0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 11
Printed in U.S.A.

Overexpression of Cyclin D1 Occurs Frequently in Human Pancreatic Endocrine Tumors*

DANIEL C. CHUNG, SUZANNE B. BROWN, FIONA GRAEME-COOK, MASAO SETO, ANDREW L. WARSHAW, ROBERT T. JENSEN, AND ANDREW ARNOLD

Gastrointestinal Unit and Department of Medicine (D.C.C.), Department of Pathology (F.G.-C.), Department of Surgery (A.L.W.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114; Center for Molecular Medicine (S.B.B., A.A.), University of Connecticut Health Center, Farmington, Connecticut 06030; Laboratory of Chemotherapy (M.S.), Aichi Cancer Center, Nagoya, Japan; and Digestive Diseases Branch (R.T.J.), National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT

The molecular pathogenesis of human pancreatic endocrine tumors (PETs) is poorly understood. Three independent animal models have pointed to the pivotal role of the G_1/S cell cycle transition in pancreatic endocrine cell proliferation. We thus hypothesized that the cell cycle regulator cyclin D1 may contribute to the pathogenesis of human PETs. Overexpression of cyclin D1 was identified in 43% of cases, and no correlation was observed with clinical phenotype. The novel observation of frequent overexpression of cyclin D1 suggests that this established oncogene may be implicated in the pathogenesis of human PETs. The absence of detectable alterations in cyclin D1 genomic structure suggests that the mechanism for its oncogenic activation in PETs may be transcriptional or posttranscriptional. (*J Clin Endocrinol Metab* **85:** 4373–4378, 2000)

PROGRESSION THROUGH the G_1 checkpoint of the mammalian cell cycle is regulated by the activity of D-type cyclins and their partners, the cyclin-dependent kinases (CDKs) -4 and -6. Of the three D-type cyclins, cyclin D1/PRAD1 has been convincingly implicated as an oncogene in human tumorigenesis. Overexpression of cyclin D1 is observed in a wide variety of malignancies, including parathyroid tumors (1), breast cancer (2), esophageal cancer (3), and centrocytic (or mantle cell) lymphomas (4). Tumor-specific alterations in cyclin D1 genomic structure, specifically gene amplification or rearrangement, have been identified as important mechanisms of clonal oncogene activation (2, 5). Functional evidence implicating cyclin D1 as a human oncogene includes the demonstration that overexpression of cyclin D1 in mammary tissue of transgenic mice is tumorigenic (6), and that expression of a cyclin D1 antisense construct *in vitro* reduces the tumorigenicity of colon and pancreatic cancer cells (7, 8).

The cyclin D1 protein forms a complex with CDK-4 or -6, and this complex phosphorylates and thereby inactivates the *RB* tumor suppressor gene product. The activity of the cyclin D1/CDK complex is regulated by the CDK inhibitor *INK4A*^{p16}. This pathway linking p16, cyclin D1, and RB is a critical regulator of cellular growth, and alterations in this pathway are identified in a majority of human neoplasms (9, 10). Furthermore, mutations among these various components often seem to be mutually exclusive, implying that a single alteration in one of the components is sufficient to disrupt the entire pathway (11, 12).

Pancreatic endocrine tumors (PETs) are a distinctive group of malignancies characterized by dramatic clinical syndromes attributable to their overproduction of biologically active pancreatic hormones. The recent identification of a novel PET suppressor gene locus on chromosome 3p25 (13) and also the multiple endocrine neoplasia type-1 (*MEN1*) gene on chromosome 11q13 (14) has enhanced our limited understanding of the molecular pathogenesis of these tumors. Several murine models of pancreatic endocrine tumorigenesis have provided additional clues. Inactivation of p53 and RB, either through overexpression of SV40 large T-antigen in pancreatic islet cells (15, 16) or by double homozygous deletions (17, 18), leads to islet tumorigenesis. Furthermore, germline expression of the R24C CDK-4 mutant that cannot bind the p16 CDK inhibitor results in marked proliferation only of pancreatic islet cells (19). These three independent models all suggest that regulators of the G_1/S transition of the cell cycle may play a pivotal role in tumorigenesis of pancreatic endocrine cells. However, mutations of p53 and Rb do not play an important pathogenic role in human PETs (20, 21). Cyclin D1, another key regulator of the G_1/S checkpoint, thus emerged as the most attractive candidate for involvement. Furthermore, cyclin D1 is implicated in the pathogenesis of parathyroid adenomas, another well-differentiated endocrine neoplasm (1), adding to the rationale that it may also play a key role in PETs. We thus sought to determine the role of the cyclin D1 oncogene in the pathogenesis of human PETs.

