14	Side-branch IPMN
	72	Cauc	M		21.3	Current	3		<1	22	10	MCN
	81	Cauc	M		27.7	Never	6			0	15	Side-branch IPMN
	58	Cauc	M		20.7	Current	20			0	5	Side-branch IPMN
	64	Cauc	F	Y	18.0	Never	5	46	3.8	0	20	Side-branch IPMN
	73	Cauc	F		24.6	Never	0			4	14	Side-branch IPMN
	69	Cauc	F		27.8	Previous	4			0 (CK)	11	Side-branch IPMN
	68	Cauc	M		26.3	Previous	7			0 (CK)	3	Side-branch IPMN
	74	Cauc	F		26.6	None	0			19 (CK)	6.5	Side-branch IPMN
	81	Cauc	M		27.5	Previous	7			1 (CK)	25	Side-branch IPMN
	58	AAM	F		32.9	None	0			14 (CK)	5	Side-branch IPMN
	74	Cauc	F		21.7	None	0			0 (CK)	25	Side-branch IPMN
	65	Cauc	F		31.4	None	0			0 (CK)	28	MCN
	79	ASAM	M		21.5	None	0			12 (CK)	23	Side-branch IPMN
	77	Cauc	F		28.5	None	0	19	1.3	0 (CK)	28	Side-branch IPMN
	80	Cauc	M		29.9	None	0			0 (CK)	25	Side-branch IPMN
Mean	**69.8**				**26.1**					**4.5 ± 7.3**		