Pancreatic Ductal Adenocarcinoma Derived From IPMN and Pancreatic Ductal Adenocarcinoma Concomitant With IPMN

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Objectives: Pancreatic ductal adenocarcinoma (PDAC) may derive from an intraductal papillary mucinous neoplasm (IPMN) of the pancreas or may develop in the pancreatic duct apart from IPMN. The purpose of this study was to define the clinicopathological features of these 2 entities and compare them with those of ordinary PDAC.

Methods: Of 765 patients who had surgical resection for IPMN, 122 were diagnosed as having PDAC derived from IPMN and 31 with PDAC concomitant with IPMN. In addition, 7605 patients with PDAC who were registered in the Japan Pancreas Society pancreatic cancer registry were compared with the above patients.

Results: Pancreatic ductal adenocarcinomas derived from IPMN and concomitant with IPMN were significantly smaller, less invasive, and less extensive than ordinary PDAC. The median survival of patients with the 2 conditions was significantly longer than for those with ordinary PDAC when compared overall or when limited to TS2 (2.0 cm < tumor size ≤ 4.0 cm) or TS3 (4.0 cm < tumor size ≤ 6.0 cm) cases.

Conclusions: These findings suggest that PDAC concomitant with IPMN and PDAC derived from IPMN may have more favorable biological behaviors or be diagnosed earlier than ordinary PDAC.

Key Words: IPMN, PDAC concomitant with IPMN, PDAC derived from IPMN

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Coauthors from Takashi Hatori to Yo Kato are listed in the order of the number of patients contributed by each coauthor used to compile this study series.

Intraductal papillary mucinous neoplasm (IPMN) is characterized by papillary proliferation of atypical mucinous epithelium in the pancreatic ductal system, and the affected pancreatic ducts are often cystically dilated.[1,2] Intraductal papillary mucinous neoplasm is a spectrum of diseases ranging from adenoma, to in situ carcinoma, to invasive carcinoma (minimally invasive carcinoma and invasive carcinoma derived from IPMN).[3] On the other hand, pancreatic ductal adenocarcinoma (PDAC) develops independently of IPMN in the pancreatic duct.[4,5] When PDAC originates in the vicinity of IPMN, the distinction between PDAC derived from IPMN and PDAC concomitant with IPMN is sometimes difficult to make. In this collective series, we developed a definition of the 2 conditions and analyzed the incidence of the conditions in patients with IPMN. In addition, we compared the clinicopathological features between (1) ordinary PDAC and PDAC derived from IPMN and (2) ordinary PDAC and PDAC concomitant with IPMN.

MATERIALS AND METHODS

The Japan Pancreas Society (JPS) formed a committee to solve the clinical and pathological problems associated with PDAC derived from IPMN and PDAC concomitant with IPMN. The committee (Drs H. Maguchi, K. Hanada, Y. Noda, M. Tada, and A. Nakaizumi as internists; Drs K. Yamaguchi, T. Hatori, Y. Shimizu, and T. Nakagori as surgeons; and Drs Y. Kato, T. Furukawa, B. Nobukawa, and S. Ban as pathologists) discussed the definition of PDAC derived from IPMN and PDAC concomitant with IPMN and proposed a new definition of 3 categories (PDAC derived from IPMN, PDAC concomitant with IPMN, and PDAC of undetermined relationship with IPMN) based on the topological relationship of the 2 conditions and the presence or absence of a histological transition (Fig. 1) between the conditions as follows:

PDAC Derived From IPMN

Pancreatic ductal adenocarcinoma is evidently derived from IPMN, based on the findings of radiologic images and macroscopic or microscopic findings, and a histological transition is present between IPMN and PDAC.

PDAC Concomitant With IPMN

Intraductal papillary mucinous neoplasm is obviously different from PDAC, according to the radiologic images and macroscopic or microscopic findings.

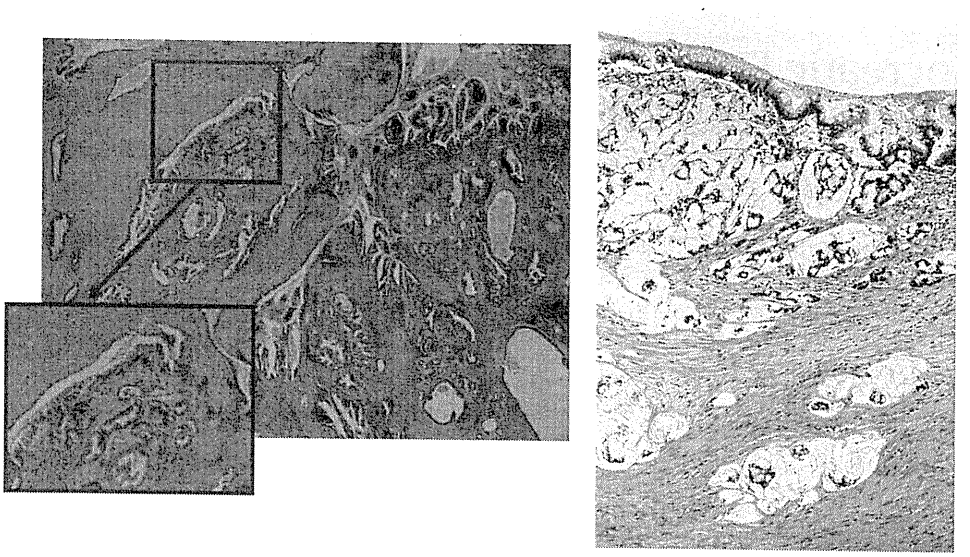

FIGURE 1. Histological transition from IPMN to tubular carcinoma or mucinous carcinoma.

TABLE 1. Intraductal Papillary Mucinous Neoplasm, PDAC Derived From IPMN, and PDAC Concomitant With IPMN

IPMN	582 cases
Adenoma	381 cases
Carcinoma	201 cases
Noninvasive	157 cases
Minimally invasive	44 cases
PDAC derived from IPMN	122 cases
PDAC concomitant with IPMN	31 cases
PDAC undetermined derived from IPMN or concomitant with IPMN	30 cases
Total	765 cases

TABLE 2. Clinical Features of Patients With PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (Overall)

	PDAC	PDAC Derived From IPMN		PDAC Concomitant With IPMN	
	(n = 7605)	(n = 122)	*P*	(n = 31)	*P*
Age,* mean (SD), yr	63.5 (9.9)	66.5 (8.4)	<0.001	67.1 (8.2)	0.021
Sex,† n (%)					
Male	4674 (61.5)	77 (63.1)	0.67	21 (67.7)	0.457
Female	2931 (38.5)	45 (36.9)		10 (32.3)	
Follow-up duration,* mean (SD), mo	17.1 (22.0)	36.7 (36.0)	<0.001	37.3 (36.9)	<0.001

P value compared with PDAC.
*Two-sample *t* test.
†χ^2 test.

Undetermined Whether PDAC Is Derived From IPMN or Concomitant With IPMN

Intraductal papillary mucinous neoplasm and PDAC are evident, but whether PDAC was derived from IPMN or whether PDAC was concomitant with IPMN could not be determined because there was no histological transition between the 2 diseases. The histological transition might not be evident (1) because serial stepwise section examination of the resected specimens was not done in all the cases, (2) because the transition might have disappeared because of the extensive and massive growth of PDAC, or (3) because the 2 diseases developed independently and collided with each other. Thus, such cases were considered as undetermined whether PDAC was derived

TABLE 3. Comparison Among IPMN Types and IPMN, PDAC Derived From IPMN, and PDAC Concomitant With IPMN

	Main Duct (+Mixed) Type, n (%)	Branch Duct Type, n (%)	*P*
IPMN (n = 582)	181 (31.1)	401 (68.9)	—
PDAC derived from IPMN (n = 122)	61 (50.0)	61 (50.0)	<0.001
Tubular adenocarcinoma (n = 81, 66.4%)	38 (46.9)	43 (53.1)	0.004
Mucinous carcinoma (n = 41, 33.6%)	23 (56.1)	18 (43.9)	0.001
PDAC concomitant with IPMN (n = 31)	3 (9.6)	28 (90.4)	0.012
Tubular adenocarcinoma (n = 31, 100%)	3 (9.6)	28 (90.4)	0.012
Mucinous carcinoma (n = 0, 0.0%)	0 (0)	0 (0)	NA

P value compared with IPMN.
NA indicates not available.

