The EMBO Journal Vol.16 No.10 pp.2621–2633, 1997

Expression of a dominant-negative mutant TGF-β type II receptor in transgenic mice reveals essential roles for TGF-β in regulation of growth and differentiation in the exocrine pancreas

Erwin P.Bottinger[1,2], John L.Jakubczak[3], Ian S.D.Roberts[4], Michelle Mumy, Philipp Hemmati, Kerri Bagnall, Glenn Merlino[3] and Lalage M.Wakefield

Laboratory of Chemoprevention and [3]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA and [4]Department of Pathological Sciences, The University of Manchester, Oxford Road, Manchester M13 9PT, UK

[2]Present address: Albert Einstein College of Medicine, Bronx, NY 10461, USA

[1]Corresponding author

E.P.Bottinger and J.L.Jakubczak contributed equally to this work

Using a dominant-negative mutant receptor (DNR) approach in transgenic mice, we have functionally inactivated transforming growth factor-β (TGF-β) signaling in select epithelial cells. The dominant-negative mutant type II TGF-β receptor blocked signaling by all three TGF-β isoforms in primary hepatocyte and pancreatic acinar cell cultures generated from transgenic mice, as demonstrated by the loss of growth inhibitory and gene induction responses. However, it had no effect on signaling by activin, the closest TGF-β family member. DNR transgenic mice showed increased proliferation of pancreatic acinar cells and severely perturbed acinar differentiation. These results indicate that TGF-β negatively controls growth of acinar cells and is essential for the maintenance of a differentiated acinar phenotype in the exocrine pancreas *in vivo*. In contrast, such abnormalities were not observed in the liver. Additional abnormalities in the pancreas included fibrosis, neoangiogenesis and mild macrophage infiltration, and these were associated with a marked up-regulation of TGF-β expression in transgenic acinar cells. This transgenic model of targeted functional inactivation of TGF-β signaling provides insights into mechanisms whereby loss of TGF-β responsiveness might promote the carcinogenic process, both through direct effects on cell proliferation, and indirectly through up-regulation of TGF-βs with associated paracrine effects on stromal compartments.

Keywords: *in vivo*/receptors/signal transduction/transforming growth factor β/transgenic mice

Introduction

The transfoming growth factors-βs (TGF-β) are multifunctional cytokines which regulate cell growth, differentiation and function (Roberts and Sporn, 1990), and recent evidence suggests that they constitute part of an important tumor suppressor pathway (Markowitz and Roberts, 1996). One or more of the three mammalian TGF-βs is expressed in nearly every tissue in the body, implicating this as a widely used regulatory system throughout development and adulthood (Flanders *et al.*, 1989; Millan *et al.*, 1991). However, despite a wealth of data on the multitude of biological activities of TGF-βs *in vitro*, in most cases the precise roles played by the TGF-βs in a particular *in vivo* setting are not known. This is in part because TGF-β action is strongly contextual. Thus, the specific effect of TGF-β on a particular cell appears to be an integrated function of the target cell type, its differentiated state and its environmental context, particularly regarding the nature of the extracellular matrix and the activities of other cytokines (Nathan and Sporn, 1991). For example, while TGF-β is a potent inhibitor of the growth of keratinocytes *in vitro*, transgenic overexpression of TGF-β in the skin can lead to an unexpected stimulation of keratinocyte proliferation in the basal state, but results in growth inhibition in a hyperplastic setting, following treatment with the phorbol ester TPA (Cui *et al.*, 1995). This demonstrates that the physiological roles of TGF-βs in a particular tissue *in vivo* may not be readily predictable from its *in vitro* effects on cells derived from that tissue.

As one approach to the molecular dissection of the *in vivo* biology of TGF-β, we wished to generate animal models in which TGF-β function is experimentally compromised in select tissues. The type I and type II TGF-β receptors (TβRI and TβRII) are activated by ligand-dependent formation of hetero-oligomeric complexes, in which TβRII transphosphorylates and activates TβRI, thereby initiating the signal transduction cascade (Wrana *et al.*, 1994). In order to eliminate the TGF-β response in target tissues, we have used transgenic overexpression of a dominant-negative mutant form of the TβRII (Brand *et al.*, 1993; Chen *et al.*, 1993), resulting in tissue-restricted functional inactivation of the TGF-β receptor complex. This approach of local functional inactivation of genes has attractive advantages when compared with germline null mutations generated by gene targeting. First, loss of function can be targeted to specific cells in selected organs with appropriate transcriptional control elements, and potential embryonic lethality can be circumvented. Because of the important roles of TGF-βs during embryonic development, null mutations in the TβRII gene are expected to result in embryonic lethality. Second, the problem of isoform redundancy, a potential issue with the three mammalian TGF-β isoforms, can be eliminated. Third, confounding systemic effects such as widespread inflammation observed in multiple organs of the TGF-β1 null mouse (Kulkarni *et al.*, 1993) can be avoided.

We report here the effects of functional inactivation of TGF-β in target tissues in mice with expression of a dominant-negative mutant TβRII (DNR) under control of a metallothionein 1 (MT1) promoter (Palmiter *et al.*,

1993). The results show that TGF-β negatively controls growth of pancreatic acinar cells and is essential for the maintenance of a differentiated acinar phenotype in the exocrine pancreas *in vivo*. Furthermore, loss of TGF-β responsiveness in DNR-positive acinar cells results in an unexpected increase in expression of TGF-β1 and TGF-β3 *in vivo* in the exocrine pancreas. This is associated with classic correlates of TGF-β overexpression such as fibrosis, angiogenesis and macrophage infiltration. These results have implications for understanding the complex roles of the TGF-β system in tumorigenesis.

Results

Generation of DNR transgenic mice and analysis of expression

The DNR transgene encodes the extracellular and transmembrane domains of TβRII and was expressed under control of a mouse MT1 promoter and MT locus control regions (LCRs) (Figure 1A) (Palmiter *et al.*, 1993). We generated two lines of transgenic mice, line AM3 with three copies, and line BF1 with one copy per haploid genome, respectively, in the strain FVB/N. Northern blotting revealed DNR RNA expression in both lines. In line AM3, the highest zinc-induced levels of DNR RNA were observed in the pancreas, liver, colon and small intestine (Figure 1B). Kidney, stomach and salivary gland had substantially lower levels of expression (Figure 1B). DNR expression was consistently lower in tissues from line BF1 mice when compared with line AM3 tissues (Figure 1C). In liver and pancreas, substantial levels of DNR were expressed without zinc induction (Figure 1C). To show the cellular localization of DNR protein *in situ*, we stained sections of pancreas and liver with anti-human TβRII(1–28) antibody. Cell surface-associated expression of DNR was observed in acinar cells in the pancreas and hepatocytes in the liver (Figure 2A and C). DNR expression was heterogeneous in both organs and was highest at 2 months of age.

Dominant-negative mutant function of DNR in hepatocytes and acinar cells

Ligand binding and receptor complex formation. Primary hepatocytes and pancreatic acinar cells were prepared from either line AM3 or from non-transgenic FVB/N mice. Ligand binding by DNR was assessed by receptor affinity labeling with [^{125}I]TGF-β1. Ligand–receptor complexes were undetectable in lysates of affinity-labeled non-transgenic FVB/N hepatocytes, indicating low levels of endogenous receptors (Figure 3A). In hepatocyte lysates from line AM3, a prominent complex of ~40 kDa represented DNR bound to [^{125}I]TGF-β1, as confirmed by immunoprecipitation of affinity-labeled lysates with anti-human TβRII(1–28) (Figure 3A). Analogous experiments showed similar results using primary acinar cell preparations (Figure 3B).