Received June 13, 2000. Revision received July 26, 2000. Accepted July 26, 2000.

Address all correspondence and requests for reprints to: Daniel C. Chung, Gastrointestinal Unit, GRJ 825, Massachusetts General Hospital, 55 Fruit Street, Boston, Massachusetts 02114. E-mail: d_chung@helix.mgh.harvard.edu.

* Supported in part by NIH Grant DK-01410-11 and by a Faculty Research Award (to A.A.) from the American Cancer Society.

Materials and Methods

Patients and tumor samples

Sixty-four surgically resected PET samples were collected from 60 patients. All were obtained from 3 tertiary care centers: Massachusetts General Hospital (58 samples), National Institutes of Health (5 samples), and University of North Carolina (1 sample). The specimens were fixed in formalin and embedded in paraffin. The tumors were classified as functional or nonfunctional, on the basis of their clinical presentation and/or serum hormone levels. Histologically, the tumors all displayed the characteristic morphology of neuroendocrine tumors, by hematoxylin and eosin staining. In equivocal cases, further confirmation of the diagnosis was obtained either with electron microscopy; staining for neuroendocrine markers such as neuron-specific enolase, chromogranin, synaptophysin, and leu-7; and/or immunohistochemistry for specific islet hormones (including insulin, gastrin, glucagon, somatostatin, vasoactive intestinal peptide, pancreatic polypeptide, and serotonin). Samples were collected in accordance with regulations of the institutional review board on human studies.

Cyclin D1 immunohistochemistry

Five-micron sections from formalin-fixed, paraffin-embedded tissues were deparaffinized with xylene and rehydrated with graded ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 5 min. Sections were heated in a microwave oven for 20 min in a citrate buffer to unmask antigen. After cooling, the sections were incubated with blocking serum (1:40 dilution of horse serum) for 2 h. The sections were then incubated overnight, at 4 C, in a humidity chamber with a 1:150 dilution of the 5D4 monoclonal antibody (22). A biotinylated horse antimouse antibody (Vector Laboratories, Inc., Burlingame, CA) was applied to the sections, and they were then incubated with the ABC Elite reagent (Vector Laboratories, Inc.). Staining was visualized with 3,3′ diaminobenzidine-hydrogen peroxidase substrate (Research Genetics, Inc., Huntsville, AL). Finally, the slides were counterstained with hematoxylin. Parallel experiments were performed with the absence of primary antibody to confirm specificity of the antibody reactions. The slides were reviewed by an expert pathologist (F. Graeme-Cook), who was blinded to the clinical features of the samples.

Southern blotting

Cyclin D1 genomic structure was analyzed with standard Southern blotting techniques, as previously described (23). Genomic DNA (7.5 μg) was digested with one or more of the following enzymes: *Bam*HI, *Eco*RI, *Xho*I, and *Hin*dIII (all New England Biolabs, Inc., Beverly, MA). The digests of genomic DNA were probed with a ^{32}P random-prime-labeled (Amersham Pharmacia Biotech, Piscataway, NJ) 1.4-kilobase (kb) cyclin D1/PRAD1 complementary DNA (cDNA) fragment (24). After visualization with autoradiography, the blots were stripped and probed with one or more of the following DNA fragments; 1.6 kb human insulin gene (American Type Culture Collection, Manassas, VA), human gastrin-5′ [656 bp PCR-generated fragment from −600 bp to exon 1 (25)], human gastrin-3′ (800 bp *Hin*dIII-*Hin*dIII fragment encoding exons 2 and 3), pancreatic polypeptide-5′ (500 bp *Bam*HI-*Eco*RI fragment located 4 kb upstream of the TATA box), and pancreatic polypeptide-3′ (800 bp *Sma*I-*Hin*dIII fragment encompassing exons 2–4). Pancreatic polypeptide plasmids were generously provided by Dr. A. Leiter (New England Medical Center, Boston, MA). Densitometry was performed using the UVP imager (UVP, Inc., Upland, CA) and NIH Image software package.

Northern blotting

Total RNA was isolated using Trizol reagent (Life Technologies, Gaithersburg, MD) from selected cases in which adequate frozen tissue was available. Fifteen micrograms of RNA were separated on a 1% agarose gel and then transferred to a Nytran nylon membrane (Schleicher & Scheull, Inc., Keene, NH) via capillary action. The membrane was then hybridized with a ^{32}P-labeled random-primed 1.4-kb cyclin D1 cDNA probe. The same blot was rehybridized with a control actin cDNA probe (CLONTECH Laboratories, Inc., Palo Alto, CA). Densitometry was performed as described above.