TABLE 4. Clinicopathological Findings of PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (Overall)

		PDAC (n = 7605)		PDAC Derived From IPMN (n = 122)			PDAC Concomitant With IPMN (n = 31)		
		n	%	n	%	*P*	n	%	*P*
Histological diagnosis	Tubular adenocarcinoma	7484	98.4	60	69.0	<0.001	20	100	0.57
	Mucinous adenocarcinoma	121	1.6	26	29.9		0	0.0	
	Tubular + mucinous	0	0.0	1	1.1		0	0.0	
	Unknown	0		35			0		
Location	Head	5204	68.6	77	67.0	0.222	14	46.7	<0.001
	Body	974	12.8	10	8.7		11	33.3	
	Tail	420	5.5	9	7.8		5	16.7	
	All segments of pancreas	115	1.5	4	3.5		0	0.0	
	Two segments of pancreas	868	11.4	15	13.0		1	3.3	
	Unknown	24		7			1		
TS	TS1	882	12.0	9	7.4	0.005	15	48.4	<0.001
	TS2	3921	53.6	65	53.7		12	38.7	
	TS3	1837	25.1	25	20.7		4	12.9	
	TS4	681	9.3	22	18.2		0	0.0	
	Unknown	284		1			0		
T	Tis	0	0.0	0	0.0	<0.001	2	6.5	<0.001
	T1	229	3.2	8	6.6		9	29.0	
	T2	281	3.9	27	22.1		0	0.0	
	T3	1915	26.8	72	59.0		14	45.2	
	T4	4714	66.0	15	12.3		6	19.4	
	Unknown	466		0			0		
N	N0	2319	33.7	65	53.3	<0.001	14	45.2	0.01
	N1	1518	22.1	39	32.0		12	38.7	
	N2	1399	20.3	15	12.3		4	12.9	
	N3	1642	23.9	3	2.5		1	3.2	
	Unknown	727		0			0		
M	M (−)	5480	72.4	118	96.7	<0.001	31	100	0.001
	M (+)	2092	27.6	4	3.3		0	0.0	
	Unknown	33		0			0		
Stage	0	0	0.0	0	0.0	<0.001	2	6.5	<0.001
	I	146	2.2	6	4.9		8	25.8	
	II	167	2.5	26	21.3		1	3.2	
	III	1278	19.0	57	46.7		10	32.3	
	IVA	2250	33.4	22	18.0		8	25.8	
	IVB	2887	42.9	11	9.0		2	6.5	
	Unknown	877		0			0		

P value compared with PDAC.

from IPMN or PDAC was concomitant with IPMN and were excluded from further comparisons to examine the details of each discrete condition.

The clinicopathological data of 765 patients who underwent surgical resection for IPMN were collected from the following 7 representative Japanese institutions:

TABLE 5. Median Survival of PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (Overall)

PDAC (n = 7605)		PDAC Derived From IPMN (n = 122)		PDAC Concomitant With IPMN (n = 31)		Results of the Log-Rank Test (*P*)	
n	MST, mo	n	MST, mo	n	MST, mo	PDAC vs PDAC Derived From IPMN	PDAC vs PDAC Concomitant With IPMN
7359	12	122	46	31	57	<0.001	<0.001

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We had 4 committee meetings where we reviewed the radiologic images and hematoxylin-eosin–stained sections of the patients and discussed the differentiation of the tumors and determined the diagnostic criteria. All 765 patients underwent surgery from February 1987 to February 2009. They consisted of 381 patients with intraductal papillary mucinous adenoma (IPMA) (49.8%), 201 with intraductal papillary mucinous carcinoma (26.3%) (157 with noninvasive IPMC and 44 with minimally invasive IPMC), 122 judged to have PDAC derived from IPMN (15.9%), 31 judged to have PDAC concomitant with IPMN (4.1%), and 30 for whom it could not be determined whether the PDAC derived from IPMN or was concomitant with IPMN (3.9%) (Table 1).

In addition, data from 7605 patients with PDAC who were registered in the JPS pancreatic cancer registry were obtained under the permission of the president of the JPS (Professor Masao Tanaka, MD, PhD, FACS, Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan). These patients underwent surgical resection in 168 Japanese institutions from November 1971 to January 2005.

Data were analyzed following the *Classification of Pancreatic Carcinoma* published by the JPS (Second English Edition, 2003, Kanehara & Co, Ltd, Tokyo, Japan).[6] Statistical analyses were done by *t* test, χ^2 test, and log-rank test. The mean

TABLE 6. Clinicopathological Features and MST (Overall Cases)

		PDAC (n = 7605)			PDAC Derived From IPMN (n = 122)			PDAC Concomitant With IPMN (n = 31)		
		n	%	MST, mo	n	%	MST, mo	%	n	%	MST, mo
All cases		7605	100.0	12	122	100.0	46	100.0	31	100.0	57
Histological diagnosis	Tubular adenocarcinoma	7484	98.4	13	60	69.0	44	69.0	20	100	51
	Mucinous adenocarcinoma	121	1.6	31	26	29.9	55	29.9	0	0.0	NA
	Tubular + mucinous	0	0.0	NA	1	1.1	30	1.1	0	0.0	NA
	Unknown	0			35				0		
TS	TS1	882	12.0	29	9	7.4	38	7.4	15	48.4	59
	TS2	3921	53.6	14	65	53.7	42	53.7	12	38.7	24
	TS3	1837	25.1	10	25	20.7	54	20.7	4	12.9	12
	TS4	681	9.3	9	22	18.2	61	18.2	0	0.0	NA
	Unknown	284			1				0		
T	Tis	0	0.0	NA	0	0.0	NA	0.0	2	6.5	138
	T1	229	3.2	45	8	6.6	50	6.6	9	29.0	42
	T2	281	3.9	25	27	22.1	39	22.1	0	0.0	NA
	T3	1915	26.8	19	72	59.0	62	59.0	14	45.2	62
	T4	4714	66.0	10	15	12.3	35	12.3	6	19.4	24
	Unknown	466			0				0		
N	N0	2319	33.7	20	65	53.3	54	53.3	14	45.2	73
	N1	1518	22.1	14	39	32.0	31	32.0	12	38.7	54
	N2	1399	20.3	11	15	12.3	47	12.3	4	12.9	24
	N3	1642	23.9	9	3	2.5	24	2.5	1	3.2	NA
	Unknown	727			0				0		
M	M (−)	5480	72.4	16	118	96.7	47	96.7	31	100	57
	M (+)	2092	27.6	9	4	3.3	36	3.3	0	0.0	NA
	Unknown	33			0				0		
Stage	0	0	0.0	NA	0	0.0	NA	0.0	2	6.5	138
	I	146	2.2	57	6	4.9	47	4.9	8	25.8	36
	II	167	2.5	36	26	21.3	42	21.3	1	3.2	54
	III	1278	19.0	23	57	46.7	60	46.7	10	32.3	60
	IVA	2250	33.4	15	22	18.0	39	18.0	8	25.8	24
	IVB	2887	42.9	9	11	9.0	44	9.0	2	6.5	12
	Unknown	877			0				0		

follow-up period was determined when the final follow-up information was obtained. Mean follow-up period of the 7605 cases with PDAC was 17.1 months, that of the 122 cases with PDAC derived from IPMN was 36.7 months, and that of the 31 cases with PDAC concomitant with IPMN was 37.3 months (Table 2). Survival curves were made by the Kaplan-Meier method. $P < 0.05$ was considered statistically significant.

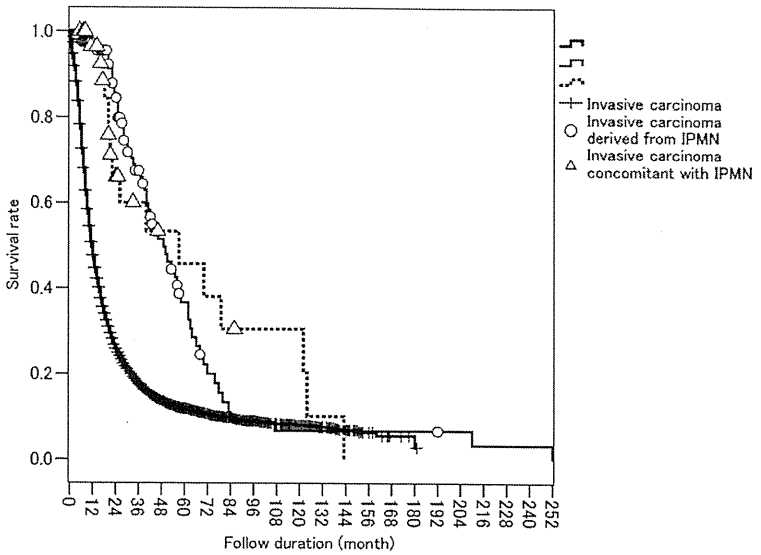
RESULTS

Clinicopathological Comparison Between PDAC and PDAC Derived From IPMN and Between PDAC and PDAC Concomitant With IPMN