Loss of TGF-β responsiveness. Primary pancreatic acinar cell cultures from control FVB/N mice showed a marked inhibition of [^{3}H]thymidine incorporation when treated with either TGF-β1, TGF-β2, TGF-β3 or activin, a member of the TGF-β superfamily structurally closely related to TGF-β (Figure 3C). In contrast, TGF-β isoforms had no effect on [^{3}H]thymidine incorporation in acinar cells from line AM3 (Figure 3C). However, activin inhibited [^{3}H]thymidine incorporation in transgenic acinar cells (Figure 3C), indicating that DNR expression only inactivates TGF-β signaling, but not signaling by other members of the TGF-β superfamily. Primary hepatocytes from control FVB/N mice were growth inhibited by TGF-β isoforms (Figure 3D), but not by activin (data not shown). None of the three TGF-β isoforms inhibited growth of hepatocytes from line AM3 (Figure 3D). In addition, induction of fibronectin protein secretion by TGF-β1, as seen in FVB/N hepatocytes, was absent in line AM3 hepatocytes (Figure 3E). These results indicate that DNR expression in cells from transgenic mice completely abrogates both the growth inhibition mediated by the three

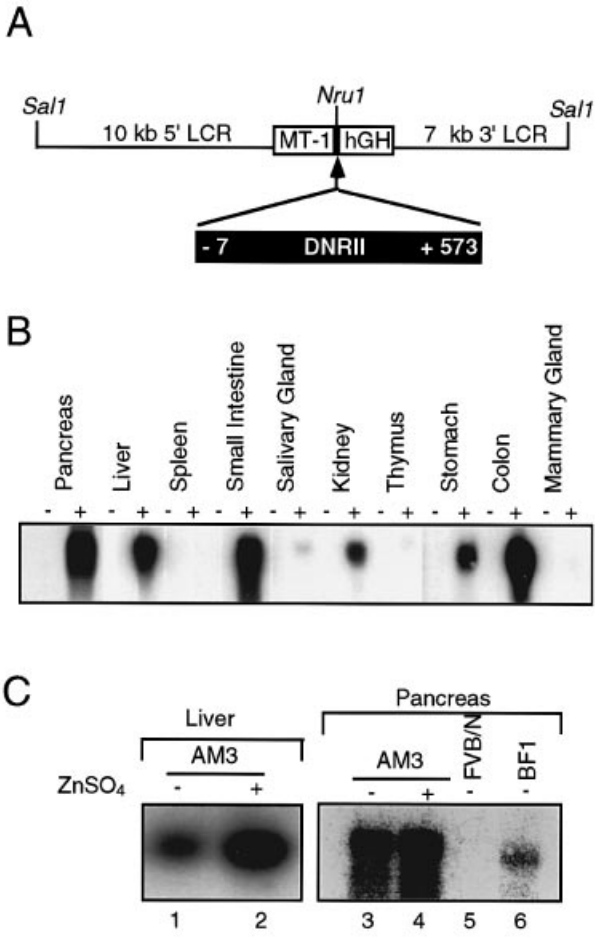

Fig. 1. Construction of the DNR transgene and transgene expression. (**A**) Schematic representation of the transgene. A 19.1 kb *Sal*I DNA fragment containing DNR was released from pMT-LCR-DNR and used for microinjections. MT-1, mouse metallothionein I promoter; hGH, poly(A) region of human growth hormone gene; 10 kb 5′ LCR and 7 kb 3′ LCR, 10 kb *Eco*RI fragment of the 5′ LCR and 7 kb *Eco*RI fragment of the 3′ LCR of the mouse MT I and II gene locus. (**B**) Tissue distribution of DNR transgene expression (line AM3). RNA (20 μg) of tissues from 2-month-old control littermate (–) and transgenic mice (+) maintained on drinking water containing 25 mM $ZnSO_4$ was hybridized with ^{32}P-labeled human DNR cDNA. (**C**) RNA (20 μg) from line AM3 liver (lanes 1 and 2) and pancreas (lanes 3 and 4), as well as pancreas from non-transgenic FVB/N (lane 5) and transgenic line BF1 (lane 6), was hybridized with a DNR probe. DNR expression in line AM3 liver and pancreas in the absence (lanes 1 and 3), or presence (lanes 2 and 4) of $ZnSO_4$ in the drinking water.

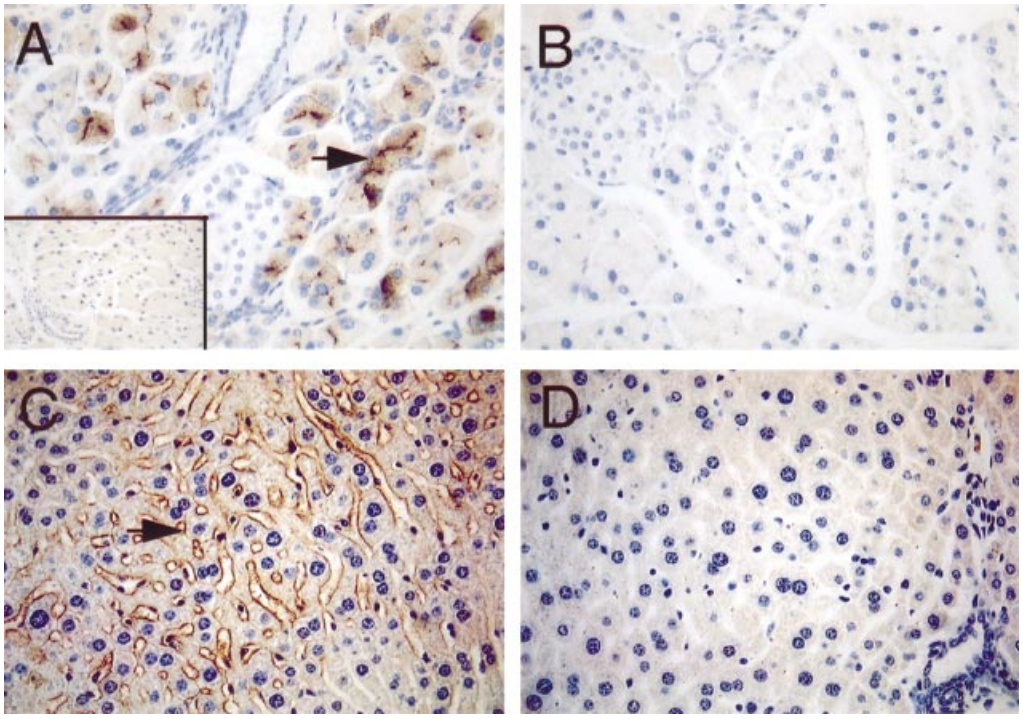

Fig. 2. Expression of DNR in pancreas and liver. Immunostaining with anti-TβRII (residues 1–28) antibody: (**A**) pancreas of 2-month-old transgenic AM3 mouse in the absence or presence (see insert) of blocking peptide; (**B**) pancreas from non-transgenic FVB/N littermate; (**C**) liver of line AM3 mouse, (**D**) liver of FVB/N littermate. Arrows denote acinar staining (A) and hepatocyte staining (C), respectively. All animals were maintained on drinking water containing 25 mM $ZnSO_4$. (A–D) all ×40.

Table I. The phenotype of line AM3

	Line AM3				Controls (FVB/N)			
Age (months)	0–4	5–8	9–14	14+	0–4	5–8	9–14	14+
Total no. of mice	26[a]	21	12	33	14	8	12	11
Ductular structures (%)	100[b]	100	100	100	0	0	0	0
Fibrosis/angiogenesis (%)	69	100	100	100	0	0	0	0
Adipose replacement (%)	27	100	100	100	0	13	25	27
Tubular complexes (%)	12	24	33	55	0	0	0	9

[a]Total number of mice per age group.
[b]Percentage of mice with pathologic lesions in this age group.

