Fluorescence In Situ Hybridization to Visualize Genetic Abnormalities in Interphase Cells of Acinar Cell Carcinoma, Ductal Adenocarcinoma, and Islet Cell Carcinoma of the Pancreas

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OBJECTIVE: To use fluorescence in situ hybridization (FISH) to visualize genetic abnormalities in interphase cell nuclei (interphase FISH) of acinar cell carcinoma, ductal adenocarcinoma, and islet cell carcinoma of the pancreas.

PATIENTS AND METHODS: Between April 4, 2007, and December 4, 2008, interphase FISH was used to study paraffin-embedded preparations of tissue obtained from 18 patients listed in the Mayo Clinic Biospecimen Resource for Pancreas Research with a confirmed diagnosis of acinar cell carcinoma, ductal adenocarcinoma, islet cell carcinoma, or pancreas without evidence of neoplasia. FISH probes were used for chromosome loci of *APC* (see glossary at end of article for expansion of all gene symbols), *BRCA2*, *CTNNB1*, *EGFR*, *ERBB2*, *CDKN2A*, *TP53*, *TYMP*, and *TYMS*. These FISH probes were used with control probes to distinguish among various kinds of chromosome abnormalities of number and structure.

RESULTS: FISH abnormalities were observed in 12 (80%) of 15 patients with pancreatic cancer: 5 of 5 patients with acinar cell carcinoma, 5 of 5 patients with ductal adenocarcinoma, and 2 (40%) of 5 patients with islet cell carcinoma. All 3 specimens of pancreatic tissue without neoplasia had normal FISH results. Gains of *CTNNB1* due to trisomy 3 occurred in each tumor with acinar cell carcinoma but in none of the other tumors in this study. FISH abnormalities of all other cancer genes studied were observed in all forms of pancreatic tumors in this investigation.

CONCLUSION: FISH abnormalities of *CTNNB1* due to trisomy 3 were observed only in acinar cell carcinoma. FISH abnormalities of genes implicated in familial cancer, tumor progression, and the 5-fluorouracil pathway were common but were not associated with specific types of pancreatic cancer.

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5FU = 5-fluorouracil; FISH = fluorescence in situ hybridization; ND-FISH = interphase FISH to detect abnormalities of chromosome number and structure; SSC = standard saline citrate

Pancreatic cancer afflicts more than 200,000 new patients worldwide each year, including more than 37,600 in the United States.¹⁻⁴ Surgery seldom cures pancreatic cancer and effective chemotherapies are largely unknown.⁵ Survival varies among patients with pancreatic cancer but is often measured in months.^{6,7} Thus, novel genetic research of pancreatic cancer is urgently needed to help establish accurate diagnoses and to develop effective treatments for this important public health problem.

This investigation used fluorescence in situ hybridization (FISH) to visualize chromosomal loci in interphase

nuclei (interphase FISH) of acinar cell carcinoma, ductal adenocarcinoma, and islet cell carcinoma of the pancreas. Acinar cell carcinoma and ductal adenocarcinoma, which are nonendocrine tumors, and islet cell carcinomas, which are neuroendocrine tumors, reportedly occur in less than 1%, 95%, and 5%, respectively, of patients with pancreatic cancer.^{3,8,9} In 2 retrospective studies of large series of patients, median survival for unresected ductal adenocarcinoma was 3 months⁸; for unresected acinar cell carcinoma, 25 months⁸; for nonfunctional islet cell carcinoma, 26 months⁹; and for functional islet cell carcinoma, 54 months.⁹

Although some genetic studies of pancreatic cancer have been published, most focus on ductal adenocarcinoma. An assortment of genetic procedures have been attempted to investigate pancreatic cancer, including family history studies,¹ conventional cytogenetic techniques,¹⁰ interphase FISH,¹¹⁻¹³ comparative genomic hybridization,¹⁴ RNA expression analyses,^{15,16} and evaluation of sequence variations.¹⁷ These studies implicate a variety of point mutations and chromosomal anomalies in most, if not all, patients with pancreatic cancer. Nevertheless, the biology of various forms of pancreatic cancer remains poorly understood, and this problem precludes efforts to establish novel genetic treatments for these disorders.

This investigation used interphase FISH to visualize genetic abnormalities in individual normal and neoplastic cells in acinar cell carcinoma, ductal adenocarcinoma, and islet cell carcinoma of the pancreas.¹⁸ This research project was specifically designed to detect genetic abnormalities that have been shown via other genetic technologies to

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A glossary of genetics terminology appears at the end of this article.

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be associated with acinar cell carcinoma of the pancreas, familial pancreatic cancer, tumor progression in pancreatic cancer, or genes linked with the molecular pathway of 5-fluorouracil (5FU) chemotherapy.

PATIENTS AND METHODS

This study was performed with approval of the Mayo Clinic Institutional Review Board, and informed consent was obtained in accordance with the Declaration of Helsinki. Between April 4, 2007, and December 4, 2008, paraffin-embedded pancreatic tissue was studied from patients listed in the Mayo Clinic Biospecimen Resource for Pancreas Research with a confirmed diagnosis of acinar cell carcinoma, ductal adenocarcinoma, islet cell carcinoma, or pancreas without evidence of neoplasia.