The mean ages of patients with PDAC derived from IPMN and PDAC concomitant with IPMN was 66.5 ($P < 0.001$) and 67.1 ($P = 0.021$) years, respectively, both of which were significantly higher than the mean age of 63.5 years of the PDAC patients. The male-to-female ratio was approximately 60% in all 3 groups (Table 2). The IPMN in the cases of PDAC derived from IPMN was significantly more frequently of the main duct type than when IPMN was detected alone, and most IPMNs in PDAC concomitant with IPMN were of the branch duct type, which was not the case when patients presented with IPMN only. Concerning the histological type, approximately one-third of the cases of PDAC derived from IPMN (41/122) were mucinous carcinomas, although most of the cases of PDAC concomitant with IPMN (28/31) were tubular adenocarcinomas, similar to ordinary PDAC (Table 3). Approximately 30% of the cases of PDAC derived from IPMN were mucinous carcinomas, which was significantly more frequent than is observed in patients with PDAC alone ($P < 0.001$; Table 4). More than 50% of the lesions of PDAC concomitant with IPMN were located in the body or tail of the pancreas, whereas approximately 70% of PDAC ($P < 0.001$) and PDAC derived from IPMN ($P = 0.002$) were in the head of the pancreas. About 50% of the cases of PDAC concomitant with IPMN were of TS1 (≤2 cm) in size, whereas approximately 10% of PDAC ($P < 0.001$) and PDAC derived from IPMN ($P < 0.001$) were of TS1. Lymph node metastasis in PDAC was significantly more frequent and more extensive than

P for No. Cases			*P* for MST		
PDAC vs PDAC Derived From IPMN	PDAC vs PDAC Concomitant With IPMN	PDAC Derived From IPMN vs PDAC Concomitant With IPMN	PDAC vs PDAC Derived From IPMN	PDAC vs PDAC Concomitant With IPMN	PDAC Derived From IPMN vs PDAC Concomitant With IPMN
			<0.001	<0.001	0.808
<0.001	0.57	0.016	<0.001	0.003	0.354
			0.354	NA	NA
			NA	NA	NA
0.005	<0.001	<0.001	0.888	0.337	0.19
			<0.001	0.031	0.116
			<0.001	0.028	0.689
			<0.001	NA	NA
			<0.001	0.002	0.127
<0.001	<0.001	<0.001	NA	NA	NA
			0.732	0.301	0.815
			0.404	NA	NA
			<0.001	0.104	0.14
			0.001	0.007	0.831
<0.001	0.01	0.87	<0.001	0.136	0.601
			<0.001	0.015	0.404
			<0.001	0.036	0.369
			0.223	0.280	0.333
<0.001	0.001	0.307	<0.001	0.001	0.789
			0.017	NA	NA
<0.001	<0.001	<0.001	NA	NA	NA
			0.676	0.141	0.917
			0.986	0.957	0.625
			0.001	0.717	0.075
			<0.001	0.018	0.778
			<0.001	0.075	0.67

(Table 6 continued from previous page. Data read horizontally)



	survival rate		
	1-year	3-year	5-year
PDAC	51%	19%	12%
PDAC derived from IPMN	97%	68%	37%
PDAC concomitant with IPMN	97%	61%	46%

* p<0.001

FIGURE 2. Survival curves of PDAC, PDAC derived from IPMN, and PDAC concomitant with IPMN (overall).

in patients with PDAC derived from IPMN and PDAC concomitant with IPMN. Distant metastasis was also more frequent in PDAC than in PDAC derived from IPMN and PDAC concomitant with IPMN. In addition, the stage at the time of the diagnosis of PDAC was more advanced than in patients diagnosed with PDAC derived from IPMN and PDAC concomitant with IPMN.

The median survival times (MSTs) of the 122 patients with PDAC derived from IPMN and of 31 with PDAC concomitant with IPMN were 46 and 57 months, respectively, both of which were significantly longer than the 12 months of the 7605 patients with PDAC (Table 5). The MST of the 7605 patients with PDAC of the tubular type was 13 months, which was significantly shorter than the 44 months of the 122 patients with PDAC derived from IPMN ($P < 0.003$) and 51 months of the 31 patients with PDAC concomitant with IPMN ($P = 0.016$) (Table 6). The MSTs of patients with PDAC of TS1, TS2, TS3, and TS4 were significantly shorter than those of patients with PDAC derived from IPMN and PDAC concomitant with IPMN. The MSTs of patients with PDAC of stage I or II were similar to those of patients with PDAC derived from IPMN and PDAC concomitant with IPMN. However, the MSTs of patients with stage III, IVA, and IVB PDAC were significantly shorter than those of patients with PDAC derived from IPMN and concomitant with IPMN. The survival curve of the patients with PDAC was more unfavorable than that for patients with PDAC derived from IPMN ($P < 0.001$) and with PDAC concomitant with IPMN ($P < 0.001$) (Fig. 2). The 1-, 3-, and 5-year survival rates of PDAC were 51%, 19%, and 12%, respectively, all of which were significantly shorter than 97%, 68%, and 37% of patients with PDAC derived from IPMN and the 97%, 61%, and 46% of patients with PDAC concomitant with IPMN.

TABLE 7. Clinical Features of PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (TS2 or TS3)

	PDAC	PDAC Derived From IPMN		PDAC Concomitant With IPMN	
	(n = 5758)	(n = 90)	*P*	(n = 16)	*P*
Age,* mean (SD), yr	63.6 (9.9)	66.0 (8.7)	0.012	69.4 (6.4)	0.002
Sex†					
Male	3520 (61.1)	60 (66.7)	0.285	11 (68.8)	0.532
Female	2238 (38.9)	30 (33.3)		5 (31.3)	
Follow-up duration,* mean (SD), mo	15.8 (20.0)	34.7 (34.9)	<0.001	16.4 (7.5)	0.759

P value compared with PDAC.

*Two-sample *t* test.

†χ^2 test.

TABLE 8. Clinicopathological Findings of PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (TS2 or TS3)

		IDC (n = 5758)		IDC Derived From IPMN (n = 90)			IDC Concomitant With IPMN (n = 16)		
		n	%	n	%	*P*	n	%	*P*
Histological diagnosis	Tubular adenocarcinoma	5686	98.7	51	73.9	<0.001	10	100	0.722
	Mucinous adenocarcinoma	72	1.3	17	24.6		0	0.0	
	Tubular + mucinous	0	0.0	1	1.4		0	0.0	
	Unknown	0		21			6		
Location	Head	4186	72.8	62	73.8	0.615	9	56.3	0.337
	Body	697	12.1	7	8.3		4	25.0	
	Tail	306	5.3	6	7.1		2	12.5	
	All segments of pancreas	24	0.4	1	1.2		0	0.0	
	Two segments of pancreas	537	9.3	8	9.5		1	6.3	
	Unknown	8		6			0		
TS	TS2	3921	68.1	65	72.2	0.404	12	75.0	0.554
	TS3	1837	31.9	25	27.8		4	25.0	
	Unknown	0		0			0		
T	Tis	0	0.0	0	0.0	<0.001	0	0.0	0.001
	T1	0	0.0	0	0.0		0	0.0	
	T2	239	4.4	26	29.4		0	0.0	
	T3	1434	26.5	52	58.4		11	68.8	
	T4	3742	69.1	11	12.2		5	31.3	
	Unknown	343		1			0		
N	N0	1629	30.9	46	51.1	<0.001	3	18.8	0.02
	N1	1251	23.8	33	36.7		9	56.3	
	N2	1160	22.0	10	11.1		3	18.8	
	N3	1225	23.3	1	1.1		1	6.3	
	Unknown	493		0			0		
M	M (−)	4177	72.8	88	97.8	<0.001	16	100	0.015
	M (+)	1560	27.2	2	2.2		0	0.0	
	Unknown	21		0			0		
Stage	0	0	0.0	0	0.0	<0.001	0	0.0	0.007
	I	0	0.0	0	0.0		0	0.0	
	II	113	2.2	25	27.8		0	0.0	
	III	925	18.0	41	45.6		7	43.8	
	IVA	1854	36.1	17	18.9		8	50.0	
	IVB	2244	43.7	6	6.7		1	6.3	
	Unknown	642		1			0		

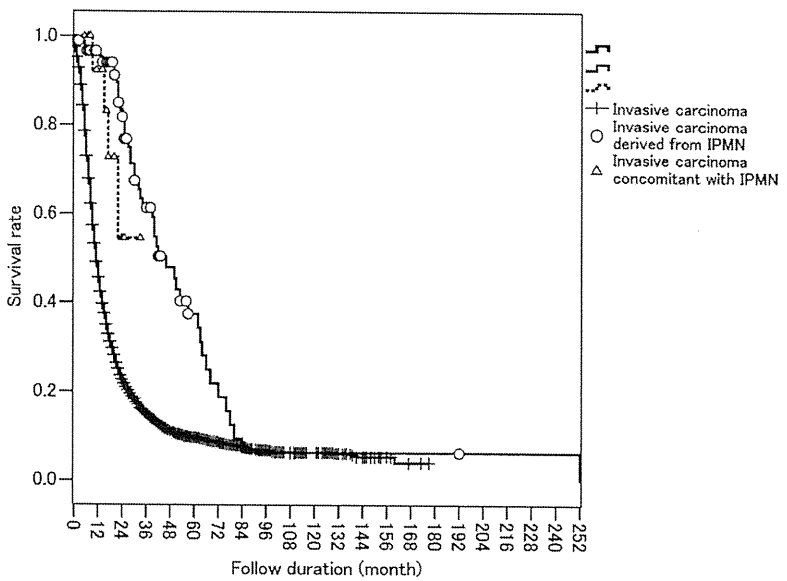
P value compared with PDAC.