TGF-β isoforms, and the TGF-β mediated induction of matrix-associated genes.

Phenotypic characterization of transgenic DNR mice

On macroscopic inspection, the pancreas of line AM3 mice of 5 months and older had a lighter color when compared with the pancreas from non-transgenic littermates. Relative pancreatic weight expressed as a percentage of total body weight was significantly decreased in line AM3 mice when compared with FVB/N mice at 8 months of age (0.82 ± 0.10% versus 1.08 ± 0.03%, $P < 0.05$). All other organs including the liver, colon and small intestine were macroscopically normal.

Histological analysis of the pancreas showed severe abnormalities, including ductular transformation, neoangiogenesis, inter- and intralobular fibrosis and adipose replacement of acini in the exocrine pancreas of both transgenic lines AM3 and BF1 (see Table I; Figures 4 and 5). The islets of Langerhans appeared normal, consistent with the absence of DNR expression in endocrine cells. The abnormalities in the exocrine pancreas were generally more severe and occurred at a younger age in line AM3 when compared with line BF1, consistent with increased levels of DNR expression in the AM3 pancreas (Figure 1C). We focused therefore on line AM3 for a detailed analysis of the phenotype in the pancreas.

The earliest feature of the phenotype was the appearance of aberrant non-acinar cells in the exocrine pancreas from 3 weeks of age. These cells increased progressively in number and formed primitive ductular structures (Table I; Figure 4B). Strong staining with AE1/AE3 antibody, a marker of ductal epithelium, was observed in most of the aberrant non-acinar cells, confirming their ductal cell phenotype (Figure 4D). In addition, acinar cells in line AM3 mice were progressively replaced by adipose cells (Table I; Figure 4F). Tubular complexes, reflecting acinoductular metaplasia (Bockman, 1981), were found in some younger line AM3 mice and appeared frequently in older transgenic mice, often transforming entire lobules (Table I;

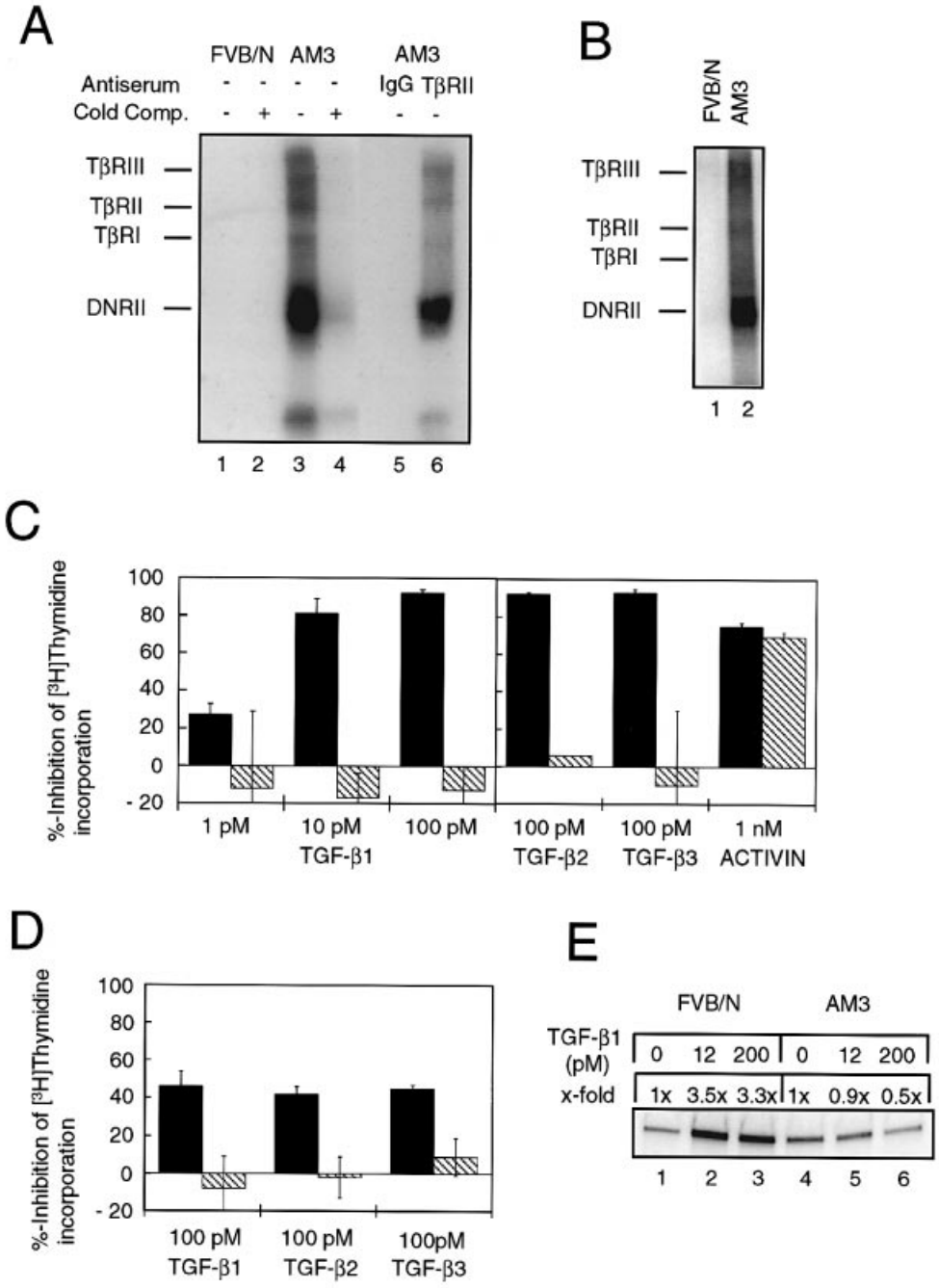

Fig. 3. Dominant-negative mutant function of DNR in primary hepatocytes and acinar cells. Primary cultures of hepatocytes or purified pancreatic acini were generated from 2-month-old transgenic line AM3 and non-transgenic FVB/N mice. (**A**) Affinity labeling with [^{125}I]TGF-β1 of hepatocytes from FVB/N (lanes 1 and 2) and line AM3 mice (lanes 3 and 4), in the absence (lanes 1 and 3), or presence (lanes 2 and 4) of a 50-fold molar excess of unlabeled TGF-β1 (cold competitor). TGF-β receptor complexes are denoted. Lanes 5 and 6, immunoprecipitation of affinity-labeled lysates from line AM3 hepatocytes with control IgG (1.5 μg/ml) (lane 5) or with anti-TβRII(1–28) (1.5 μg/ml) (lane 6). (**B**) Immunoprecipitation with anti-TβRII(1–28) of affinity-labeled lysates from FVB/N (lane 1) or line AM3 (lane 2) acinar cells. TGF-β receptor complexes are denoted. (**C**) Growth inhibition as measured by [^{3}H]thymidine incorporation after treatment with TGF-β1, -β2, -β3 or activin, as indicated, in acinar cells from non-transgenic FVB/N (black bars) or transgenic line AM3 mice (hatched bars). (**D**) Growth inhibition as measured by [^{3}H]thymidine incorporation after treatment with TGF-β1, -β2 or -β3, as indicated, in primary hepatocytes from non-transgenic FVB/N (black bars) or transgenic line AM3 mice (hatched bars). (**E**) Effect of TGF-β1 on secretion of fibronectin by metabolically labeled primary hepatocytes from FVB/N or line AM3 mice (visualized by autoradiography). Band intensities were quantified by densitometry, and levels of expression are denoted as x-fold above baseline expression (1.0×) in untreated cells (lanes 1 and 4).