Each of these specimens was selected and reviewed by one of the authors of this article (T.C.S.) using appropriate cytology, histology, and electron microscopic examination. The clinical history of each of these patients was reviewed by another of the authors (R.R.M.). The selection of specimens was arbitrary for patients with ductal adenocarcinoma and islet cell carcinoma but required the availability of paraffin-embedded blocks. Because of the rarity of acinar cell carcinoma, we studied all such tumor specimens with available paraffin-embedded blocks or biopsy specimens in the Mayo Clinic pancreatic registry. Specimens classified as pancreatic tissue without neoplasia were selected on the basis of the availability of paraffin-embedded pancreatic tissue and lack of pathological evidence of malignant neoplasia.

FISH PROBES

FISH probes used in this study are described in Table 1 and Figure 1. We used fluorescence-labeled DNA probes for genes associated with acinar cell carcinoma (*APC* and *CTNNB1*; see glossary at end of article for expansion of all gene symbols), familial pancreatic cancer (*CDKN2A* and *BRCA2*), tumor progression in solid tumors (*TP53*, *EGFR* and *ERBB2*), and the pathway for 5FU (*TYMS* and *TYMP*). Suitable FISH controls were used with each of these cancer genes. FISH probes for cen 7, *EGFR*, *CDKN2A*, cen 9, *LAMP1*, and *TP53* were purchased from a commercial company (Abbott Molecular, Des Plaines, IL). Probes for *CTNNB1*, *BCL6*, *APC*, *EGR1*, *BRCA2*, *ERBB2*, *TYMS*, *BCL2*, *BID*, and *TYMP* were manufactured by the Mayo Clinic team (J.G.K., E.C.T.) specifically for this investigation.

FISH PROCESS

Paraffin-embedded tissues were cut into 5- μ m sections and placed on positively charged microscope slides and then baked in a 90°C oven for 15 minutes. Slides were deparaf-

finized in xylene for 30 minutes, dehydrated in 100% ethanol for 5 minutes, and then air dried. Slides were placed in 10 mM citric acid at 80°C for 45 minutes, pretreated in 2 $\times$ standard saline citrate (SSC) (300 mmol/L of sodium chloride and 30 mmol/L of sodium citrate) at 37°C for 5 minutes, digested in 0.2% pepsin working solution (pepsin from porcine gastric mucosa lyophilized powder, 2500-3500 U/mg protein [E1%/280] [Sigma-Aldrich, St Louis, MO] in 0.9% sodium chloride solution) at 37°C for 48 minutes, dehydrated with an ethanol series (70%, 85%, and 100%) for 2 minutes each, and then air dried.

Probe mixtures were diluted with LSI (locus-specific indicator)/WCP (whole chromosome paint) hybridization buffer (Abbott Molecular). A total of 5 to 10 μ L of each probe mix was placed on the designated hybridization area and sealed with rubber cement. A ThermoBrite denaturation-hybridization system (Abbott Molecular) set at 80°C was used for codenaturation of probe and target DNA for 5 minutes, before hybridization at 37°C for a minimum of 8 hours. The rubber cement and coverslip were removed and the slides were placed in 0.1% NP-40/2 $\times$ SSC solution at 72°C for 2 minutes. Slides were removed and rinsed in an ambient solution of 0.1% NP-40/2 $\times$ SSC for 1 minute. Next, 10 μ L of 4',6'-diamidino-2-phenylindole dihydrochloride (Abbott Molecular) was applied to each slide.

SCORING FISH SIGNALS

For each specimen, 200 consecutive qualifying interphase nuclei were examined with a fluorescent microscope equipped with filters to view SpectrumOrange, SpectrumGreen, and 4',6'-diamidino-2-phenylindole dihydrochloride. On the basis of prior unpublished studies, we determined that the occurrence of the same abnormal FISH pattern in at least 10% of interphase nuclei is consistent with a neoplastic clone.¹⁹ Any specimen with an abnormal FISH pattern in less than 10% of nuclei may be normal or have a neoplastic clone that is not detectable by these FISH probes. All specimens were randomly analyzed independently in a blinded fashion by at least 2 technologists (K.J.B., S.A.S., D.L.K.). FISH results for different technologists were similar; thus, the mean of their results was used as the final percent of normal and abnormal nuclei for each patient.

INTERPRETING FISH SIGNALS

FISH probes were applied to each specimen in pairs to simultaneously observe a locus for a pancreatic cancer gene in one color (eg, orange) and a control locus in another color (eg, green) (Tables 1 and 2; Figure 1). Each set of FISH probes was hybridized to different loci on the same chromosome. This FISH strategy is referred to as ND-FISH because it permits detection of gains and losses of FISH signals and distinguishes between abnormalities of

TABLE 1. **Eight Sets of Gene Targets and Control Loci for Interphase FISH Studies in Pancreatic Cancer**

Logic for target gene	Target gene	Locus	Control	Locus
Acinar cell carcinoma	<i>CTNNB1</i>	3p22	<i>BCL6</i>	3q27
Acinar cell carcinoma	<i>APC</i>	5q22	<i>EGR1</i>	5q31
Familial pancreatic cancer	<i>CDKN2A</i>	9p21	cen 9	D9Z1
Familial pancreatic cancer	<i>BRCA2</i>	13q13	<i>LAMP1</i>	13q34
Overexpressed in pancreatic cancer	<i>EGFR</i>	7p12	cen 7	D7Z1
Cancer progression	<i>ERBB2</i>	17q12	<i>TP53</i>	17p13
Capecitabine target	<i>TYMS</i>	18p11	<i>BCL2</i>	18q21
Capecitabine target	<i>TYMP</i>	22q13	<i>BID</i>	22q11

See glossary at end of this article for expansion of all gene symbols. FISH = fluorescence in situ hybridization.

chromosome number and structure (Table 2).²⁰ Using ND-FISH with the probes in this study permits detection of copy number changes involving segments of chromosomes 3, 5, 7, 9, 13, 17, 18, and 22 in interphase nuclei. For purposes of this article, the results of each cancer gene for each patient were classified as gain-aneuploidy, loss-aneuploidy, gain-structural, loss-structural, gain-near-tetraploidy, or gain-amplification.