Clinicopathological Comparison of TS2 or TS3 PDAC and TS2 or TS3 PDAC Derived From IPMN and Concomitant With IPMN

Next, we compared the tumors that were TS2 (2.0 cm < tumor size ≤ 4.0 cm) or TS3 (4.0 cm < tumor size ≤ 6.0 cm) in size because TS2 and TS3 tumors were the most frequent sizes diagnosed in patients with PDAC, PDAC derived from IPMN, and PDAC concomitant with IPMN of this series. A total of 5578 patients had TS2 or TS3 PDAC, 90 patients had TS2 or TS3 PDAC derived from IPMN and 16 had TS2 or TS3 PDAC concomitant with IPMN (Table 7). These 3 groups of PDAC were compared to examine whether the type TS2 or TS3

TABLE 9. Median Survival of PDAC, PDAC Derived From IPMN, and PDAC Concomitant With IPMN (TS2 or TS3)

PDAC (n = 5578)		PDAC Derived From IPMN (n = 90)		PDAC Concomitant With IPMN (n = 16)		Results of the Log-Rank Test (*P*)	
n	MST, mo	n	MST, mo	n	MST, mo	PDAC vs PDAC Derived From IPMN	PDAC vs PDAC Concomitant With IPMN
5578	11	90	46	16	24	<0.001	0.002



	Survival rate			
	1-year	3-year	5-year	
PDAC	50%	16%	10%	*
PDAC derived from IPMN	96%	62%	38%	**
PDAC concomitant with IPMN	93%	60%	N.A.	

*p<0.001 ** p=0.002

FIGURE 3. Survival curves of PDAC, PDAC derived from IPMN, and PDAC concomitant with IPMN (TS2 or TS3).

PDAC tumors were different from the TS2 or TS3 PDAC derived from IPMN or the TS2 or TS3 PDAC concomitant with IPMN. The T number in the TS2 or TS3 PDAC was significantly greater than for PDAC derived from IPMN and TS2 or TS3 PDAC concomitant with IPMN (Table 8). Lymph node and distant metastases were significantly more frequent and more extensive in TS2 or TS3 PDAC than in TS2 or TS3 PDAC derived from IPMN and TS2 or TS3 PDAC concomitant with IPMN. Distant metastasis was also more frequent in TS2 or TS3 PDAC than in TS2 or TS3 PDAC derived from IPMN and TS2 or TS3 PDAC concomitant with IPMN. The stages of TS2 or TS3 PDAC were more advanced than those of TS2 or TS3 PDAC derived from IPMN and TS2 or TS3 PDAC concomitant with IPMN.

The MST of patients with TS2 or TS3 PDAC was 11 months, being significantly shorter than the 46 months ($P < 0.001$) of the patients with TS2 or TS3 PDAC derived from IPMN and 24 months ($P = 0.002$) of those with TS2 or TS3 PDAC concomitant with IPMN (Table 9). The MSTs of PDAC derived from IPMN and concomitant with IPMN were longer than those of ordinary PDAC for each stage. The survival curves of patients with TS2 or TS3 PDAC were more unfavorable than those of TS2 or TS3 PDAC derived from IPMN ($P < 0.001$) and of TS2 or TS3 PDAC concomitant with IPMN ($P = 0.002$; Fig. 3). The 1-, 3-, and 5-year survival rates of ordinary TS2 or TS3 PDAC were 50%, 16%, and 10%, respectively, whereas those of TS2 or TS3 PDAC derived from IPMN were 96%, 62%, and 38%, respectively, and those of TS2 or TS3 PDAC concomitant IPMN were 93%, 60%, and NA (not available).

DISCUSSION

The definition of PDAC derived from IPMN and PDAC concomitant with IPMN was proposed in this study mainly with regard to the topological relationship between the 2 lesions and the presence or absence of a histological transition between the 2 conditions. This was a multi-institutional study, and we could not use *mucin profiles* and molecular biological examination for the differentiation. A total of 765 patients with IPMN were classified into 5 categories, that is, 381 (50%) with IPMA, 201 (26%) with IPMC (157 with noninvasive and 44 with minimally invasive disease), 122 (16%) with PDAC derived from IPMN, 31 (4%) with PDAC concomitant with IPMN, and 30 (4%) with PDAC of undetermined status with regard to IPMN. When the 2 groups composed of PDAC derived from IPMN and PDAC

TABLE 10. Pancreatic Ductal Adenocarcinoma, PDAC Derived From IPMN, and PDAC Concomitant With IPMN

	PDAC		PDAC Derived From IPMN		PDAC Concomitant With IPMN
Age, yr	64	<	67	≒	67
Sex, M/F	M: 60%	≒	M: 60%	≒	M: 60%
Site	H ≫ B, T		H ≫ B, T		H > B, T
Type (IPMN)			MPD Br		Br
Histological diagnosis	Tub		Muc (30%)		Tub
Tis	big	>	smaller	>	smallest
T	big	>	smaller	>	smallest
N (+)	70%	>	50%	≒	50%
M (+)	30%	>	3%	≒	0%
Stage	Advanced	>	Earlier	>	Earliest
MST, mo	12	<	46	≒	57

concomitant with IPMN were compared with ordinary PDAC, the mean ages of the 2 groups were higher than those of the non-IPMN PDAC group (Table 10). Mucinous carcinoma was more frequently seen in the group of PDAC derived from IPMN than in the other 2 groups. Pancreatic ductal adenocarcinoma concomitant with IPMN was more frequently located in the body or tail of the pancreas than were PDAC derived from IPMN and ordinary PDAC. Pancreatic ductal adenocarcinoma derived from IPMN and concomitant with IPMN were significantly smaller than ordinary PDAC in size and showed less invasive and extensive growth than ordinary PDAC. The median survivals of the 2 groups were significantly longer than that of patients with typical PDAC when compared overall and when limited to TS2 or TS3 cases.

Intraductal papillary mucinous neoplasm progresses from adenoma to carcinoma (noninvasive, then minimally invasive, and finally to PDAC derived from IPMN).[1–3,7,8] Yamaguchi et al[5] first reported PDAC concomitant with IPMN in 2002. Thereafter, this combination has been reported mainly in Japan,[9,10] and the development of pancreatic cancer apart from IPMN has been also reported during the follow-up of branch duct IPMN.[11,12] When IPMN and PDAC are present near each other, the distinction between PDAC derived from IPMN and PDAC concomitant with IPMN is difficult to make. There has been some confusion about the definition of the 2 conditions. In this series, we proposed diagnostic criteria of the 2 diseases based on the topological relationship and the presence or absence of a transitional area between the 2 diseases. In this series, we did not perform *mucin profiles* and molecular biological examinations because the present study was a multi-institutional analysis. If we added molecular biology to the criteria, we might have been able to differentiate the 2 conditions more precisely, decreasing the number of patients included in the "undetermined" group.

The reported incidence of PDAC concomitant with IPMN was 9%[5] or 4%[10] in 2 series of surgically resected IPMN. Ingkakul et al[13] reported that 22 (9.3%) of 236 patients with IPMN had concomitant PDAC synchronously or metachronously, and their multivariate analysis revealed that worsening of diabetes mellitus and an abnormal serum CA 19-9 level are 2 significant predictors of the presence of PDAC in IPMN. The development of independent PDAC has been reported in the follow-up of patients with IPMN.[11,12,14] Tada et al[11] reported that PDAC developed in 5% of patients with IPMN during a 3.8-year follow-up. Uehara et al[12] showed an 8% incidence of PDAC developing in 60 patients with branch duct IPMN during the mean follow-up period of 87 months. The 5-year rate of development of PDAC was 6.9%, and the incidence of PDAC was 1.1% per year. Tanno et al[14] showed that 4 (4.5%) of 89 patients with branch duct IPMN developed PDAC during a median follow-up of 64 months. When the new definition is applied, the incidence of PDAC concomitant with IPMN in the present series was 4.1%, which was lower than in the previous reports. This difference might come from the strict definition in this series and the multi-institutional collection of surgically resected cases.

Some have reported that the clinical outcome of patients with PDAC derived from IPMN is better than that for patients with ordinary PDAC because PDAC derived from IPMN is diagnosed at an earlier phase[15] or because the clinicopathological features of PDAC derived from IPMN are different from those of ordinary PDAC.[16–18] A global genomic analysis of IPMN showed significant molecular features that were different from ordinary PDAC.[19] In this series, the clinical outcome of patients with PDAC derived from IPMN was better than that of ordinary PDAC when compared overall and when limited to TS2 or TS3 tumors in size. Patients with IPMN related to PDAC (PDAC derived from IPMN and PDAC concomitant with IPMN) showed a longer MST than those with ordinary PDAC in each stage. Therefore, the biological behavior of PDAC derived from IPMN may be different from ordinary PDAC.