Figure 4G). Within the pancreas of transgenic lines AM3 and BF1, the severity of the described abnormalities correlated well with the level of DNR expression in affected lobules. Older animals (>14 months) were also examined for the presence of eosinophilic foci of acinar cells, considered a marker of focal hyperplasia in rats (Eustis *et al.*, 1990). Eosinophilic foci were not observed in non-transgenic FVB/N mice ($n = 12$), but were found in 21% (7/33) ($P = 0.001$) of line AM3 pancreas (Figure 4H).

To better define the abnormalities in the transgenic

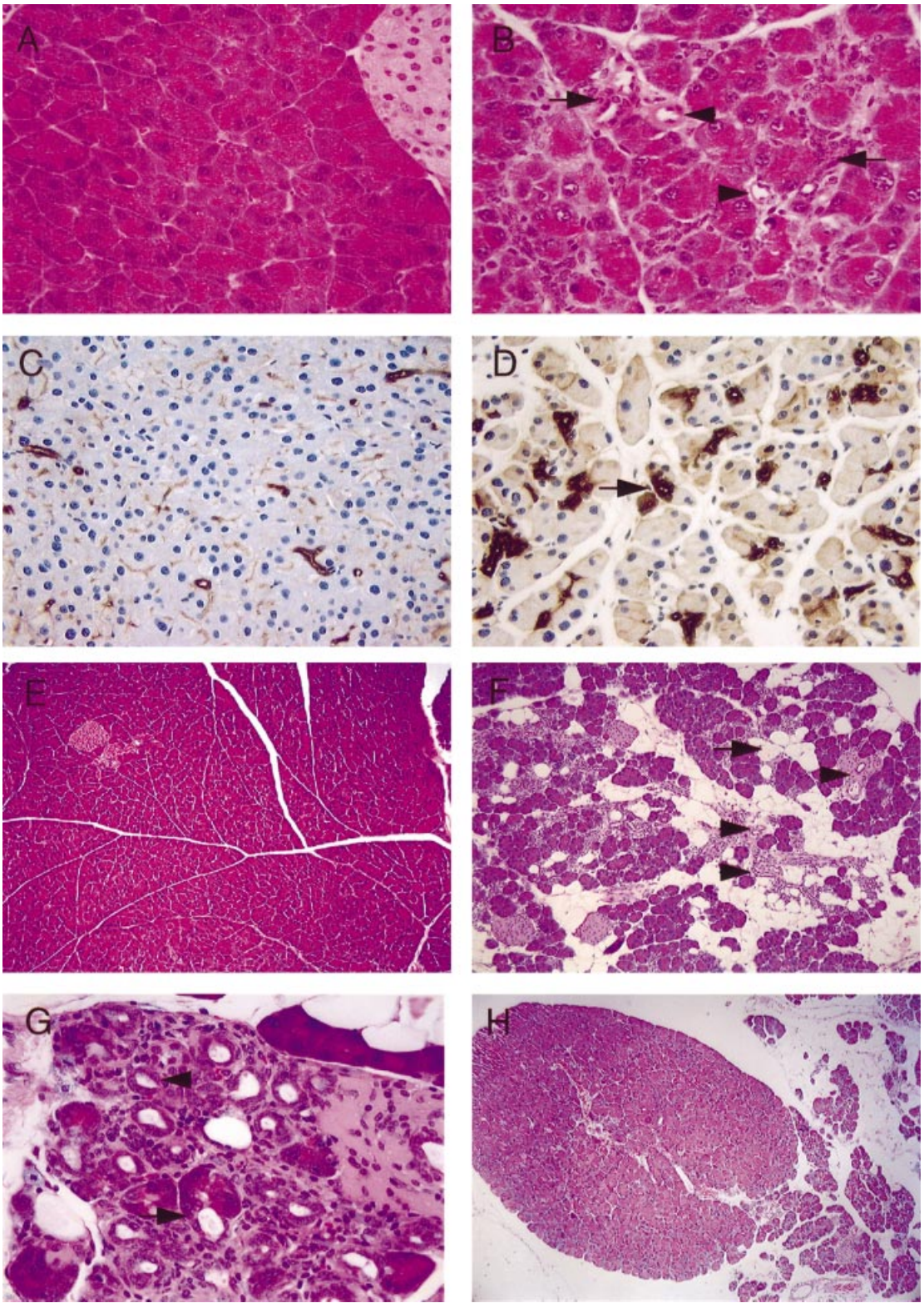

Fig. 4. Phenotype of mouse pancreas. (A and B) (×40) H&E staining of sections of the pancreas in 6-week-old non-transgenic FVB/N (**A**) and transgenic line AM3 mice (**B**); the arrows depict aberrant ductular cells and ductules; arrowheads denote microvessels. (C and D) (×40) Anti-cytokeratin immunostaining for markers of ductal epithelial cells in the pancreas of 6-week-old control FVB/N (**C**) and line AM3 mice (**D**); the arrow shows staining of an aberrant ductule. (E and F) (×10) H&E staining of pancreas sections of 15-month-old FVB/N (**E**) and transgenic line AM3 mice (**F**); the arrow depicts adipose replacement of acini, arrowheads show ductular and tubular structures. (**G**) (×40) H&E staining of a pancreas section of a 14-month-old transgenic line AM3 mouse; the arrow depicts an acinus undergoing dedifferentiation into a tubular complex. Most acini in this lobule have dedifferentiated into tubular complexes (see arrowhead). (**H**) (×5) Low power view of H&E staining of a pancreas section of a 19-month-old line AM3 mouse showing an eosinophilic focus of acinar cells (oval structure).