STATISTICAL ANALYSES

Because this investigation involved small sample sizes, no formal statistical analyses were done to test observed variations in results among various forms of pancreatic cancer.

RESULTS

Table 3 summarizes the clinical findings for each of the 18 patients in this investigation. This series of patients included 5 with acinar cell carcinoma, 5 with ductal adenocarcinoma, 5 with islet cell carcinoma, and 3 involv-

ing pancreatic tissue without neoplasia. Of the patients, 11 (61%) were women and 7 (39%) were men. At the time of this study, 13 patients (72%) were alive and 5 (28%) were dead (3 died of acinar cell carcinoma and 2 of ductal adenocarcinoma). For the 5 patients who died, survival ranged from 8.4 months for a patient with ductal adenocarcinoma to 46.6 months for a patient with acinar cell carcinoma. None of the patients had a family history of pancreatic cancer. Eight patients (44%) had a history of smoking. Three patients (17%) were treated with 5FU. Except for patient 17 who received only chemotherapy and radiation, each of the patients had undergone pancreatic surgical resection. Among the 5 patients with islet cell carcinoma, 4 (80%) had nonfunctional disease and 1 (20%) had functional disease that was classified as an insulinoma. Except for patients 2 and 17, all patients were in the early stages of disease.

SPECIMENS

All FISH studies were performed on pancreatic tissue, except for patient 3, for whom we studied a tumor involving

FIGURE 1. Illustration of the chromosomal loci for each of the cancer-control sets of fluorescence-labeled DNA and in situ hybridization probes used in this study. For expansion of gene symbols, see glossary at end of article.

TABLE 2. Interpreting ND-FISH Signal Patterns and Distinguishing Among Chromosomal Abnormalities

Observed FISH signal pattern	Cancer gene result	Chromosomal abnormality	Examples	Study classification
Two cancer genes and control targets	Normal	None	Normal	Normal
Three cancer genes and control targets	Gain	Aneuploidy	Trisomy	Gain-aneuploidy
Four cancer genes and control targets	Gain	Aneuploidy	Tetrasomy	Gain-aneuploidy
Lacks a cancer gene and control target	Loss	Aneuploidy	Monosomy	Loss-aneuploidy
Additional cancer gene and normal complement of control targets	Gain	Structural	Unbalanced translocation	Gain-structural
Lack of cancer gene and normal complement of control targets	Loss	Structural	Deletion, unbalanced translocation	Loss-structural
Additional copies of all cancer genes and control targets	Gain	Cell fusion	Approximately 92 chromosomes	Near-tetraploidy
Six or more cancer gene signals and normal complement of control targets	Gain	Gene amplification	Amplification	Gain-amplification

FISH = fluorescence in situ hybridization; ND-FISH = interphase FISH to detect abnormalities of chromosome number and structure.

the spleen. In addition to pancreatic tissue, a lymph node specimen involving neoplastic cells was analyzed from patient 17. Each specimen in this investigation was a paraffin-embedded tissue block except for specimen 17, which was a paraffin-embedded needle biopsy specimen. For patient 17, FISH studies on the pancreas were unsuccessful, but the FISH process worked satisfactorily for tumor biopsies of a lymph node involving neoplastic cells collected near the pancreas.

OVERALL FISH RESULTS

FISH results for each patient are shown in Table 4. FISH was successful for 6 or more of the 8 chromosomes studied for each patient. FISH was unsuccessful for *CDKN2A* and cen 9 in 4 patients (22%) and for *APC* and *EGR1* in 2 patients (11%). Among the successfully studied patients, the most frequent FISH abnormality detected involved chromosome 3, followed by chromosomes 17, 18, 5, 9, 22, 7, and 13.

TABLE 3. Clinical Findings for 18 Patients With Pancreatic Cancer or Pancreatic Tissue Without Neoplasia^a