Statistical analysis

Correlations between immunohistochemical staining for cyclin D1 and clinical features were analyzed using the χ^2-test and Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Tumor samples

Sixty-four tumor samples were collected from 60 patients. Among the 64 samples, there were 22 insulinomas, 14 gastrinomas, 3 parathyroid hormone-related peptide (PTHrP)-secreting tumors, 2 ACTH-secreting tumors, 1 glucagonoma, 1 carcinoid, 1 VIPoma, 1 somatostatinoma, 18 nonfunctional tumors, and 1 endocrine tumor with indeterminate hormonal function. Four patients had multiple tumors available for analysis. In 1 patient with a primary insulinoma, nonfunctional hepatic metastases were resected 9 yr later. One patient with a primary ACTH-producing tumor had a recurrence that was resected 18 months later. One patient with a nonfunctional tumor had a PTHrP- producing retroperitoneal recurrence resected 8 yr later. Finally, a patient with a primary insulinoma had a recurrent insulinoma resected 3 yr later. Disease stage in 28 patients was advanced, defined by extrapancreatic spread to surrounding soft tissue, lymph nodes, or liver; and the remaining 32 patients had disease that was localized (benign).

Among the 60 patients, 25 were male and 35 were female. The mean age at the time of surgical resection among the men was 51.0 ± 17.6 yr (range, 11–83 yr); and among the women, 52.1 ± 14.6 yr (range, 24–80 yr). Four of the patients developed disease in association with the MEN-1 syndrome. Two of these tumors were insulinomas, and 1 was a gastrinoma. The hormonal function of the fourth one was indeterminate, because it represented one of multiple tumors obtained from a patient with hyperinsulinemia and hypergastrinemia; and immunohistochemical studies for various hormones failed to define the tumor's specific hormonal subtype.

Cyclin D1 immunohistochemistry

Overexpression of cyclin D1 protein was determined by immunohistochemical staining with the 5D4 monoclonal antibody to human cyclin D1 (22). As a positive control, strong nuclear staining for cyclin D1 was observed in a breast cancer specimen with a known amplification of the cyclin D1 gene (data not shown) (26). No cyclin D1 staining was seen in normal pancreatic islets (Fig. 1A). However, there was strong nuclear immunoreactivity for cyclin D1 in 43% (26 of 60) of the cases. The prevalence of this strong staining was graded on the following scale: A (<25% positive nuclei), B (25–50% positive nuclei), and C (>50% positive nuclei). Among the 26 positive samples, 13 (21% of total cases) were in category A, 7 (12% of total cases) in category B, and 6 (10% of total cases) in category C. Representative examples are illustrated in Fig. 1, B, C, and D. Among the 4 cases in which multiple tumor samples were examined, the staining pattern was identical in 3 of the cases. In the fourth case, the primary nonfunctional tumor had no staining, but the recurrent PTHrP-secreting tumor was positive (category A).

No correlation was observed between any degree of cyclin D1 staining and clinical parameters, including age, sex, or