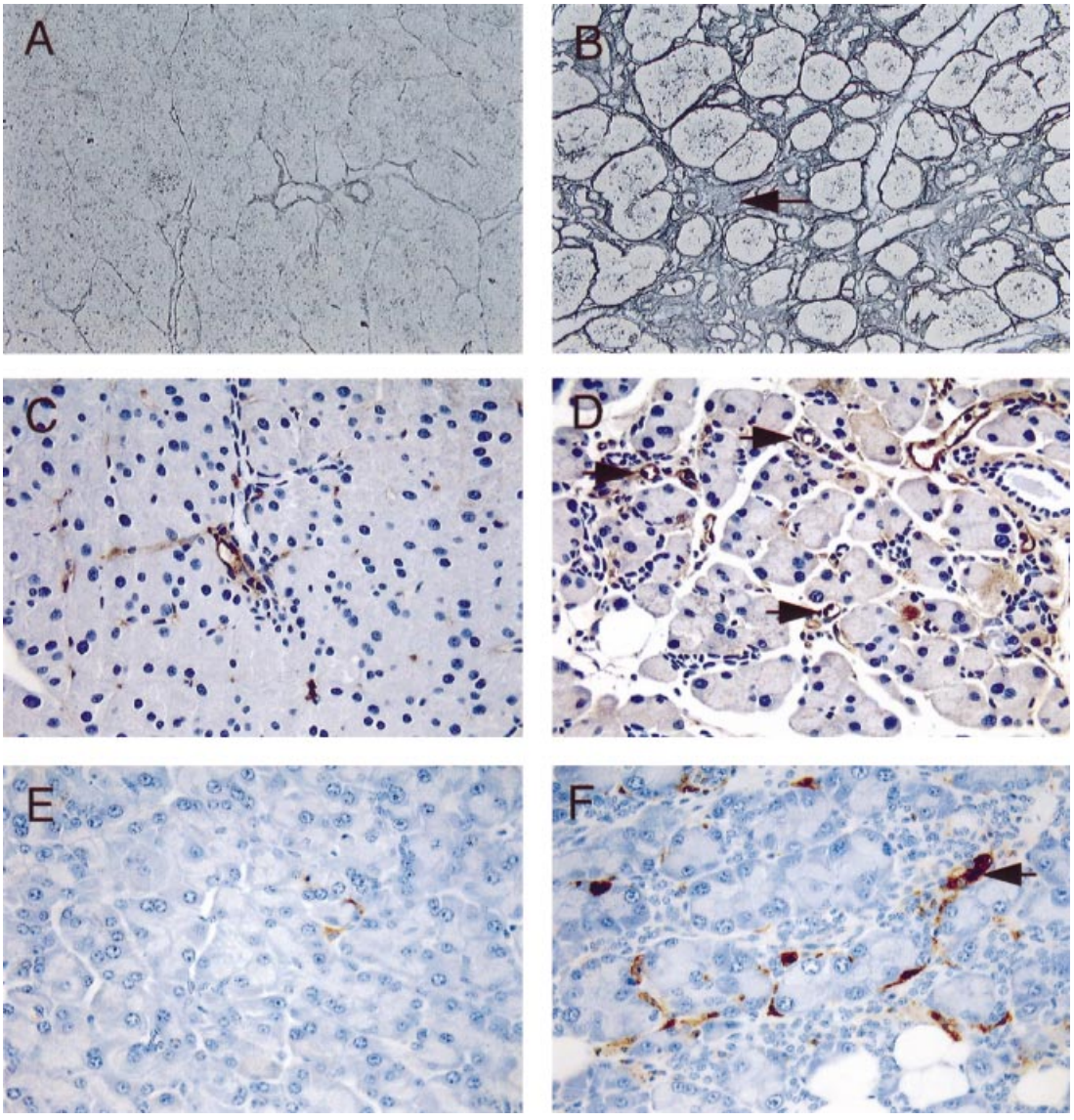

Fig. 5. Stromal abnormalities in transgenic mouse pancreas. (A and B) (×40) Reticulin staining of pancreas sections of 8-month-old FVB/N (**A**) and line AM3 mice (**B**); the arrow shows collagen deposition. (C and D) (×40) Anti-von Willebrand factor immunostaining of pancreas sections of 3-month-old FVB/N (**C**) and line AM3 mice (**D**); the arrows depict aberrant microvessels. (E and F) (×40) Immunostaining for macrophages in pancreas sections of 3-month-old FVB/N (**E**) and line AM3 mice (**F**); the arrow denotes Mac-2-positive macrophages.

Table II. Rates of proliferation and apoptosis in the exocrine pancreas of non-transgenic FVB/N and transgenic line AM3 mice

	6 weeks		n^c	8 months		n
	PCNA[a]	Apoptosis[b]		PCNA	Apoptosis	
FVB/N	185 ± 93	0 ± 0	5	12.7 ± 4.5	0.7 ± 0.6	3
AM3	678 ± 258[d]	7 ± 2[d]	7	74.7 ± 27.3[d]	4.5 ± 1.2[d]	6

[a]PCNA-positive acinar cells/mm^2.
[b]Apoptotic cells/mm^2.
[c]Number of mice.
[d]$P < 0.05$.

pancreas, we applied specific staining techniques. Inter- and intralobular fibrosis developed by 5 months of age in line AM3 mice, and increased progressively (Table I). Reticulin staining of pancreas sections for collagen demonstrated significant expansion of the extracellular matrix in 8-month-old line AM3 mice when compared with FVB/N mice (18.6 ± 5.8% versus 3.5 ± 0.5% reticulin-stained section surface, respectively; $P < 0.05$) (Figure 5A and B). Consistent with this, RNA expression levels of collagen 1, fibronectin and tissue inhibitor of metalloproteinase 1 (TIMP-1) were increased in line AM3 pancreas when compared with non-transgenic pancreas at 1, 3, 6 and 10

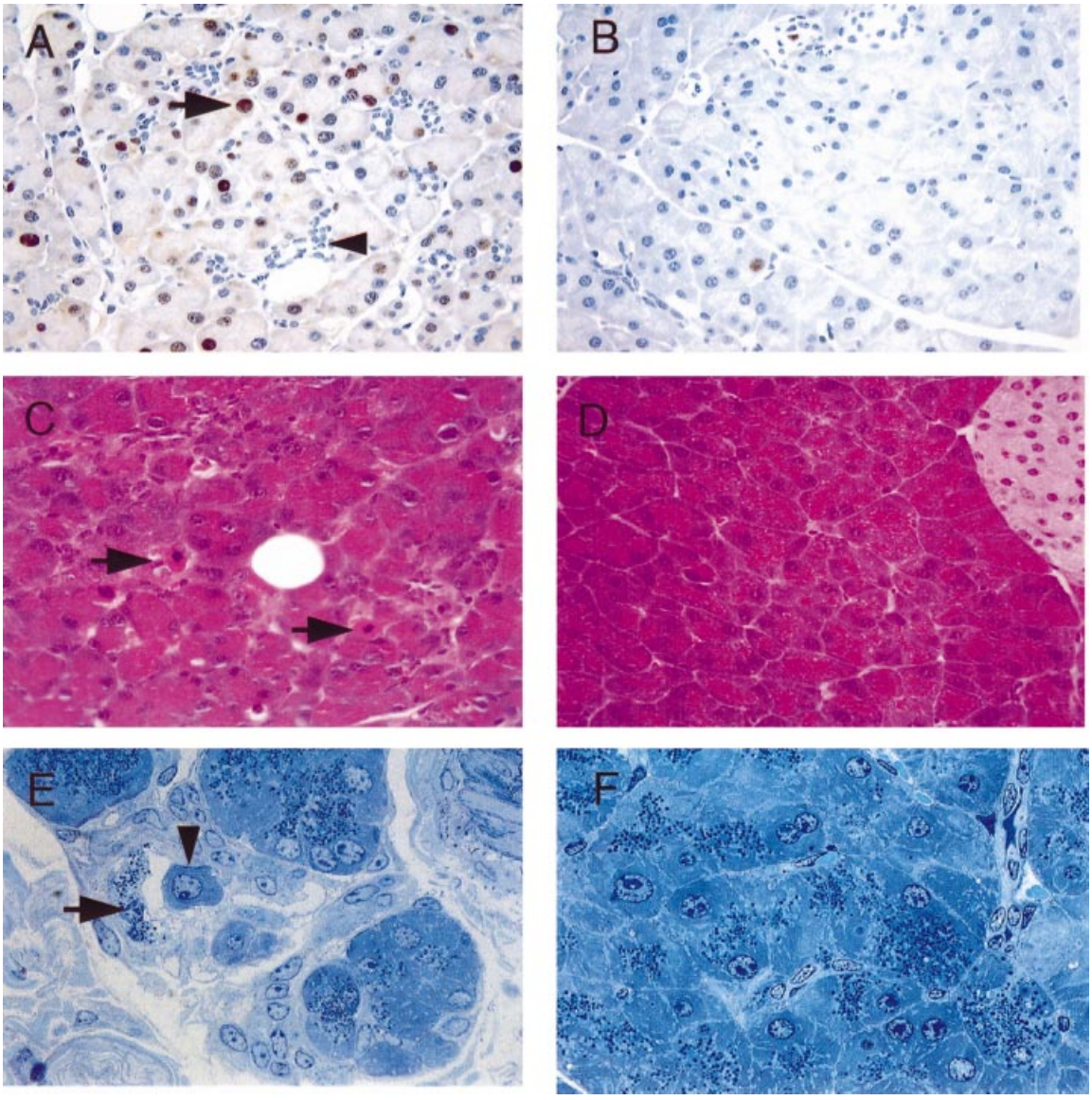

Fig. 6. Proliferation, apoptosis and differentiation in the transgenic pancreas. (**A**) PCNA immunostaining in the pancreas of 6-week-old transgenic line AM3 mice; the arrow denotes a PCNA-positive acinar cell nucleus; the arrowhead shows lack of PCNA staining in non-acinar cells (×40). (**B**) PCNA staining in non-transgenic FVB/N mice (×40). (**C**) H&E staining of a pancreas section of 6-week-old transgenic line AM3 mice; the arrows show apoptotic cell bodies (×40). (**D**) Control FVB/N mice (×40). (**E**) Toluidine blue staining of 0.5 μm semi-thin sections of the pancreas of 3-month-old transgenic line AM3 mice (×100); the arrow shows ductular cells containing zymogen granules and the arrowhead denotes a remnant acinar cell in the ductular structure. (**F**) Control FVB/N mice (X100).

months of age (data not shown). Increased angiogenesis (see also Figure 4B) and macrophage infiltration were revealed by immunostaining for von Willebrand factor VIII (Figure 5D) and Mac-2 antigens (Figure 5F), respectively.