Diagnosis	Patient No./age at diagnosis (y)/sex	Follow-up (mo)	Ever a smoker?	Family history of pancreatic cancer?	Status	Survival (mo)	5FU (mo)	Treatment	Histopathologic stage and comments
Acinar cell carcinoma of pancreas	1/61.5/F	1.7	No	No	Alive	5.9	1.2	Resection	IB
	2/28.1/F	19.4	Yes	No	Dead	46.6		Resection	IV
	3/82.8/M	21.7	No	No	Dead	26.5		Resection	IIA
	17/62.8/M	2.4	No	No	Alive	36.0	>24	Chemotherapy	III
	18/47.0/F	1.1	No	No	Dead	13.9		Resection	IIA
Ductal adenocarcinoma of pancreas	5/80.0/F	0.9	Yes	No	Alive	9.9		Resection	IIB
	7/43.8/F	1.6	Yes	No	Dead	8.4		Resection	IIB
	8/79.4/F	4.0	No	No	Alive	12.9	1.5	Resection	IIA
	12/72.3/M	2.0	No	No	Dead	15.5		Resection	IIB
	15/48.1/M	1.1	Yes	No	Alive	3.6		Resection	IIB
Islet cell carcinoma of pancreas	6/68.7/F	1.9	No	No	Alive	21.7		Resection	IIB, nonfunctional, metastasis to lymph node
	10/51.6/M	2.0	Yes	Unknown	Alive	15.3		Resection	IIB, nonfunctional, metastasis to lymph node, MEN1
	11/54.7/F	2.1	Yes	No	Alive	16.3		Resection	IB, nonfunctional
	13/61.9/M	2.0	Yes	No	Alive	1.5		Resection	IIA, nonfunctional, highly invasive
	14/78.2/M	1.3	Yes	No	Alive	5.5		Resection	IA, insulinoma
Pancreatic tissue without evidence of neoplasia	4/50.3/F	8.9	No	No	Alive	107.9		Resection ^b	IB, nonfunctional, metastasis to distant sites
	9/55.4/F	1.0	No	No	Alive	0.9		Resection ^c	
	16/49.7/F	1.1	No	No	Alive	12.8		Resection ^c	

^a 5FU = 5-fluorouracil; MEN1 = multiple endocrine neoplasia type 1.

^b Islet cell carcinoma: pathology findings for the tissue used in this investigation were normal.

^c Nesidioblastosis with islet cell hypertrophy without evidence of neoplasia on pathology.

TABLE 4. Results of Interphase FISH Studies for 8 Sets of Gene Targets and Control Probes on Chromosomes 3, 5, 7, 9, 13, 17, 18, and 22^a

	Acinar cell carcinoma (patient No./specimen)					Ductal adenocarcinoma (patient No./specimen)					Islet cell carcinoma (patient No./specimen)					Pancreatic tissue without neoplasia (patient No./specimen)		
	1/P	2/P	3/S	17/LN	18/P	5/P	7/P ^b	8/P	12/P	15/P	6/P	10/P	11/P	13/P	14/P ^b	4/P	9/P	16/P
Genes possibly linked with acinar cell carcinoma^c																		
<i>CTNNB1</i>																		
Sig	3-5	1	3-4	3-6	3-4	N	3-5	1	N	N	N	N	N	N	4	N	N	N
%	74	70	33	20	90	N	75	40	N	N	N	N	N	N	75	N	N	N
<i>BCL6</i>																		
Sig	3-5	N	3-4	3-9	3-4	N	3-5	N	N	N	N	N	N	N	4	N	N	N
%	74	N	33	86	90	N	75	N	N	N	N	N	N	N	75	N	N	N
<i>APC</i>																		
Sig	N	1	US	N	3-4	US	N	N	N	N	N	N	N	1	3-4	N	N	N
%	N	54	US	N	91	US	N	N	N	N	N	N	N	58	75	N	N	N
<i>EGR1</i>																		
Sig	3-8	N	US	N	3-4	US	N	3	N	N	N	N	N	N	3-4	N	N	N
%	87	N	US	N	91	US	N	21	N	N	N	N	N	N	75	N	N	N
Possible familial genes^c																		
<i>CDKN2A</i>																		
Sig	1	N	US	3-4	N	1	US	1	N	US	N	N	N	1	US	N	N	N
%	74	N	US	83	N	21	US	54	N	US	N	N	N	71	US	N	N	N
cen 9																		
Sig	N	N	US	3-4	3-4	N	US	N	N	US	N	N	N	1	US	N	N	N
%	N	N	US	83	84	N	US	N	N	US	N	N	N	71	US	N	N	N
<i>BRCA2</i>																		
Sig	1	N	1	N	N	N	3-4	N	N	N	N	N	N	N	3	N	N	N
%	85	N	53	N	N	N	50	N	N	N	N	N	N	N	85	N	N	N
<i>LAMP1</i>																		
Sig	1	N	1	N	N	N	3-4	N	N	N	N	N	N	N	3	N	N	N
%	85	N	53	N	N	N	50	N	N	N	N	N	N	N	85	N	N	N
Tumor progression in solid tumors^c																		
<i>EGFR</i>																		
Sig	N	N	AMP	3-4	N	N	3-4	N	N	N	N	N	N	N	3-4	N	N	N
%	N	N	78	74	N	N	61	N	N	N	N	N	N	N	79	N	N	N
cen 7																		
Sig	N	N	AMP	3-4	N	N	3-4	N	N	N	N	N	N	N	3-4	N	N	N
%	N	N	78	74	N	N	61	N	N	N	N	N	N	N	79	N	N	N
<i>TP53</i>																		
Sig	N	N	1	3	N	1	3-4	1	N	1	N	N	N	N	3	N	N	N
%	N	N	59	14	N	42	78	74	N	61	N	N	N	N	90	N	N	N
<i>ERBB2</i>																		
Sig	N	N	1	N	N	N	3-4	N	N	N	N	N	N	N	3	N	N	N
%	N	N	59	N	N	N	78	N	N	N	N	N	N	N	90	N	N	N
Genes in 5FU pathway^c																		
<i>TYMS</i>																		
Sig	N	1	1	N	N	N	3-4	N	2-3	N	N	N	N	N	3	N	N	N
%	N	72	56	N	N	N	44	N	44	N	N	N	N	N	73	N	N	N
<i>BCL2</i>																		
Sig	1	1	1	N	N	N	N	N	1	1	N	N	N	N	3	N	N	N
%	77	72	56	N	N	N	N	N	44	37	N	N	N	N	73	N	N	N
<i>TYMP</i>																		
Sig	1	N	1	N	N	N	3-4	N	N	1	N	N	N	N	3	N	N	N
%	78	N	75	N	N	N	75	N	N	68	N	N	N	N	78	N	N	N
<i>BID</i>																		
Sig	1	N	N	N	N	N	3-4	N	N	2-4	N	N	N	N	3	N	N	N
%	78	N	N	N	N	N	75	N	N	68	N	N	N	N	78	N	N	N