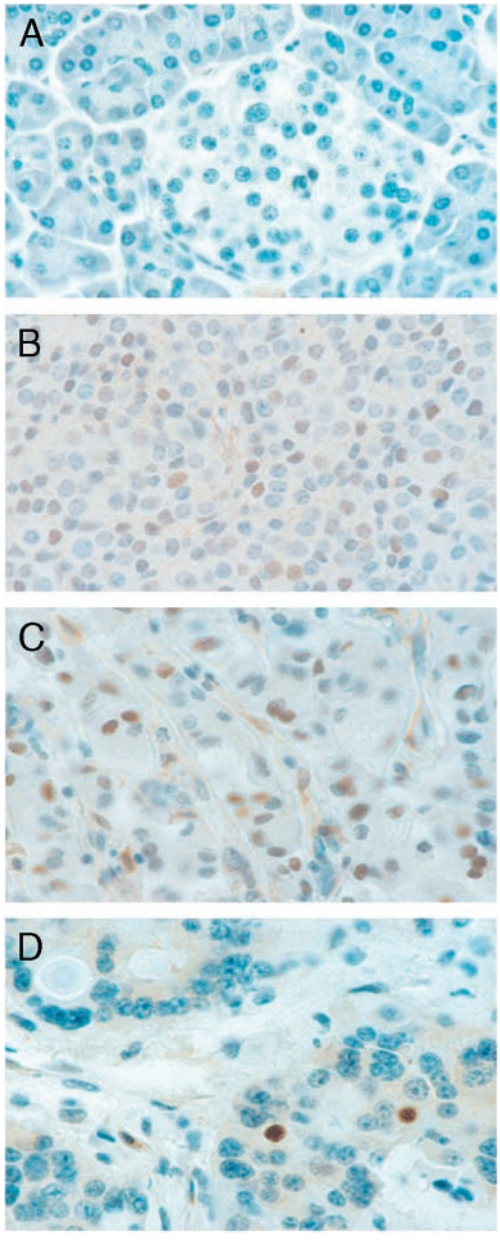

FIG. 1. Immunohistochemical detection of cyclin D1 protein expression in human PETs. A, Normal pancreatic islet, demonstrating absence of nuclear staining for cyclin D1; B, malignant ACTH-producing tumor, demonstrating strong nuclear staining (*dark* signal intensities) for cyclin D1 (this tumor was categorized as group C); C, benign nonfunctional tumor, demonstrating strong nuclear staining for cyclin D1 (group C); D, benign insulinoma with strong nuclear staining in scattered nuclei (group A).

hormonal subtype (Table 1). With regard to tumor stage, a trend was observed between cyclin D1 staining and stage of disease. Sixteen of twenty-eight (57%) patients with advanced disease had tumors with positive cyclin D1 staining, and 10 of 32 (31%) patients with benign disease had tumors that stained positively. However, this correlation was not statistically significant ($P = 0.067$). Two chromosomal loci that are frequently deleted in human PETs are 3p25 and 11q13, as we have previously reported (13, 27). No known tumor suppressor gene lies at 3p25, but both the *MEN1* and cyclin D1 (*CCND1)* genes localize to 11q13. No correlation was observed between loss of heterozygosity (LOH) at either of these loci and cyclin D1 staining. Finally, there was no consistent correlation between clinical MEN-1 status and cyclin D1 staining. Of the four tumors analyzed from MEN-1 patients, one sample (gastrinoma) stained positive for cyclin D1 (category C).

Cyclin D1 gene expression

Levels of cyclin D1 RNA in PETs were determined through Northern blotting. Figure 2 illustrates levels of cyclin D1 gene expression in selected human PETs for which tissue was available. Lane 1 represents RNA obtained from normal pancreas, and cyclin D1 levels are very low. In contrast, elevated cyclin D1 RNA levels are detected in lanes 2, 4, 7, 8, and 9. Densitometric analysis of cyclin D1 RNA normalized to actin RNA revealed the levels of up-regulation, relative to normal pancreas, to be 7.7, 3.2, 3.2, 11.4, and 3.3, respectively. All of these samples are positive for cyclin D1 staining, by immunohistochemistry. The tumors in lanes 5 and 6 stained negatively for cyclin D1, by immunohistochemistry, and cyclin D1 RNA levels are not elevated. Lane 3 is the one sample that was weakly positive, by immunohistochemistry, but failed to demonstrate elevated RNA levels.

Cyclin D1 genomic structure

Chromosomal alterations, including amplification or rearrangement, can account for activation of cyclin D1 in many tumor types. Southern blotting studies were performed on 36 of the tumor samples in which high-molecular-weight genomic DNA was available. Among the 36 cases, there was a representative distribution among all grades of cyclin D1 staining, by immunohistochemistry. Eighteen (50%) were negative, 9 (25%) were in group A, 4 (11%) were in group B, and 5 (14%) were in group C. A representative example of these blots is illustrated in Fig. 3A. The blot was stripped and rehybridized with a control probe for human gastrin (Fig. 3B). Densitometry failed to identify any increase in cyclin D1 gene dosage in these tumors.

Translocations involving the cyclin D1 oncogene can also underlie activation of this gene. Previous studies have described rearrangements that pair cyclin D1 with genes expressed specifically in the tissue type from which the tumor is derived, such as the PTH gene with cyclin D1 in parathyroid tumors (28). We thus sought to determine whether such gene translocations were also present in PETs. The islet-specific genes for the hormones gastrin, insulin, and pancreatic polypeptide were considered as candidate partner genes in such a rearrangement. Among 36 cases, Southern blotting was performed on *Bam*HI digests of tumor DNA using probes for cyclin D1 and also on *Eco*RI, *Xho*I, and *Hin*dIII digests with an insulin, 2 gastrin, and 2 pancreatic polypeptide gene probes, as described. In all the cases examined, there were no tumor-specific gene rearrangements identified.