TGF-β regulates growth and affects differentiation of acinar cells in the exocrine pancreas in vivo

Proliferation and apoptosis. To address possible mechanisms underlying the observed pancreatic phenotype, we analyzed proliferative and apoptotic rates in the pancreas. Immunohistochemistry for proliferating cell nuclear antigen (PCNA) demonstrated a large increase in the proportion of acinar cells in cell cycle in line AM3 pancreas when compared with control FVB/N pancreas at 6 weeks (Table II; Figure 6A and B) and 8 months of age (Table II). This was accompanied by large numbers of apoptotic cells in line AM3 pancreas, but not in FVB/N pancreas (Table II; Figure 6C and D). The aberrant ductular epithelial cells present in line AM3 pancreas showed no evidence of increased proliferative activity, as demonstrated by the low frequency of PCNA staining in these cells (1.2 ± 0.4% at 6 weeks of age), when compared with acinar cells (35.0 ± 5.1% at 6 weeks of age) (Figure 6A). These results indicated that inactivation of TGF-β signaling in acinar cells causes an increase in proliferation and apoptosis throughout adult life in transgenic mice. In contrast, rates of proliferation and apoptosis in the liver were not significantly different between line AM3 mice and FVB/N littermates (data not shown).

Differentiation and dedifferentiation. The accumulation of largely non-proliferating ductular cells and duct-like

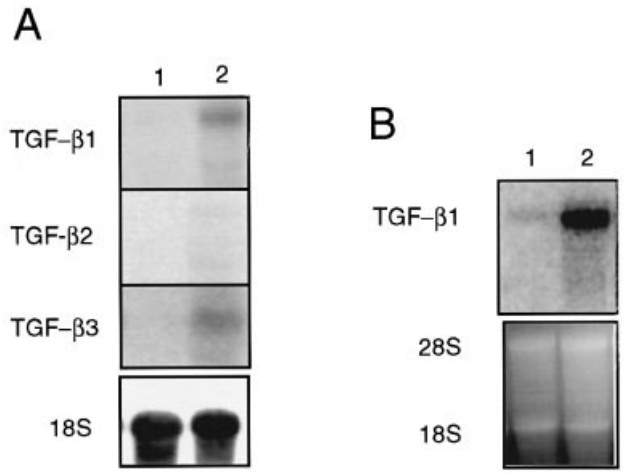

Fig. 7. Expression of TGF-β isoforms in the transgenic pancreas. (**A**) RNA (10 μg) from the pancreas of 3-month-old non-transgenic FVB/N (lane 1) and transgenic AM3 (lane 2) mice was hybridized with TGF-β1, TGF-β2 and TGF-β3 cDNA probes. Hybridization with a ribosomal 18S probe shows equal loading. (**B**) RNA (10 μg) from primary acinar cell cultures generated from control FVB/N (lane 1) and line AM3 mice (lane 2) hybridized with a TGF-β1 probe. Ethidium bromide-stained agarose gel demonstrating 28S and 18S ribosomal complexes.

structures with the loss of acini suggested that normal differentiation and/or maintenance of a differentiated phenotype were disturbed in the transgenic pancreas. Toluidine blue staining of semi-thin (0.5 μm) sections of line AM3 pancreas showed that acini were replaced by ductular structures harboring transitional cells, characterized by ductal morphology, but containing zymogen granules (Figure 6E). Within the same structure, cells with remnant acinar morphology were present (Figure 6E). Dedifferentiation of acini into ductal structures as so-called 'tubular complexes' has been described in association with neoplastic and inflammatory conditions in the pancreas (Bockman, 1981). Tubular complexes were present in severely affected pancreatic lobules in line AM3 mice with increasing age (Table I; Figure 4G). These findings suggest that inactivation of TGF-β signaling in acinar cells *in vivo* is associated with dedifferentiation of acini into duct-like structures and tubular complexes. In addition, ductal cells may accumulate in association with high rates of acinar cell turnover (see Table II) in the transgenic pancreas.

Inactivation of TGF-β signaling results in a selective increase of expression of TGF-β isoforms

Because increased expression of matrix-associated genes and angiogenesis *in vivo* have been associated with increased TGF-β activity (Roberts *et al.*, 1986), we examined levels of expression of TGF-β isoforms. By Northern blot analysis, expression of TGF-β1 and TGF-β3 RNA were markedly increased in line AM3 pancreas, and TGF-β2 RNA levels were slightly increased (Figure 7A). Similar results were obtained at 1, 2, 6 and 10 months of age. When compared with primary acinar cells from FVB/N control littermates, primary acinar cells from line AM3 mice had increased levels of TGF-β1 RNA (Figure 7B). Immunostaining with TGF-β isoform-specific antibodies showed little staining for TGF-β1, TGF-β2 and TGF-β3 in the exocrine pancreas of FVB/N mice (Figure 8). However, TGF-β1 protein was markedly increased in acinar cells and aberrant non-acinar cells, and TGF-β3 protein was markedly increased exclusively in acinar cells in the pancreas of line AM3 (Figure 8). TGF-β2 staining was not changed (Figure 8). These results suggest that inactivation of TGF-β signaling in acinar cells *in vivo* leads to increased expression of TGF-β1 and TGF-β3 isoforms.

Discussion

Using a dominant-negative mutant receptor approach in transgenic mice, we have demonstrated for the first time the feasibility of inactivating TGF-β in select tissues in the whole animal. Specifically, we have shown that TGF-βs play an essential role in maintaining epithelial homeostasis and the differentiated phenotype in the exocrine pancreas, as demonstrated by severely perturbed cellular proliferation parameters and development of characteristic histologic abnormalities in the exocrine pancreas of two transgenic lines. While hepatocytes and acinar cells derived from the transgenic mice showed no growth inhibition or gene induction in response to any of the three TGF-β isoforms, growth inhibition induced by activin, the TGF-β superfamily member most closely related to TGF-β, was not affected. This confirms that the dominant-negative effect of this construct is confined to TGF-β1, 2 and 3.

Mechanisms underlying the disruption of homeostasis

Our data clearly indicate that TGF-β is an essential negative regulator of pancreatic acinar cell growth *in vivo*, consistent with *in vitro* data (Logsdon *et al.*, 1992). We observed no changes in hepatocyte proliferation, apoptosis or collagen deposition *in vivo* in the liver of DNR transgenic mice when compared with normal mice, despite total loss of responsiveness to TGF-β in DNR transgenic hepatocytes *in vitro*. This suggests that TGF-β may not be a direct inhibitor of hepatocyte proliferation in the normal liver, and that the relative contribution of endogenous TGF-βs to epithelial homeostasis may vary from organ to organ. Since it has been shown that transgenic overexpression of active TGF-β1 in the liver can suppress the early proliferative response after partial hepatectomy (Bottinger *et al.*, 1996), it is possible that TGF-β may play a role in growth control in the liver when proliferative homeostasis is perturbed. We are currently investigating this possibility. However, it is also possible that, despite the *in vitro* data, *in vivo* expression of the DNR construct in hepatocytes may not be adequate to inactivate the function of TβRII.