We first reported that the clinical outcome of patients with PDAC concomitant with IPMN was better than that of ordinary PDAC because PDAC concomitant with IPMN was detected at an earlier stage because of the presence of IPMN.[5] In this series, we compared the clinical course of PDAC concomitant with IPMN and that of ordinary PDAC when compared overall and when limited to TS2 or TS3 tumors in size. The clinical course of PDAC concomitant with IPMN was better than ordinary PDAC. Thus, the biological behavior of PDAC concomitant with IPMN may also be different from that of ordinary PDAC.

Concerning pancreatic carcinogenesis, 2 main pathways have been considered: (1) from PanIN to PDAC[20–22] and (2) from IPMN to mucinous carcinoma.[23,24] Others have reported that mucinous carcinoma of the pancreas often originates from IPMN.[23,24] In the present series, mucinous carcinoma was more frequently present in PDAC derived from IPMN than PDAC alone or PDAC concomitant with IPMN. In minimally invasive foci of IPMC, IPMC invaded the stroma in the form of mucinous carcinoma in about a half of the patients.[3] With regard to the histological type, approximately one-third of the PDAC derived from IPMN (41/122) was mucinous carcinoma, although most of PDAC concomitant with IPMN (28/31) was tubular adenocarcinoma, which is similar to ordinary PDAC. These facts may support the hypothesis that most of the mucinous carcinoma of the pancreas originates from IPMC.

This series is a collective series of surgically resected IPMN, PDAC derived from IPMN, and PDAC concomitant with IPMN, and there are some biases that resulted from this limitation. In this series, the PDAC derived from IPMN or concomitant with IPMN were less invasive and showed less extensive growth than those of ordinary PDAC. The overall survival rates of PDAC derived from IPMN and PDAC concomitant with IPMN were significantly better than those of ordinary PDAC. Even when limited to TS2 or TS3 tumors, PDAC derived from IPMN and PDAC concomitant with IPMN showed less aggressive growth than TS2 or TS3 PDAC. Therefore, PDAC derived from IPMN and concomitant with IPMN may have more favorable biological features than ordinary PDAC. Further examination of the natural history of PDAC derived from IPMN and concomitant with IPMN is therefore necessary before any definitive conclusions can be made about the origins, behavior, and lethality of the different types of pancreatic cancer.

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Adenoviral Therapy Is More Effective in Gemcitabine-resistant Pancreatic Cancer than in Gemcitabine-sensitive Cells

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Abstract. *Background: Although gemcitabine is the standard treatment for pancreatic cancer, this particular type of cancer develops rapidly and has intrinsic chemoresistance. Chemoresistance plays a critical role in tumor progression, invasion and migration. Nevertheless, the effect of adenoviral therapy on chemoresistant cancer cells has not been studied. In this study, we compared the efficacy of adenoviral therapy in parental and chemoresistant pancreatic cancer cells. Materials and Methods: To establish gemcitabine-resistant cells, pancreatic cancer SUIT2 cells were exposed to increasing concentrations of gemcitabine. Both parental and chemoresistant cells were infected with adenoviruses expressing either green fluorescent protein (Ad-GFP) or the hepatocyte growth factor antagonist, NK4 (Ad-NK4). To investigate the transduction efficacy, GFP expression and NK4 concentrations were measured and an invasion assay was used to investigate the efficacy of the adenoviral therapy. Results: The 50% inhibitory concentration of gemcitabine was <10 nM in the parental SUIT-2 cells, while it was >1 μM in gemcitabine-resistant cells. A large number of gemcitabine-resistant cells were GFP-positive compared with only a small number of parental cells ($p<0.05$). The NK4 expression level was significantly higher in gemcitabine-resistant cells than in parental cells ($p<0.05$). The supernatant from Ad-NK4-infected gemcitabine-resistant cells significantly inhibited the invasion of cancer cells compared with that from Ad-NK4-infected parental cells ($p<0.05$). Conclusion: Both the efficiency of transduction and the therapeutic efficacy of adenoviral therapy were higher in gemcitabine-resistant cells than in parental cells, suggesting that adenoviral gene therapy is more effective in patients with gemcitabine- resistant pancreatic cancer.*

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Key Words: Virotherapy, pancreatic cancer, chemoresistance, gemcitabine, PDAC, GFP.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal type of human cancer. The prognosis of patients with PDAC is extremely poor, with an overall 5-year survival rate of only 4.4% (1). The limited efficacy of conventional systemic therapies is one reason for this poor prognosis. Gemcitabine, a pyrimidine nucleoside analogue, is currently the standard treatment for pancreatic cancer. Although gemcitabine yields great clinical benefit in patients with advanced pancreatic cancer, the response rates and survival benefits when gemcitabine is used alone are very low (2). Therefore, many therapeutic regimens that combine gemcitabine with other cytotoxic agents such as 5-fluorooracil (3, 4), irinotecan (5), exatecan (6), cisplatin (7, 8), and oxaliplatin (9) were developed and evaluated in clinical trials; however, these combination therapies have not improved overall survival (2). One reason for the low efficacy of gemcitabine and gemcitabine-based combination therapies is chemoresistance. PDAC is either intrinsically chemoresistant, or rapidly becomes tolerant to gemcitabine. Recent studies show that chemoresistance plays a critical role in tumor progression, invasion and migration, and the malignant potential of chemoresistant cells is higher than that of chemosensitive cells (10, 11). Therefore, new therapeutic approaches are needed.

Adenovirus-based cancer gene therapy is a novel approach for treating tumors that are resistant to established therapies (12). Adenoviral therapy shows promising results both *in vitro* and *in vitro*, and many clinical trials have been conducted (13-17). Nevertheless, the relationship between adenoviral therapy and chemoresistance has not been studied. In this study, we established gemcitabine-resistant pancreatic cancer cells and compared the efficiency of transduction and the efficacy of adenoviral therapy in both chemoresistant and chemosensitive pancreatic cancer cells.

Materials and Methods

Cell lines and the establishment of gemcitabine-resistant cells. The human pancreatic cancer cell line SUIT-2 was a kind gift from Dr. H. Iguchi (National Shikoku Cancer Center). The human fibroblast cell line, MRC-5, which secretes biologically active hepatocyte growth factor (HGF), was obtained from the RIKEN Cell Bank (Ibaragi, Japan). Cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with streptomycin (100 μg/ml), penicillin (100 U/ml) and 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 90% air. To establish a gemcitabine-resistant pancreatic cancer cell line, SUIT2 cells were exposed to increasing concentrations of gemcitabine as previously described (18).

Construction of recombinant adenoviruses. A recombinant adenoviral vector expressing human HGF antagonist NK4 (Ad-NK4) and a control vector expressing the bacterial β-galactosidase gene (Ad-lacZ) were constructed as previously described (19-21). In brief, Ad-NK4 was generated by homologous recombination of the pJM17 plasmid and the shuttle plasmid vector pSV2+ containing an expression cassette and the CMV early promoter/enhancer, followed by human *NK4* cDNA and a polyadenylation signal. An adenovirus expressing green fluorescent protein (Ad-GFP) was generated, amplified and titrated as previously described (22, 23).

Propidium iodide (PI) assay. The 50% inhibitory concentration was evaluated by PI fluorescence intensity. Cells were plated in 24-well tissue culture plates and cultured for 24 hours. Several different concentrations of gemcitabine were used and the cells were incubated for a further 48 hours. PI (30 μM) and digitonin (600 μM) were then added to each well. Fluorescence intensity, corresponding to the total number of cells, was measured using an Infinite F200 (Tecan Trading AG, Switzerland) apparatus fitted with 535 nm excitation and 620 nm emission filters. The results were converted to survival rates by comparing treated cells with untreated cells. All experiments were performed in triplicate wells.

Assessment of transgene distribution by evaluation of GFP expression. Parental and gemcitabine-resistant cells were seeded in 6-well plates and cultured in DMEM supplemented with 10% FBS for 24 hours. Cells were infected with Ad-GFP at 10 multiplicities of infection (MOI). The culture medium was replaced with fresh medium 1 hour after transfection. After 24, 48 or 72 hours of infection, the GFP-positive and GFP-negative cells were observed and counted under a fluorescence microscope.