Discussion

Overexpression of cyclin D1 protein was identified in 43% of human PETs. This novel observation strongly suggests

TABLE 1. Cyclin D1 staining

	Negative	Positive				Total
		A	B	C	A, B, or C	
Stage[a]						
Benign	22	6	2	2	10	32
Advanced	12	7	5	4	16	28
Total	34	13	7	6	26	60
Tumor type[b]						
Insulinoma	14	5	2	1	8	22
Gastrinoma	8	3	2	1	6	14
PTHrPoma	2	1	0	0	1	3
ACTHoma	0	0	0	2	2	2
Carcinoid	0	0	1	0	1	1
VIPoma	0	1	0	0	1	1
Glucagonoma	0	1	0	0	1	1
Somatostatinoma	1	0	0	0	0	1
Nonfunctional	10	3	2	3	8	18
Unknown	1	0	0	0	0	1
Total	36	14	7	7	28	64
MEN1 status[b]						
MEN1 +	3	0	0	1	1	4
Sporadic	33	14	7	6	27	60
Total	36	14	7	7	28	64
11q13 LOH[b]						
LOH +	11	5	0	0	5	16
LOH −	13	5	4	5	14	27
Uninformative	12	4	3	2	9	21
Total	36	14	7	7	28	64

[a] Analyzed by individual case (60 total).
[b] Analyzed by individual tumor (64 total).

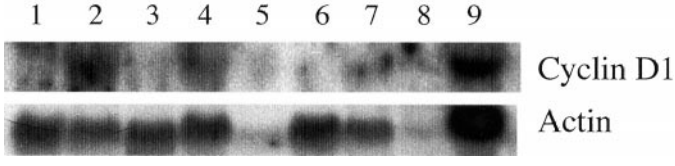

FIG. 2. Northern blot analysis of cyclin D1 mRNA expression in human PET samples. A, Blot hybridized with cyclin D1 cDNA probe. Lane 1 represents normal pancreas. Samples 2, 3, 4, 7, 8, and 9 were positive by cyclin D1 immunohistochemistry, and samples 5 and 6 were negative by immunohistochemistry. B, Blot rehybridized with a control probe for β-actin.

that the cyclin D1 oncogene is involved in the pathogenesis of these tumors. Overexpression of cyclin D1 protein was identified in both benign and advanced lesions, suggesting that it may be activated as an early event in tumor pathogenesis. In addition, there was no correlation with hormonal subtype, implying its broad importance in PETs. These findings add considerably to our limited understanding of the underlying molecular pathogenesis of PETs, which comes from studies demonstrating the involvement of the *MEN1* tumor suppressor gene (14, 29–31.) and also a novel tumor suppressor gene locus on chromosome 3p25 (13). Genome-wide allelotyping studies in human PETs also suggest a role for yet-unidentified tumor suppressor genes on chromosomes 3q, 11p, 16p, and 22q (27).

Previously described mechanisms of cyclin D1 oncogene activation include amplification or rearrangement of the cyclin D1 locus on chromosome 11q13. Southern blotting studies failed to implicate either of these two mechanisms in PETs. Potential alternatives for cyclin D1 gene activation include transcriptional and posttranscriptional mechanisms. Cyclin D1 transcription can be stimulated by growth factors such as insulin-like growth factor-I and transforming growth factor-α (32, 33), as well as by β-catenin acting through TCF-binding sites in the cyclin D1 promoter (34). In addition, regulation of cyclin D1 protein levels can occur on the translational level, through the activity of eukaryotic elongation initiation factor-4E (35). In this series of PETs, elevated levels of cyclin D1 RNA are seen in tumors with positive staining for cyclin D1 protein, suggesting that transcriptional up-regulation of the cyclin D1 gene or posttranscriptional stabilization of cyclin D1 messenger RNA (mRNA) may be the predominant mechanism. One growth factor that may potentially up-regulate cyclin D1 gene transcription is insulin-like growth factor II, which is overexpressed in a murine model of PETs (36). However, additional mechanisms cannot be ruled out, and the identification of one tumor sample with strong protein immunostaining, but normal RNA levels, suggests that posttranslational mechanisms may also play a role in a small subset of tumors.