Concomitant with increased proliferation, we observed increased apoptosis in the transgenic acini throughout adult life. Induction of apoptosis has been shown to accompany enhanced cell proliferation resulting from many forms of perturbation of the cell cycle, including oncogene expression or inactivation of negative regulators of the cell cycle (Evan *et al.*, 1992; Jacks *et al.*, 1992; Howes *et al.*, 1994; Naik *et al.*, 1996; White, 1996). Since TGF-β actually induces apoptosis in many epithelial cell types (Oberhammer *et al.*, 1992; Hsing *et al.*, 1996), the increased apoptosis observed in DNR-positive acini most likely represents an appropriate response to the abnormal

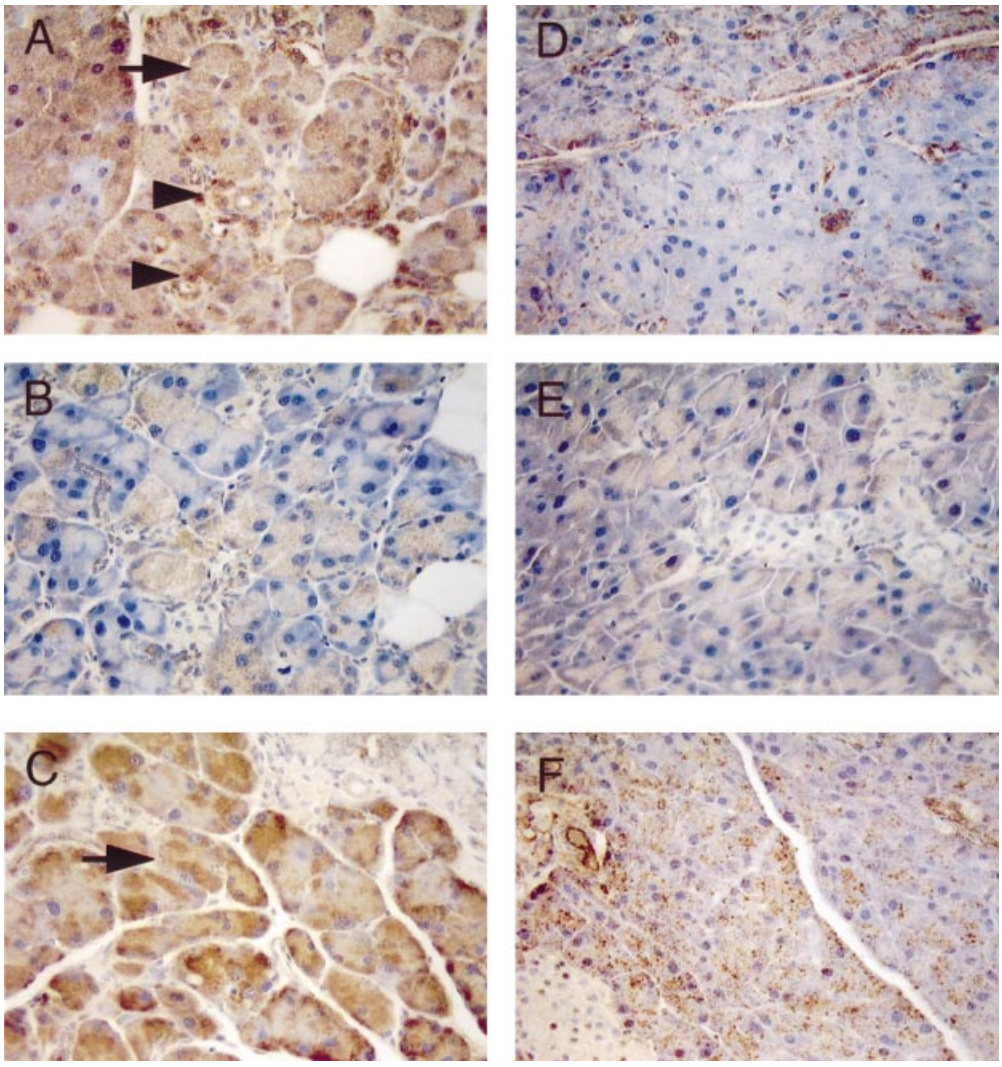

Fig. 8. *In situ* expression of TGF-β isoforms. Immunostaining for TGF-β1 (**A** and **D**), TGF-β2 (**B** and **E**) and TGF-β3 (**C** and **F**) in pancreas sections of 6-month-old transgenic line AM3 mice (A–C) and non-transgenic FVB/N littermates (D–E) (all ×40). Arrows show staining for TGF-β1 (A) and TGF-β3 (C) in acini in lobules with histologic changes; the arrowhead in (A) depicts TGF-β1 staining in aberrant non-acinar cells.

proliferation and differentiation, as opposed to a direct effect of loss of TGF-β function in these cells.

The increased numbers of primitive ductules in the transgenic pancreas at all ages, and the presence of tubular complexes, suggest that loss of negative growth regulation by TGF-β in the acinar cells may be incompatible with the acquisition or maintenance of the differentiated phenotype. It is well documented that acinar cells, which derive from ductal cells during normal development (Githens, 1993), can retrodifferentiate into cells with the ductal phenotype, forming ductules and tubular complexes in experimental models and human diseases associated with abnormal growth in the pancreas (Logsdon, 1995). Furthermore, when acinar cells are stimulated to grow *in vitro*, they dedifferentiate into a more fetal-like phenotype with ductal characteristics, while on cessation of growth, differentiated acinar markers are re-acquired (De Lisle and Logsdon, 1990). Thus acinar dedifferentiation can be a direct consequence of increased proliferation in this normally quiescent compartment.

Several lines of evidence support the conclusion in our transgenic model that the abnormally proliferating acinar cells may dedifferentiate into cells with a ductal phenotype. First, we have observed the presence of intermediate cell types with features of both acinar and ductal morphology (Figure 6). Similar intermediate cell types consistent with acinoductular retrodifferentiation have been seen using ultrastructural analysis in the pancreas of MT-TGF-α transgenic mice (Bockman and Merlino, 1992). Second, the affected lobules in the transgenic animals are characterized by a net loss of acinar cells, despite greatly increased proliferation in the acinar compartment. In contrast, a net gain of ductular cells occurs in these lobules without increased proliferation in the ductal compartment. These observations are most consistent with acinoductular metaplasia. It should be emphasized here that the ductular cells do not express the DNR transgene. Dedifferentiated ductular cells in tubular complexes of pancreas of MT-TGF-α transgenic mice also fail to express transgenic TGF-α, suggesting that the MT1 promoter is not expressed in ducts and ductules (Jhappan *et al.*, 1990). Hence, these cells may re-acquire responsiveness to TGF-β-mediated growth inhibition. Finally, the absence of inflammation or necrosis in most of the affected lobules suggests that

increased acinar proliferation and dedifferentiation result from loss of TGF-β function in the DNR transgenic pancreas, rather than as a secondary consequence of other pathologic changes.

Replacement of pancreatic acini within lobules by adipose tissue is often observed in humans and animals accompanying atrophy of acini from any cause including aging (Seifert, 1984; Eustis *et al.*, 1990). In the DNR transgenic pancreas, where acinar homeostasis is severely disturbed by abnormal proliferation, differentiation and apoptosis, the sometimes dramatic replacement of acini by intralobular adipose tissue is probably a non-specific consequence of the resulting acinar atrophy, and not directly related to loss of TGF-β function.