Real-time PCR and reverse transcription-PCR assays. The adenovirus DNA content of the infected cells was determined using real-time PCR, SYBR® Premix Ex Taq 2 (Takara, Tokyo, Japan) and a Chromo4™ System (Bio-Rad, Hercules, CA, USA). PCR conditions were as follows: 40 cycles at 95.5°C for 5 seconds, 60°C for 20 seconds, with +0.1°C/second up to 95°C for melting analysis. Each sample was run in triplicate. The primers used for the *NK4* gene were: 5'-GCAATTAAAACATGCGCTGA-3' and 3'-ATTGACAGTGC CCCTGTAGC-5' (24). The number of viral DNA copies was calculated from a standard curve obtained for the purified adenovirus vector and was further adjusted according to the protein concentration of each lysate. The mRNA levels for the Coxsackie virus and adenovirus receptor (CAR), β3-integrin, β5-integrin, clathrin, and dynamin 2 were quantified by real-time reverse transcription-PCR using a QuantiTect SYBR Green reverse transcription-PCR kit (Qiagen, Tokyo, Japan), 10 ng of total RNA, and primers specific for CAR, 5'-GGCGCTCCTGCTGTGC-3' and 5'-CTTTGGCTTTTTC AATCATCTTC-3'; β3-integrin, 5'-GAGGATGACTGTGTCGTCAG-3' and 5'-CTGGCGCGTTCTTCCTCAAA-3'; β5-integrin, 5'-CCTGTCCATGAAGGATGACTTG-3' and 5'-CTCATTGAAGCT GTCCACTCTG-3'; clathrin, 5'-CGGTTGCTCTTGTTACGG-3' and 5'-CGGTTGCTCTTGTTACGG-3'; and dynamin 2, 5'-AGGAGTACT GGTTTGTGCTGACTG-3' and 3'-GTGCATGATGGTCTTTGGCA TGAG-5'. The reaction mixture was first incubated at 50°C for 30 minutes to allow reverse transcription. PCR was initiated with one cycle of 95°C for 15 minutes to activate the modified Taq polymerase, followed by 40 cycles at 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and one cycle at 60°C for 15 seconds, with +0.1°C/second up to 95°C for melting analysis. Each sample was run in triplicate. The levels of CAR, β3-integrin, β5-integrin, clathrin, and dynamin 2 mRNA were normalized to those of 18S rRNA amplified using the specific primers 5'-GTAACCCGTTGAACCCCATT-3' and 5'-GCGATGATGGCTAACCTACC-3', and expressed as a ratio compared with untreated controls.

Measurement of NK4 expression levels. After infection with Ad-NK4, the conditioned media were collected and changed every 24 hours. The HGF concentration in the conditioned media was measured by using a human HGF ELISA Kit (IMMUNIS HGF EIA; Institute of Immunology, Tokyo, Japan) according to the manufacturer's recommendations.

Invasion assay. The invasiveness of pancreatic cancer cells was quantified as the number of cells invading through Matrigel-coated transwell inserts (Becton Dickinson) as previously described (25). In brief, transwell inserts (8 μm pores) were coated with Matrigel (20 μg/well; Becton Dickinson). Gemcitabine-sensitive and -resistant SUIT-2 cells were infected with Ad-lacZ or Ad-NK4 at MOI of 100, and culture media were collected 2 days after infection. Fresh, untreated gemcitabine-sensitive SUIT-2 cells were seeded into the upper chambers of the 24-well plates at a density of $1\times10^5/cm^2$ in 250 μl of DMEM supplemented with 10% FBS and cultured in 750 μl of conditioned media from the gemcitabine-sensitive SUIT-2 cells or gemcitabine-resistant SUIT-2 cells infected with Ad-lacZ or Ad-NK4. MRC-5 cells (1×10^5 cells/well), which secrete HGF, were seeded into the lower chambers of the 24-well plates. After 48 hours of incubation, any cells that had invaded to the lower surface of the Matrigel-coated membrane were counted in three randomly selected fields under a light microscope.

Statistical analysis. Values were expressed as the mean±SD. Comparisons between all groups were made using one-way ANOVA, and Student's *t*-test was used for comparisons between two groups. The level of statistical significance was set at $p<0.05$. To confirm the induction results, experiments were repeated at least three times.

Results

Establishment of gemcitabine-resistant cells. SUIT2 cells were exposed to increasing concentrations of gemcitabine to establish a gemcitabine-resistant pancreatic cancer cell line. As shown in Figure 1A, gemcitabine-resistant cells showed a round morphology when compared with the parent

SUIT-2 cells. The 50% inhibitory concentration for gemcitabine was <10 nM in the parental SUIT-2 cells, while it was >1 μM in gemcitabine-resistant cells (Figure 1B).

GFP expression in Ad-GFP-infected parental cells and gemcitabine-resistant cells. To investigate differential expression of transgenes delivered by the adenoviral vector between parental and gemcitabine-resistant cells, we examined the expression levels of GFP in Ad-GFP-transfected cells. Parental and gemcitabine-resistant SUIT-2 cells were cultured for 24 hours after seeding and then infected with Ad-GFP at 10 MOI. At 24, 48 or 72 hours after infection, the GFP-positive and GFP-negative cells were observed and counted under a fluorescence microscope. Figure 2A shows that a large number of gemcitabine-resistant SUIT-2 cells were GFP-positive, while only a low number of parental SUIT-2 cells were GFP-positive on each day after infection (Figure 2B, $p<0.05$). This suggests that the expression level of adenovirus-delivered transgenes in gemcitabine-resistant cells was higher than that in the parental cells.

NK4 expression in Ad-NK4-infected parental and gemcitabine resistant cells. We next used Ad-NK4 to investigate differences in the efficacy of the adenoviral therapy between parental and gemcitabine-resistant cells. NK4 acts as a competitive HGF antagonist and inhibits pancreatic cancer cell migration and invasion (26, 27). After infection with Ad-NK4, we collected the conditioned media every 24 hours and measured the NK4 expression levels by ELISA. The NK4 levels in gemcitabine-resistant cells were significantly higher than those in gemcitabine-sensitive cells at 1, 2 and 3 days after infection (Figure 3A, $p<0.05$).

Efficacy of the adenoviral therapy in Ad-NK4-infected parental cells and gemcitabine resistant cells. SUIT-2 cells were seeded in the upper chambers of a transwell plate, and MRC-5 cells, which secrete HGF, were seeded in the lower chambers with the culture supernatant from Ad-NK4 or Ad-LacZ-infected parental or gemcitabine-resistant cells, and invasive cells were counted 48 hours later. As shown in Figure 3B, the number of cells that invaded across the membrane after culture with the supernatant from Ad-NK4-infected cells was lower than that of cells cultured with the supernatant from untreated or Ad-LacZ-infected cells, which is consistent with the previous data (28). The number of cells that invaded across the membrane after culture with the supernatant from Ad-NK4-infected gemcitabine-resistant cells was lower than that of cells cultured with the supernatant from Ad-NK4-infected parental cells (Figure 3C, $p<0.05$).

Adenoviral mRNA expression and adenoviral DNA content of Ad-NK4-infected parental and gemcitabine-resistant cells. To clarify the mechanisms underlying the increased efficacy of adenoviral therapy in gemcitabine-resistant cells, we investigated the levels of adenoviral mRNA expression and the adenoviral DNA content of Ad-NK4-infected parental and gemcitabine-resistant cells. Parental and resistant cells were infected with Ad-NK4 at 20 MOI, and *NK4* mRNA expression and viral DNA content were quantified 48 hours later. As shown in Figure 4A, the expression level of *NK4* mRNA in gemcitabine-resistant cells was higher than that in parental cells ($p<0.05$). The viral DNA content of gemcitabine-resistant cells was also higher than that of parental cells (Figure 4B, $p<0.05$).

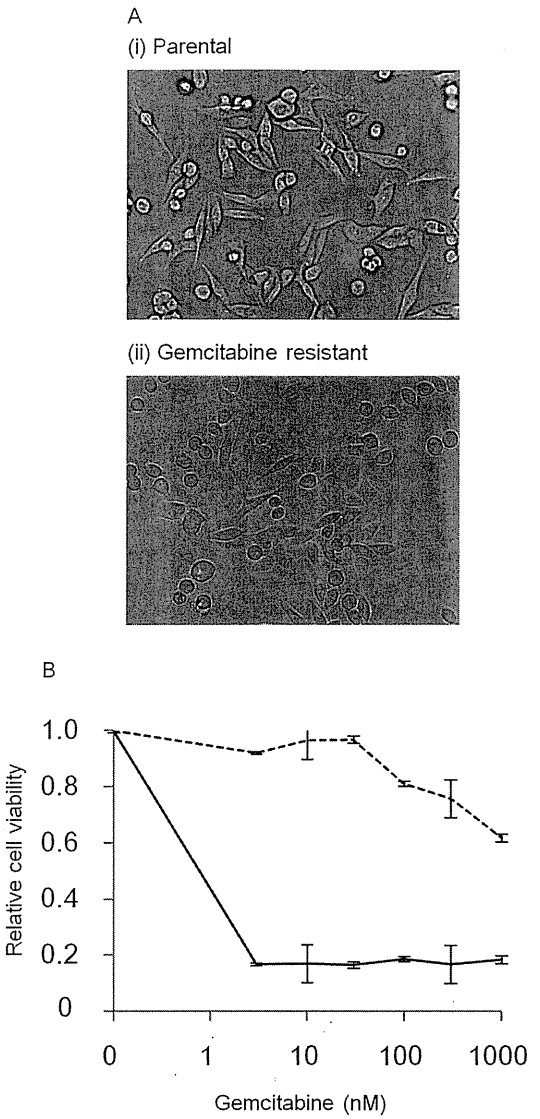

Figure 1. *Gemcitabine-resistant and parental SUIT-2 cells. A: Morphology of (i) parental cells and (ii) gemcitabine-resistant cells (×200 magnification). B: The 50% inhibitory concentration of parental and gemcitabine-resistant cells. Solid line, parental cells; dotted line, gemcitabine-resistant cells.*