Immunoreactivity against cyclin D1 was not observed uniformly in all tumor cells. Nevertheless, the absence of any cyclin D1 staining in normal, nonneoplastic islet cells underscores the significance of the strong staining observed in these tumors. The heterogeneous staining pattern is consistent with the known variations in nuclear cyclin D1 levels, depending upon the specific phase of the cell cycle and the short half-life of cyclin D1 protein (37). Furthermore, transgenic animals that constitutively overexpress cyclin D1 under the epithelial specific EBV ED-L2 promoter demonstrate a similar heterogenous staining pattern in targeted tissues (38).

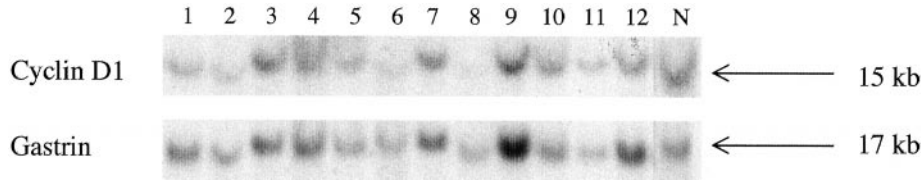

FIG. 3. Representative Southern blot analysis of cyclin D1 genomic DNA in human PETs. Lanes 1–12 are representative *Bam*HI-digested tumor samples; lane 13 is a *Bam*HI-digested normal nontumor DNA control. A, Blot hybridized with a human cyclin D1 cDNA probe. B, Same blot stripped and rehybridized with a probe (human gastrin-5′) for the human gastrin gene. There is no evidence for cyclin D1 gene rearrangement or amplification.

Cyclin D1 lies in a pathway that also includes the *INK4A*^{P16} and *RB* tumor suppressor gene products. There seems to be little redundancy among these three components, such that a mutation in one gene is sufficient to disrupt the entire pathway (11, 12). A previous study failed to identify *RB* gene alterations in a large series of PETs (21), and data from a group of 28 PETs also failed to identify any *INK4A*^{P16} gene deletions (27). Thus, the cyclin D1 oncogene seems to be a critical target in this particular pathway. A recent study indicated that *INK4A*^{P16} gene alterations, including homozygous deletions or methylation defects, may play a role in a subset of gastrinomas and nonfunctional PETs (39). Although the status of the cyclin D1 oncogene was not determined in these tumors, the identification of such alterations highlights the importance of this p16-cyclin D1-RB pathway in the development of human PETs.

Cyclin D1 overexpression has been associated with a poor prognosis in pancreatic (40), esophageal (41), breast (42), and colorectal (43) cancer; and in some cases, this correlation was independent of tumor stage. However, no such correlation was observed in this series of endocrine tumors. Interestingly, cyclin D1 overexpression was observed in both early- and late-stage tumors, suggesting that activation of this oncogene is a potentially early event in tumor development. In some models, including hepatic and parathyroid tumorigenesis, cyclin D1 is also up-regulated early in benign proliferative stages (44, 45); and, just as in PETs, this involvement is consistent with maintenance of a well-differentiated tumor cell phenotype.

The identification of a potentially key role for cyclin D1 in the tumorigenesis of pancreatic endocrine cells is consistent with previously described animal models that implicate other regulators of the G_1/S cell cycle transition. However, none of those factors, including p53, Rb, and CDK-4, have been shown to be relevant to human PETs. These observations highlight the important lessons and limitations of these particular animal models.

In summary, cyclin D1 overexpression is identified frequently in a large series of human PETs. This overexpression does not seem to be a consequence of an alteration in cyclin D1 genomic structure. Interestingly, such overexpression is identified in many benign, early-stage tumors. Thus, the cyclin D1 oncogene may play an important pathogenic role in human PETs, perhaps quite early in tumor development.

Acknowledgment

We thank Gretchen Warland, Dr. Robin Kirby, Dr. Carolyn Compton, and Dr. Loyal Tillotson for assistance in tissue procurement and slide preparation; Yuchiao Chang for biostatistical assistance; and Dr. Andrew Leiter for sharing the pancreatic polypeptide vectors.