^a See glossary at the end of this article for expansion of all gene symbols. AMP = more than 6 fluorescence in situ hybridization [FISH] signals relative to the normal control site; 5FU = 5-fluorouracil; LN = lymph node; N = normal (2 FISH signals); P = pancreas; S = spleen; Sig = FISH signals; US = unsuccessful.

^b FISH results for patients 7 and 14 suggest these tumors are near-tetraploid as they each have 3 more signals for nearly all chromosomal loci studied. Thus, these FISH results are different from those of other patients in this series.

^c The normal number of FISH signals in any interphase nucleus is 2. The observation of only 1 FISH signal suggests loss of a chromosomal locus. The observation of 3 or more signals suggests gain of a chromosomal locus.

TABLE 5. Gains and Losses of FISH Signals for Each Cancer Locus and Its Associated Cytogenetic Abnormality^a

Patient No.	<i>CTNNB1</i>	<i>APC</i>	<i>CDKN2A</i>	<i>BRCA2</i>	<i>EGFR</i>	<i>TP53</i>	<i>ERBB2</i>	<i>TYMS</i>	<i>TYMP</i>
Acinar cell carcinoma of pancreas									
1	Gain-aneuploidy	Normal	Loss-structural	Loss-aneuploidy	Normal	Normal	Normal	Normal ^b	Loss-aneuploidy
2	Loss-structural	Loss-structural	Normal	Normal	Normal	Normal	Normal	Loss-aneuploidy	Normal
3	Gain-aneuploidy	US	US	Loss-aneuploidy	Gain-AMP	Loss-aneuploidy	Loss-aneuploidy	Loss-aneuploidy	Loss-structural
17	Gain-aneuploidy	Normal	Gain-aneuploidy	Normal	Gain-aneuploidy	Gain-structural	Normal	Normal	Normal
18	Gain-aneuploidy	Gain-aneuploidy	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ductal adenocarcinoma of pancreas									
5	Normal	US	Loss-structural	Normal	Normal	Loss-structural	Normal	Normal	Normal
7	Gain ~4n	Normal	US	Gain ~4n	Gain ~4n	Gain ~4n	Gain ~4n	Gain ~4n ^b	Gain ~4n
8	Loss-structural	Normal	Loss-structural	Normal	Normal	Loss-structural	Normal	Normal	Normal
12	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal ^b	Normal
15	Normal	Normal	US	Normal	Normal	Loss-structural	Normal	Normal ^b	Loss-structural
Islet cell carcinoma of pancreas									
6	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Normal	Loss-structural	Loss-aneuploidy	Normal	Normal	Normal	Normal	Normal	Normal
14	Gain ~4n	Gain ~4n	US	Gain ~4n	Gain ~4n	Gain ~4n	Gain ~4n	Gain ~4n	Gain ~4n
Normal pancreas tissue									
4	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

^a See glossary at end of this article for expansion of all gene symbols. ~4n = near-tetraploidy; AMP = more than 6 fluorescence in situ hybridization (FISH) signals relative to the normal control site; Gain = gain of FISH signal; Loss = loss of FISH signal; US = unsuccessful.

^b Patients 1, 7, 12, and 15 each had loss of the control locus *BCL2*.

Each of the 5 patients with acinar cell carcinoma had 3 to 6 FISH abnormalities in 14% to 91% of nuclei. Each of the 5 patients with ductal adenocarcinoma had 1 to 6 FISH abnormalities in 21% to 78% of nuclei. Of the 5 patients with islet cell carcinoma, 2 (40%) had 2 to 7 FISH abnormalities in 58% to 90% of nuclei; the remaining 3 patients with this condition (60%) had normal FISH results. FISH results were normal for specimens of pancreatic tissue without neoplasia.

FISH RESULTS AND CHROMOSOMAL ABNORMALITIES

For each patient and each cancer gene, the results of gains and losses of FISH signals as well as their associated chromosomal abnormalities are summarized in Table 5.

Near-Tetraploidy. Patients 7 and 14 had 3 to 4 FISH signals for nearly all FISH probes, suggesting that these tumors had gains of cancer genes due to near-tetraploidy.

***CTNNB1*.** Gains of *CTNNB1* due to chromosome 3 aneuploidy were seen only in patients 1, 3, 17, and 18 with acinar cell carcinoma. Losses of *CTNNB1* due to chromosome 3 structural abnormalities were seen in patient 2 with acinar cell carcinoma and patient 8 with ductal adenocarcinoma.