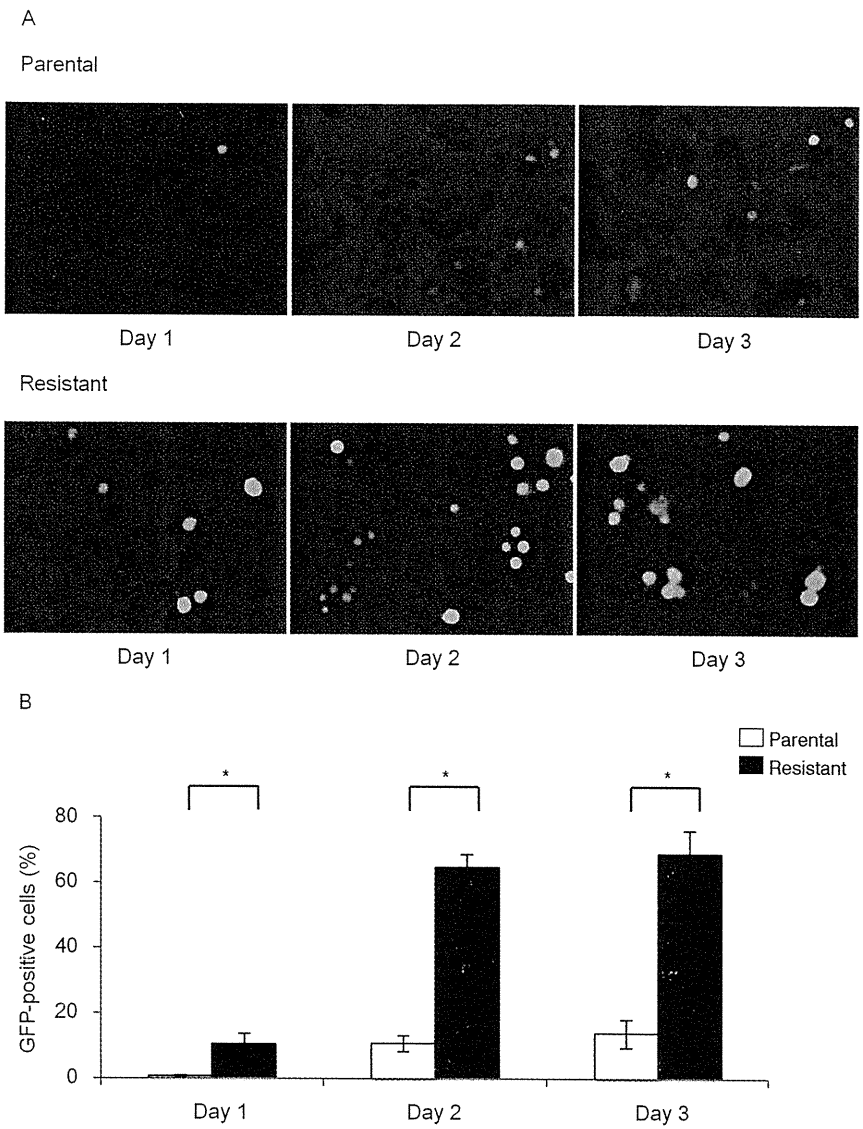

Figure 2. *GFP expression in Ad-GFP-infected parental and gemcitabine-resistant cells. Parental and gemcitabine-resistant cells were seeded and cultured for 24 h. Cells were infected with Ad-GFP at 10 multiplicities of infection (MOI). At 24, 48 or 72 h after infection, the GFP-positive and GFP-negative cells were observed and counted under a fluorescence microscope. A: GFP-positive and GFP-negative cells observed by fluorescence microscopy (×200 magnification). B: The percentage of GFP-positive cells. Each value represents the mean±SD of triplicate measurements. *p<0.05.*

Levels of CAR, β3-integrin, β5-integrin, clathrin and dynamin 2 mRNA in parental and gemcitabine-resistant cells. The viral DNA and mRNA expression levels in gemcitabine-resistant cells were higher than in those in parental cells. Therefore, to investigate the efficiency of adenoviral cell attachment and endocytosis in gemcitabine-resistant and parental cells, we quantified the levels of CAR, β3- and β5-integrin, clathrin and dynamin 2 mRNA using qRT-PCR (Figure 4C). These molecules are required for adenoviral cell attachment and endocytosis. We found no difference in the expression levels of any of these mRNAs between gemcitabine-resistant and parental cells.

Discussion

The results of this study show that: i) the uptake of adenoviral genes and the efficiency of transduction were higher in gemcitabine-resistant cells than in gemcitabine-sensitive cells; ii) adenoviral gene therapy is more effective against gemcitabine-resistant cells than against gemcitabine-sensitive cells; and iii) the levels of CAR, β3-integrin, β5-integrin, dynamin and clathrin mRNA expression were not different between gemcitabine-resistant cells and gemcitabine-sensitive cells.

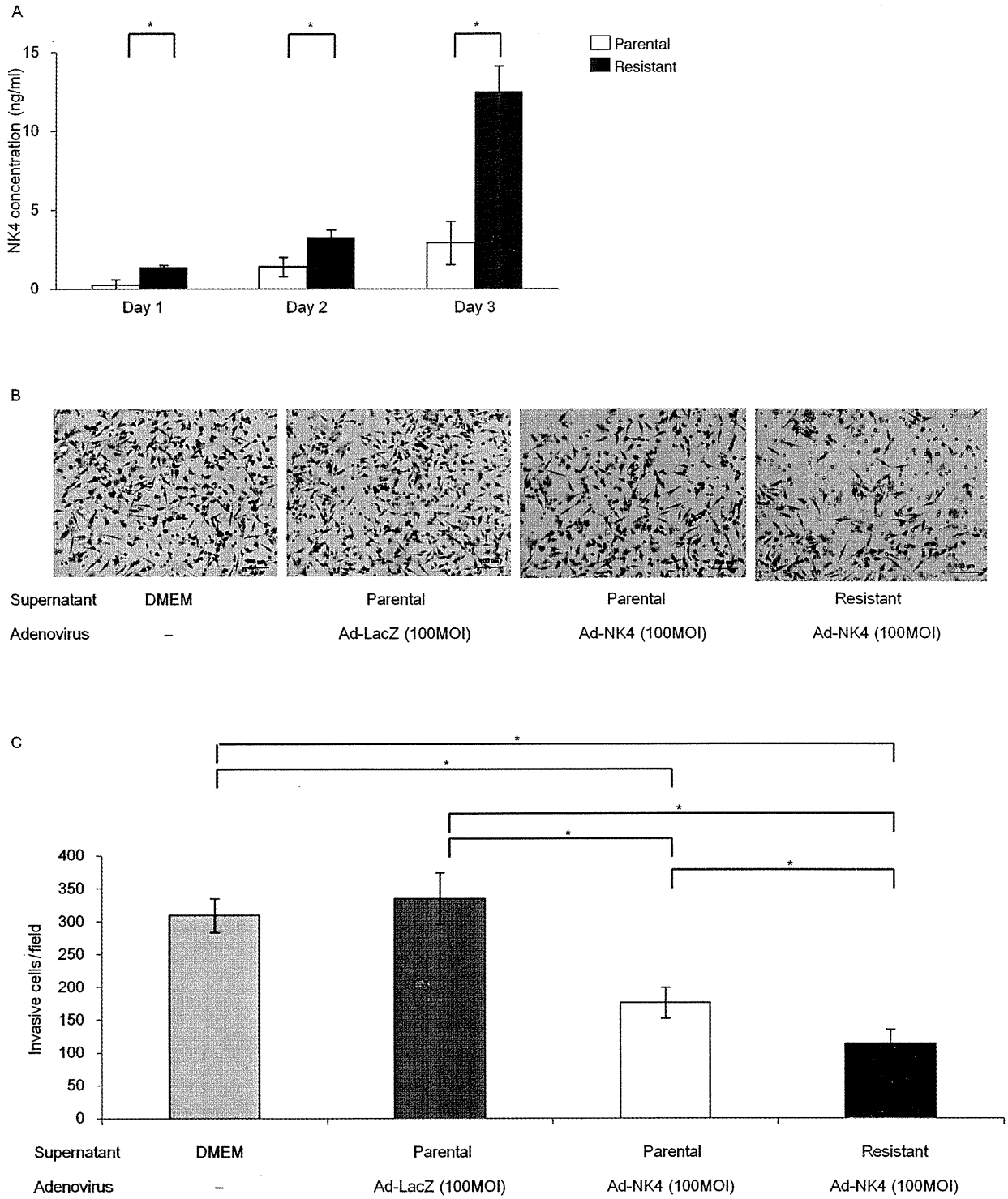

Figure 3. *Differences in the efficacy of the adenoviral therapy between parental and gemcitabine-resistant cells. A: The concentration of NK4 in each cell type. Parental and resistant cells were infected with Ad-NK4 at 20 MOI, and 48 hours after infection, the supernatants were collected and NK4 levels in culture media were measured by ELISA. Each value represents the mean±SD of triplicate measurements. *p<0.05. B, C: Invasion assay. Parental and gemcitabine-resistant cells were infected with Ad-lacZ or Ad-NK4 at MOI of 100 and culture media were collected 2 days after infection. Fresh, untreated parental SUIT-2 cells were seeded into the upper chamber of a transwell and cultured with each of the conditioned media. MRC-5 cells (1×10^5 cells/well), which secrete HGF, were seeded in the lower chamber. After 48 h, cells that had invaded to the lower surface of the Matrigel-coated membrane were counted. Each value represents the mean±SD of triplicate measurements. *p<0.05.*