References

1. **Arnold A.** 1993 Molecular genetics of parathyroid gland neoplasia. J Clin Endocrinol Metab. 77:1108–1112.
2. **Gillett C, Fantl V, Smith R, et al.** 1994 Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res. 54:1812–1817.
3. **Jiang W, Kahn SM, Tomita N, Zhang YJ, Lu SH, Weinstein IB.** 1992 Amplification and expression of the human cyclin D gene in esophageal cancer. Cancer Res. 52:2980–2983.
4. **Rosenberg CL, Wong E, Petty EM, et al.** 1991 PRAD1, candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. Proc Natl Acad Sci USA. 88:9638–9642.
5. **Motokura T, Arnold A.** 1993 PRAD1/cyclin D1 proto-oncogene: genomic organization, 5′ DNA sequence, and sequence of a tumor-specific rearrangement breakpoint. Genes Chromosom Cancer. 7:89–95.
6. **Wang T, Cardiff R, Zukerberg L, Lees E, Arnold A, Schmidt EV.** 1994 Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature. 369:669–671.
7. **Arber N, Doki Y, Han EK, et al.** 1997 Antisense to cyclin D1 inhibits the growth and tumorigenicity of human colon cancer cells. Cancer Res. 57:1569–1574.
8. **Kornmann M, Arber N, Korc M.** 1998 Inhibition of basal and mitogen-stimulated pancreatic cancer cell growth by cyclin D1 antisense is associated with loss of tumorigenicity and potentiation of cytotoxicity to cisplatinum. J Clin Invest. 101:344–352.
9. **Schutte M, Hruban RH, Geradts J, et al.** 1997 Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res. 57:3126–3130.
10. **Bartek J, Bartkova J, Lukas J.** 1997 The retinoblastoma protein pathway in cell cycle control and cancer. Exp Cell Res. 237:1–6.
11. **Bartkova J, Lukas J, Guldberg P, et al.** 1996 The p16-cyclinD/Cdk4-pRb pathway as a functional unit frequently altered in melanoma pathogenesis. Cancer Res. 56:5475–5483.
12. **Shapiro GI, Edwards CD, Kobzik L, et al.** 1995 Reciprocal Rb inactivation and p16INK4 expression in primary lung cancers and cell lines. Cancer Res. 55:505–509.
13. **Chung DC, Smith AP, Louis DN, Graeme-Cook F, Warshaw AL, Arnold A.** 1997 A novel pancreatic endocrine tumor suppressor gene locus on chromosome 3p with clinical prognostic implications. J Clin Invest. 100:404–410.
14. **Chandrasekharappa SC, Guru SC, Manickam P, et al.** 1997 Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science. 276:404–407.
15. **Hanahan D.** 1985 Heritable formation of pancreatic β-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature. 315:115–122.
16. **Asa SL, Lee YC, Drucker DJ.** 1996 Development of colonic and pancreatic endocrine tumours in mice expressing a glucagon-SV40 T antigen transgene. Virchows Arch. 427:595–606.
17. **Williams BO, Remington L, Albert DM, Mukai S, Bronson RT, Jacks T.** 1994 Cooperative tumorigenic effects of germline mutations in Rb and p53. Nat Genet. 7:480–484.
18. **Harvey M, Vogel H, Lee E, Bradley A, Donehower L.** 1995 Mice deficient in both p53 and Rb develop tumors primarily of endocrine origin. Cancer Res. 55:1146–1151.
19. **Rane SG, Dubus P, Mettus RV, et al.** 1999 Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet hyperplasia. Nat Genet. 22:44–52.
20. **Yoshimoto K, Iwahana H, Fukuda A, Sano T, Saito S, Itakura M.** 1992 Role of p53 mutations in endocrine tumorigenesis: mutation detection by polymerase chain reaction-single strand conformation polymorphism. Cancer Res. 52:5061–5064.
21. **Chung DC, Smith AP, Louis DN, Graeme-Cook F, Warshaw AL, Arnold A.** 1997 Analysis of the retinoblastoma tumour suppressor gene in pancreatic endocrine tumours. Clin Endocrinol (Oxf). 47:523–528.