***APC*.** Gain of *APC* due to aneuploidy was seen only in patient 18 with acinar cell carcinoma. Losses of *APC* due to chromosome 5 structural abnormalities were seen in patient 2 with acinar cell carcinoma and patient 13 with islet cell carcinoma.

***CDKN2A*.** Gain of *CDKN2A* due to chromosome 9 aneuploidy was seen in patient 17 with acinar cell carcinoma. Losses of *CDKN2A* due to chromosome 9 structural abnormalities were seen in patient 1 with acinar cell carcinoma and in patients 5 and 8 with ductal adenocarcinoma. Loss of *CDKN2A* due to chromosome 9 aneuploidy was seen in patient 13 with islet cell carcinoma.

FIGURE 2. Representative microphotographic images ($\times 1000$) of normal and abnormal cell nuclei from paraffin-embedded pancreatic cancer specimens processed by fluorescence in situ hybridization (FISH). A, Patient 9 with pancreatitis showing normal FISH results for *BRCA2* (orange) and *LAMP1* (green). B, Patient 14 with islet cell carcinoma showing 3 FISH signals for *APC* (green) and *EGR1* (orange) as part of a near-tetraploid condition. C, Patient 3 with acinar cell carcinoma showing amplification (≥ 15 target signals) of *EGFR* (orange) and cen 7 (green). D, Patient 5 with ductal cell adenocarcinoma showing 1 *TP53* (orange) and 2 *ERBB2* (green) signals, suggesting a deletion or unbalanced translocation.

BRCA2. No patients had gain of *BRCA2*. Losses of *BRCA2* due to chromosome 13 aneuploidy were seen in patients 1 and 3 with acinar cell carcinoma.

EGFR. Gains of *EGFR* due to chromosome 7 aneuploidy were seen only in patient 17 with adenocarcinoma. Gains of *EGFR* due to amplification on chromosome 7 involving more than 15 FISH signals were seen in patient 3 with acinar cell carcinoma. Losses of *EGFR* were not observed.

TP53. Gains of *TP53* due to a chromosome 17 structural abnormality were seen in patient 17 with acinar cell carcinoma. Losses of *TP53* due to chromosome 17 aneuploidy were seen in patient 3 with acinar cell carcinoma. Losses of *TP53* due to chromosome 17 structural abnormalities were seen in patients 5, 8, and 15 with ductal adenocarcinoma.

ERBB2. Gains of *ERBB2* were not observed. Losses of *ERBB2* due to chromosome 17 aneuploidy were seen only in patient 3 with acinar cell carcinoma.

TYMS and BCL2. Gains of *TYMS* were not observed. Losses of *TYMS* due to chromosome 18 aneuploidy were seen in patients 2 and 3 with acinar cell carcinoma. It is interesting to note that patients 1, 7, 12, and 15 each had loss of the *TYMS* cancer gene control locus *BCL2* due to chromosome 18 structural abnormalities.

TYMP. Gains of *TYMP* were not observed. Loss of *TYMP* was seen in 2 patients with acinar cell carcinoma: patient 1 had loss of *TYMP* due to aneuploidy and patient 3 had loss of *TYMP* due to a structural abnormality. Loss of *TYMP* due to a chromosome 22 structural abnormality was also seen in patient 15 with ductal adenocarcinoma.

DISCUSSION

Interphase FISH studies were successful in 276 (96%) of the 288 probes attempted in this investigation. We attribute this success to recent improvements in processing paraffin-embedded tissues for FISH studies.^{21,22} Interphase FISH detected the neoplastic clone in 12 (80%) of 15 patients. Thus, we clearly studied gene loci involved in the biology of pancreatic cancer. Pancreatic specimens from the 3 patients without neoplasia were normal (ie, no false-positive instances). Interphase FISH permitted visualization of specific gene loci in individual nondividing cell nuclei (Figure 2). In addition, this method was useful for distinguishing among abnormalities of chromosomal loci resulting from monosomy, trisomy, polyploidy, amplification, deletion, and translocation (Table 5). Moreover, this method was

useful for estimating the percentage of neoplastic nuclei in any given tissue sample (Table 4).

CTNNB1 AND APC ABNORMALITIES

We selected *CTNNB1* and *APC* for this investigation because these genes have been associated with oncogenesis in acinar cell carcinoma and pancreatoblastomas by somatic mutation analysis.¹⁶ The oncogene *CTNNB1* produces a β -catenin protein that is important in the cadherin-mediated cell adhesion system and acts as a downstream activator in the *Wnt* (wingless type) signaling pathway.²³ The tumor suppressor gene *APC* promotes phosphorylation of *CTNNB1* and has been associated with germ line mutations in familial adenomatous polyposis.¹⁷

The *CTNNB1* locus and its control *BCL6* locus are at 3p22 and 3q27, respectively. In the current study, each of the 5 acinar cell carcinoma specimens had FISH abnormalities of *CTNNB1*, 4 (80%) of which involved gain of *CTNNB1* due to trisomy 3. The only patients with other forms of pancreatic cancer in this study who had gains of *CTNNB1* were patient 7 with ductal adenocarcinoma and patient 14 with islet cell carcinoma; the overall FISH pattern for each of these 2 tumors was consistent with no net gain of chromosome 3 in the setting of near-tetraploidy. The observations in this study, together with other published investigations based on different genetic technologies,¹⁷ support the hypothesis that acinar cell carcinoma is genetically different from ductal adenocarcinoma and islet cell carcinoma. Moreover, these studies suggest that the gene for β -catenin may be specifically involved in the biology of acinar cell carcinoma.