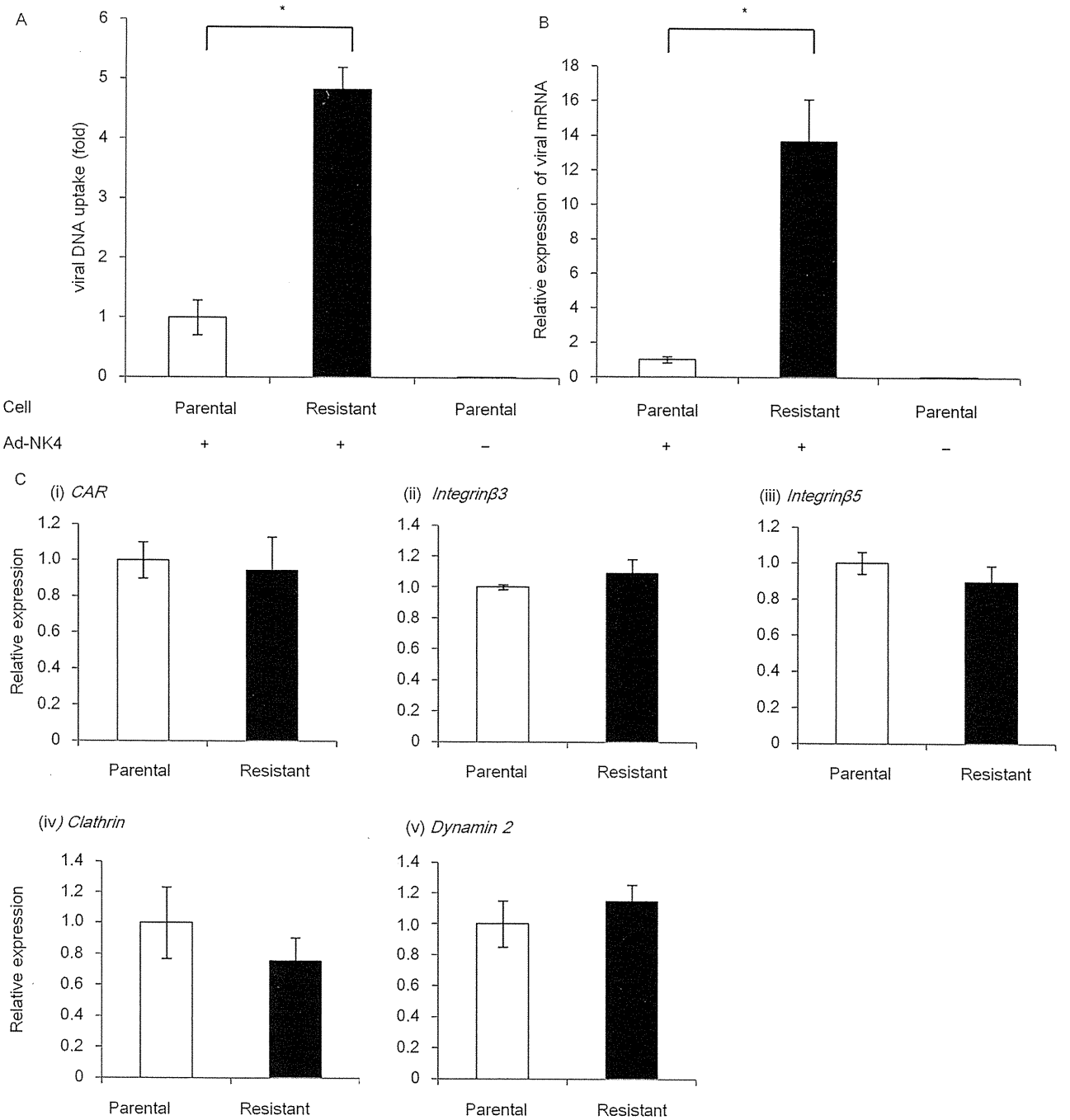

Figure 4. *The adenoviral mRNA levels and adenoviral DNA content of Ad-NK4-infected parental and gemcitabine-resistant cells. Parental and resistant cells were infected with Ad-NK4 at 20 MOI, and NK4 mRNA expression and viral DNA content were quantified 48 h after infection. Each value represents the mean±SD of triplicate measurements. *p<0.05. A: The expression level of adenoviral mRNA. B: Adenoviral DNA content. C: The levels of CAR, β3 and β5integrins, clathrin and dynamin 2 mRNA in parental cells and gemcitabine-resistant cells. Each value represents the mean±SD of triplicate measurements.*

Although gemcitabine is currently the standard treatment for pancreatic cancer, the response rates and survival benefits are very low. In recent studies, several strains of gemcitabine-resistant cells were established and their characteristics of including molecular markers related to gemcitabine resistance and associated with the acquisition of an epithelial–mesenchymal-like phenotype by cancer cells or cancer stem cells, were investigated (29-31). However, the relationship

between chemoresistance and adenoviral therapy has not been studied. We first investigated this using Ad-GFP to examine the association between chemoresistance and adenoviral therapy. Surprisingly, the expression level of GFP in gemcitabine-resistant SUIT-2 cells was significantly higher than that in the parent cells. We also found that there were significant differences in the efficacy of the adenoviral therapy between parental and gemcitabine-resistant cells. These data suggest that adenoviral gene therapy is a good treatment option for pancreatic cancer that has developed tolerance to gemcitabine.

HGF is a multi-domain glycoprotein first identified as a potent mitogen for adult rat hepatocytes in primary culture and which has high affinity for the c-Met receptor (32), which is frequently overexpressed in pancreatic cancer. The interaction between HGF and the c-Met receptor increased the rate of proliferation, invasion, migration and angiogenesis of cancer cells (33-36). NK4 is composed of the *N*-terminal hairpin and subsequent four-kringle domains of HGF, and it acts as a dose-dependent, competitive HGF antagonist. NK4 inhibits pancreatic cancer cell migration and invasion *in vitro* and suppresses growth, invasion, and metastasis of human pancreatic carcinoma *in vivo* (21, 26, 37). Adenoviruses expressing NK4 have been developed, and these viruses have similar antitumor effects (37, 38). In this study, we used Ad-NK4 to investigate the efficacy of adenoviral gene therapy in parental and gemcitabine-resistant cells. The expression level of the *NK4* transgene delivered by the adenoviral vector was higher in the supernatant of gemcitabine-resistant cells than in the supernatant of parental cells, which is consistent with the results of our experiments using Ad-GFP. In addition, the supernatant from Ad-NK4-infected gemcitabine-resistant cells, which contained a higher concentration of NK4, inhibited pancreatic cancer cell invasion to a greater extent than that from Ad-NK4-infected parental cells. These data also suggest that adenoviral gene therapy is more effective in pancreatic cancer cells that have acquired gemcitabine resistance.

The expression level of *NK4* mRNA and the viral DNA content of Ad-NK4-infected gemcitabine-resistant cells were higher than those of parental cells, suggesting that the difference in transgene expression level between parent and gemcitabine-resistant cells was caused by differences in the efficiency of cellular uptake of adenoviral particles. Attachment and internalization are required for an adenovirus to enter a host cell (39, 40). During the first step, the fiber protein of all adenovirus serotypes (except subgroup B) binds to a primary receptor, CAR. During the second step, the CAR-docked particles activate αvβ3 and αvβ5integrins and their co-receptors, triggering endocytosis. The adenoviral particles are then rapidly internalized. Endocytosis of adenoviral particles is mediated by clathrin and involves the large GTPase, dynamin (39). Although we quantified the levels of CAR, β3 and β5integrins, clathrin and dynamin 2 mRNA in both parental and gemcitabine-resistant cells, we found no differences. Furthermore, we performed additional experiments, including flow cytometry to detect CAR expression on the cell surface and a promoter assay to check the CMV promoter (data not shown), but found no reasonable explanation for this mechanism. Therefore, we were unable to identify the mechanism by which transgene expression in gemcitabine-resistant cells is more efficient than in parental cells. However, there may be some unknown, causal mechanism that awaits future study.

In conclusion, our results show that both the efficiency of transduction and efficacy of adenoviral therapy were higher in gemcitabine-resistant cells than in gemcitabine-sensitive cells. This suggests that adenoviral gene therapy should be more effective in patients with pancreatic cancer that has acquired gemcitabine resistance, and that adenoviral therapy may be a good therapeutic choice, especially for patients who do not respond to 'conventional' gemcitabine therapy.

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