22. **Banno S, Yoshikawa K, Nakamura S, et al.** 1994 Monoclonal antibody against PRAD1/cyclin D1 stains nuclei of tumor cells with translocation or amplification at BCL-1 locus. Jpn J Cancer Res. 85:918–926.
23. **Arnold A, Staunton CE, Kim HG, Gaz RD, Kronenberg HM.** 1988 Monoclonality and abnormal parathyroid hormone genes in parathyroid adenomas. N Engl J Med. 318:658–662.
24. **Motokura T, Bloom T, Kim HG, et al.** 1991 A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature. 350:512–515.
25. **Tillotson LG, Wang T, Brand SJ.** 1994 Activation of gastrin transcription in pancreatic insulinoma cells by a CACC promoter element and a 70-kDa sequence-specific DNA binding protein. J Biol Chem. 269:2234–2240.
26. **Oyama T, Kashiwabara K, Yoshimoto K, Arnold A, Koerner F.** 1998 Frequent overexpression of the cyclin D1 oncogene in invasive lobular carcinoma of the breast. Cancer Res. 58:2876–2880.
27. **Chung DC, Brown SB, Graeme-Cook F, et al.** 1998 Localization of putative tumor suppressor loci by genome-wide allelotyping in human pancreatic endocrine tumors. Cancer Res. 58:3706–3711.
28. **Arnold A, Kim H, Gaz RD, et al.** 1989 Molecular cloning and chromosomal mapping of DNA rearranged with the parathyroid hormone gene in a parathyroid adenoma. J Clin Invest. 83:2034–2040.
29. **Toliat M-R, Berger W, Ropers HH, Neuhaus P, Wiedenmann B.** 1997 Mutations in the *MEN1* gene in sporadic neuroendocrine tumours of gastroenteropancreatic system. Lancet. 350:1223.
30. **Zhuang Z, Vortmeyer AO, Pack S, et al.** 1997 Somatic mutations of the *MEN1* tumor suppressor gene in sporadic gastrinomas and insulinomas. Cancer Res. 57:4682–4686.
31. **Wang EH, Ebrahimi S, Wu A, Kashefi C, Passaro E, Sawicki MP.** 1998 Mutation of the *MEN1* gene in sporadic pancreatic endocrine tumors. Cancer Res. 58:4417–4420.
32. **Furlanetto RW, Harwell SE, Frick KK.** 1994 Insulin-like growth factor-I induces cyclin-D1 expression in MG63 human osteosarcoma cells *in vitro*. Mol Endocrinol. 8:510–517.
33. **Yan Y-X, Nakagawa H, Lee M-H, Rustgi AK.** 1997 Transforming growth factor-α enhances cyclin D1 transcription through the binding of early growth response protein to a cis-regulatory element in the cyclin D1 promoter. J Biol Chem. 272:33181–33190.
34. **Tetsu O, McCormick F.** 1999 Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature. 398:422–426.
35. **Rosenwald IB, Lazaris-Karatzas A, Sonenberg N, Schmidt EV.** 1993 Elevated levels of cyclin D1 protein in response to increased expression of eukaryotic initiation factor 4E. Mol Cell Biol. 13:7358–7363.
36. **Christofori G, Naik P, Hanahan D.** 1994 A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. Nature. 369:414–418.
37. **Lukas J, Pagano M, Staskova Z, Draetta G, Bartek J. Cyclin D.** 1994 1 protein oscillates and is essential for cell cycle progression in human tumor cell lines. Oncogene. 9:707–718.
38. **Mueller A, Odze R, Jenkins TD, et al.** 1997 A transgenic mouse model with cyclin D1 overexpression results in cell cycle, epidermal growth factor receptor, and p53 abnormalities. Cancer Res. 57:5542–5549.
39. **Muscarella P, Melvin WS, Fisher WE, et al.** 1998 Genetic alterations in gastrinomas and nonfunctioning pancreatic neuroendocrine tumors; an analysis of p16/MTS1 tumor suppressor gene inactivation. Cancer Res. 58:237–240.
40. **Gansauge S, Gansauge F, Ramadani M, et al.** 1997 Overexpression of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis. Cancer Res. 57:1634–1637.
41. **Naitoh H, Shibata J, Kawaguchi A, Kodama M, Hattori T.** 1995 Overexpression and localization of cyclin D1 mRNA and antigen in esophageal cancer. Am J Pathol. 146:1161–1169.
42. **McIntosh GG, Anderson JJ, Milton I, et al.** 1995 Determination of the prognostic value of cyclin D1 overexpression in breast cancer. Oncogene. 11:885–891.
43. **Arber N, Hibshoosh H, Moss SF, et al.** 1996 Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. Gastroenterology. 110:669–674.
44. **Ramljak D, Jones AB, Diwan BA, Perantoni AO, Hochadel JF, Anderson LM.** 1998 Epidermal growth factor and transforming grown factor-α-associated overexpression of cyclin D1, Cdk4, and c-Myc during hepatocarcinogenesis in Helicobacter hepaticus-infected A/JCr mice. Cancer Res. 58:3590–3597.
45. **Zukerberg LR, Yang WI, Gadd M, et al.** 1995 Cyclin D1 (PRAD1) protein expression in breast cancer: approximately one-third of infiltrating mammary carcinomas show overexpression of the cyclin D1 oncogene. Mod Pathol. 8:560–567.