One patient with acinar cell carcinoma and another patient with ductal adenocarcinoma had loss of a *CTNNB1* locus due to a chromosome 3 structural abnormality. Apparently, loss of *CTNNB1* is not unique to acinar cell carcinoma.

The *APC* locus and its control *EGR1* locus are at 5q22 and 5q31, respectively. Abnormalities of the *APC* locus were seen in 2 patients with acinar cell carcinoma and 2 with islet cell carcinoma. FISH abnormalities were due to near-tetraploidy in 1 patient, chromosome 5 structural abnormalities in 2 patients, and chromosome 5 aneuploidy in 1 patient. Thus, our observations for *APC* were not unique to acinar cell carcinoma or to a given kind of chromosomal abnormality.

ABNORMALITIES OF FAMILIAL CANCER GENES

We selected *CDKN2A* and *BRCA2* for this investigation because these genes have been implicated in familial pancreatic cancer.²⁴ None of the patients in our study had a family history of pancreatic cancer, suggesting that they had sporadic forms of this disease. We realize that interphase FISH is not designed to detect point mutations of

CDKN2A and *BRCA2*, which are associated with germ line inheritance in familial disorders.²⁴ Nevertheless, interphase FISH can detect chromosomal abnormalities that might be associated with these genes in pancreatic cancer tumors.

The loci for *CDKN2A* and its control cen 9 are at 9p21 and the centromere of chromosome 9, respectively. We observed abnormalities of *CDKN2A* in 5 (45%) of 11 successfully studied specimens from various forms of pancreatic cancer. Loss of a *CDKN2A* locus due to monosomy 9 occurred in 1 patient with islet cell carcinoma. One patient with acinar cell carcinoma and 2 patients with ductal adenocarcinoma had loss of a *CDKN2A* locus due to a chromosome 9 structural abnormality. The only patient in this series with gain of *CDKN2A* due to aneuploidy had acinar cell carcinoma; this observation was consistent with tetrasomy 9. Thus, FISH abnormalities of *CDKN2A* were common in this series but were not unique to any specific form of pancreatic cancer.

The *BRCA2* locus and its control *LAMP1* locus are at 13q13 and 13q34, respectively. We observed FISH abnormalities of *BRCA2* in 4 patients. Loss of a *BRCA2* locus due to monosomy 13 was observed in 2 patients with acinar cell carcinoma. In contrast, only 1 patient with ductal adenocarcinoma and 1 patient with islet cell carcinoma had gains of *BRCA2*, but in each of these patients the FISH abnormalities were near-tetraploid. Because sample size in this investigation was small, further research is needed to establish the degree of possible association between loss of *BRCA2* and acinar cell carcinoma.

ABNORMALITIES OF GENES ASSOCIATED WITH TUMOR PROGRESSION

We selected *EGFR*, *TP53*, and *ERBB2* for this study because these genes are frequently overexpressed in the progression of pancreatic cancer tumors.²⁵ The *EGFR* locus and its control cen 7 locus are at 7p12 and the chromosome 7 centromere, respectively. Abnormalities in *EGFR* were observed in 4 patients: 2 with acinar cell carcinoma, 1 with ductal adenocarcinoma, and 1 with islet cell carcinoma. Gain of *EGFR* was due to near-tetraploidy in 2 patients, chromosome 7 trisomy or tetrasomy in 1, and amplification in 1. Patient 3 with acinar cell carcinoma had amplification of both *EGFR* and cen 7; this was the only patient who had evidence of gene amplification in our series. However, amplification of *EGFR* has also been reported in ductal adenocarcinoma by others,²⁶ indicating that our observation is not unique to acinar cell carcinoma.

The *TP53* and *ERBB2* loci are both on chromosome 17 at 17p13 and 17q12, respectively. Thus, we used the *TP53* signal as a control for *ERBB2* and the *ERBB2* signal as a control for *TP53*. Abnormalities of *TP53*, *ERBB2*, or both of these gene loci were observed in 2 patients with aci-

nar cell carcinoma, 4 with ductal adenocarcinoma, and 1 with islet cell carcinoma. One patient had loss of *TP53* and *ERBB2* due to monosomy 17, 3 patients had gain of *TP53* due to structural abnormalities, and 2 patients had gains due to near-tetraploidy.

The results for *EGFR*, *TP53*, and *ERBB2* indicate that FISH abnormalities in sporadic pancreatic cancer are due to different cytogenetic abnormalities and are not unique to any specific type of pancreatic cancer.

GENETIC ABNORMALITIES OF THE THYMIDINE SYNTHESIS PATHWAY

We developed FISH probes for *TYMS* and *TYMP* for this investigation because these genes are involved in the molecular pathway of thymidine synthesis and these loci are common targets for drugs, such as 5FU, used to treat pancreatic cancer.²⁷ Among the 15 pancreatic cancer specimens in this study, 7 (47%) had abnormalities of *TYMS*, *TYMP*, or both of these genes. This result suggests that *TYMS* and *TYMP* may well be involved in the biology of pancreatic cancer.

The *TYMS* locus and its control *BCL2* locus are at 18p11 and 18q21, respectively. Two patients with acinar cell carcinoma had loss of a *TYMS* locus due to monosomy 18. In addition, 2 patients, 1 with ductal adenocarcinoma and 1 with islet cell carcinoma, had gains due to near-tetraploidy.

It is interesting to note that abnormalities of the control *BCL2* locus occurred in 4 patients (22%) in this study. Patient 7 with ductal adenocarcinoma was near-tetraploid but had loss of *BCL2* due to chromosome 18 structural abnormalities. Patient 1 with acinar cell carcinoma and patients 12 and 15 with ductal adenocarcinoma also each had loss of *BCL2* due to chromosome 18 structural abnormalities. The well-known oncogene *BCL2* is involved in other malignant disorders, especially lymphoma.²⁸ Thus, further studies of *BCL2* may be warranted to evaluate its role in pancreatic cancer.

The *TYMP* locus and its control *BID* locus are at 22q13 and 22q11, respectively. One patient with acinar cell carcinoma had loss of *TYMP* due to monosomy 22. One patient with acinar cell carcinoma and 1 patient with ductal adenocarcinoma had loss of *TYMP* due to chromosome 22 structural abnormalities. One patient with ductal adenocarcinoma and 1 patient with islet cell carcinoma had multiple copies of *TYMP* due to near-tetraploidy.

FISH abnormalities of *TYMP* and *TYMS* were observed among patients with different forms of pancreatic cancer in this study. In addition, these FISH abnormalities were due to various kinds of chromosomal abnormalities.

INTERPHASE FISH AND CHEMOTHERAPY WITH 5FU

We are aware of published cases²⁷ and know of anecdotal instances of successful treatment with 5FU for patients

with acinar cell carcinoma. An antimetabolite, 5FU inhibits synthesis of thymine nucleotides through inhibition of thymidylate synthetase and thymidine phosphorylase function.²⁹ Capecitabine is an oral form of 5FU. Both of these drugs have been used with and without modulating agents such as leucovorin, methotrexate, and oxaliplatin with uncertain efficacy in pancreatic cancer. Thus, we used FISH probes for *TYMS* and *TYMP* loci in this investigation to evaluate the potential value of interphase FISH in clinical practice to identify patients who might benefit from treatment with 5FU.

Of the patients in this series, 3 (17%) had received treatment with 5FU, capecitabine, or both of these drugs (Table 3). Patient 1 with acinar cell carcinoma and patient 8 with ductal adenocarcinoma received 5FU as adjuvant treatment. We are unable to evaluate the effectiveness of 5FU treatment because all these patients had undergone surgery for their pancreatic cancer.

Only patient 17 in this series received substantial treatment with 5FU products. This patient, who has acinar cell carcinoma, received both capecitabine and 5FU treatment and achieved a partial remission (Table 3). On initial treatment with gemcitabine and erlotinib for 2 cycles, the patient's disease progressed. After treatment with 2 cycles of capecitabine and thalidomide, the tumor was reduced. This patient was then treated with capecitabine and radiation, and the size of the tumor was further reduced. The tumor did not change in size for several months but then began to grow again. Thus, this patient began treatment with FOLFOX (folinic acid, 5FU, and oxaliplatin); tumor size was again reduced after 1 cycle. The reduced tumor size remained stable over 16 cycles of FOLFOX treatment before the patient began to show evidence of resistance to treatment. Consequently, alternative treatments for this patient are being considered. This patient has survived 3 years from diagnosis. The FISH results for *TYMS* and *TYMP* were normal for this patient, perhaps explaining in part why the patient responded to 5FU and capecitabine. The accuracy of this hypothesis would need to be tested in a prospective study enrolling numerous patients with acinar cell carcinoma who have been treated with 5FU.

CONCLUSION

Interphase FISH with probes for the 9 cancer genes used in this study can detect neoplastic clones in paraffin-embedded tissue specimens of acinar cell carcinoma, ductal adenocarcinoma, and islet cell carcinoma. Interphase ND-FISH can help discriminate among different kinds of numeric and structural abnormalities of chromosomes in pancreatic cancer. Gains of *CTNNB1* due to chromosome 3 aneuploidy were uniquely observed in acinar cell carcinoma.

noma. Abnormalities of *APC*, *CDKN2A*, *BRCA2*, *EGFR*, *TP53*, *ERBB2*, *TYMP*, and *TYMS* were common among patients in this study but were not associated with any specific form of pancreatic cancer.

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Glossary of Genetics Terminology

APC = adenomatosis polyposis coli
BCL2 = B-cell CLL/lymphoma 2
BCL6 = B-cell CLL/lymphoma 6
BID = BH3 interacting domain death agonist
BRCA2 = breast cancer 2, early onset
CDKN2A = cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
 cen 7 = α -satellite DNA for the centromeric region of chromosome 7
 cen 9 = α -satellite DNA for the centromeric region of chromosome 9
CTNNB1 = catenin (cadherin-associated protein), β -1, 88kDa
EGFR = epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
EGR1 = early growth response 1
ERBB2 = v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
LAMP1 = lysosomal-associated membrane protein 1
TP53 = tumor protein p53
TYMP = thymidine phosphorylase
TYMS = thymidylate synthetase

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