TGF-β autoregulation and carcinogenesis

Profound changes were observed in the stromal compartment of the DNR transgenic pancreas, including neoangiogenesis, fibrosis and low-level infiltration by macrophages. All these are similar to changes seen in other systems when TGF-β activity is increased (Roberts *et al.*, 1986; Pierce *et al.*, 1989), and consistent with the fibrosis and cellular infiltrates observed in the exocrine pancreas of mice transgenic for TGF-β1 expressed under the control of an insulin promoter (Lee *et al.*, 1995). We therefore examined the pancreas for expression of the three isoforms of TGF-β and found strikingly increased levels of TGF-β1 and TGF-β3 in transgenic acinar cells, indicating that inactivation of TGF-β signaling results in increased expression of TGF-β1 and TGF-β3 in these cells.

Previous *in vitro* studies in several TGF-β-responsive epithelial and fibroblast cell lines have indicated complex patterns of auto- and cross-regulation among TGF-β isoforms (Bascom *et al.*, 1989; Kim *et al.*, 1990). Here we show for the first time *in vivo* that lack of response to TGF-β and overexpression of TGF-β may be mechanistically related, and this may have important implications for understanding the roles of the TGF-β system at different stages in the carcinogenic process. While TGF-β is a growth inhibitor for most epithelia (Roberts and Sporn, 1990), and transgenic overexpression of TGF-β protects the mammary gland from tumorigenesis induced by chemical carcinogens or oncogenes (Pierce *et al.*, 1995; G.Merlino, J.Jakubczak and G.Smith, unpublished data), expression of TGF-β isoforms is actually increased in many advanced human cancers (Gorsch *et al.*, 1992; Friess *et al.*, 1993; Gold and Korc, 1994; Gold *et al.*, 1994). The elevated TGF-β is proposed to promote tumor progression, primarily through paracrine effects on stromal elements, such as increased angiogenesis and decreased immune surveillance. These observations are reconciled by the hypothesis that the TGF-β system has tumor suppressor activity early in tumorigenesis when the TGF-β response is intact, but has oncogenic effects later if the TGF-β response in the tumor cell is lost.

Recently, it has been shown that TβRII is inactivated or deleted in several different types of human tumor (Markowitz and Roberts, 1996). Our data suggest this may have two deleterious consequences, namely (i) loss of response of the tumor cell to the growth inhibitory effects of TGF-β, and (ii) consequent up-regulation of the expression of TGF-β protein, which can then further promote tumorigenesis through unwanted paracrine effects on adjacent, TGF-β-responsive stromal tissue. Clearly, it will be important to determine whether the up-regulation of TGF-β that we see in the pancreatic acinar cells when TGF-β responsiveness is abolished, is also observed in other cell types.

Implications for pancreatic cancer

Our observations of perturbed proliferation and differentiation in the transgenic pancreas may have important implications for our understanding of pancreatic carcinogenesis in humans. Pancreatic cancers in humans exhibit ductal morphology in most cases, but it is controversial whether the duct cell is the origin of pancreatic cancer. Tumors with ductal morphology in several rodent models of pancreatic carcinogenesis may have their origin in hyperproliferative and dysplastic acinar cells (Flaks, 1984; Scarpelli *et al.*, 1984). Recent studies using transgenic mice with overexpression of mitogenic oncogenes such as TGF-α, c-myc and SV40 T antigen in acinar cells directly support the potential of acinar cells to form tumors with ductal characteristics (Ornitz *et al.*, 1987; Jhappan *et al.*, 1990; Sandgren *et al.*, 1990, 1991). Furthermore, cells of mixed acinar–ductular morphology have been observed in human pancreatic carcinoma specimens (Parsa *et al.*, 1985). These data are consistent with the hypothesis that pancreatic cancer could arise from dedifferentiated acinar cells (Logsdon, 1995). Thus loss of TGF-β function and associated acinar dedifferentiation may represent a first step on the pathway to pancreatic neoplasia. Interestingly, recent genetic studies have shown that >50% of pancreatic cancers in humans have defects in the gene *DPC-4* (Hahn *et al.*, 1996), which encodes an Mad-related protein involved in TGF-β signaling (Lagna *et al.*, 1996; Zhang *et al.*, 1996). Furthermore, expression of TβRI was attenuated in some pancreatic cancers with resistance to TGF-β-mediated growth inhibition (Baldwin *et al.*, 1996). This is further evidence for the critical role which TGF-β plays in pancreatic homeostasis.

Eosinophilic foci of acinar cells were observed in a significant number (21%) of DNR mice. In rats, eosinophilic foci indicate acinar cell hyperplasia and may represent early stages of the neoplastic process (Eustis *et al.*, 1990). Hence, eosinophilic foci exclusively found in pancreas of DNR mice may represent early hyperplastic lesions with potential for malignant progression. The lack of such neoplastic progression in the pancreas of DNR transgenic mice, despite persistent hyperproliferation, focus formation and dedifferentiation in TGF-β-unresponsive acini, indicates that additional defects may be required for carcinogenesis to proceed. Alternatively, it may reflect the fact that transgene expression is lost when the cells dedifferentiate to a ductular morphology, and the growth inhibitory response is restored. However, mice bitransgenic for both the DNR and the hepatocyte growth factor (HGF) in the acinar cells do develop pancreatic cancer, which is not seen in mice expressing either transgene alone (J.L.Jakubczak and G.Merlino, unpublished data). These preliminary results indicate that loss of TGF-β function in the exocrine pancreas may enhance susceptibility to tumorigenesis, and that the DNR transgenic mice may represent a useful model for studying the role of TGF-β in pancreatic carcinogenesis *in vivo*.

Materials and methods

Construction of plasmids and transgenic mice

Human TβRII sequences between nucleotides –7 and +573, encoding extracellular and transmembrane domains followed by a stop codon at the 3′ end, were generated by PCR, ligated into the pCR vector (Invitrogen) to generate the construct pCRDNR and sequenced in their entirety. A 600 bp *Eco*RI fragment of pCRDNR containing DNR cDNA was blunt-ended and subcloned into the *Nru*I site of pMT-LCR2999B4 (Palmiter *et al.*, 1993) to generate pMT-LCR-DNR. Expression of the transgene is driven by a mouse MT1 promoter, and regulated by the presence of MT LCRs (Palmiter *et al.*, 1993). A 19.1 kb *Sal*I MT-LCR-DNR linear fragment (Figure 1A) was isolated and used for microinjections of zygote pronuclei. Transgenic mice were generated using inbred FVB/N zygotes as described previously (Jhappan *et al.*, 1990). Of 19 mice born, two were positive for MT-LCR-DNR and were bred into lines AM3 and BF1. Both lines displayed autosomal inheritance of the transgene. Tail genomic DNA was prepared and tested either by Southern blotting (founder mice) or PCR analysis (subsequent generations) (Jhappan *et al.*, 1990). Animals were cared for in accordance with NIH guidelines. When indicated, the activity of the MT1 promoter was induced by maintaining the animals on drinking water containing 25 mM $ZnSO_4$.

RNA analysis

RNA was isolated from mouse tissues by guanidium isothiocyanate extraction and column purification using the RNeasy kit (Qiagen) as described (Bonham and Danielpour, 1996). For Northern blot analysis, RNA was electrophoresed on 1% agarose gels and transferred to a filter. Filters were then hybridized with ^{32}P-labeled probes: human TβRII from pCRDNR, mouse TGF-β1, mouse TGF-β2 and mouse TGF-β3.

Preparation of pancreatic acini and primary hepatocyte cultures

Pancreatic acini were isolated from adult non-transgenic FVB/N and line AM3 mice as described (Logsdon and Williams, 1983). For proliferation studies, insulin (1 μM) and epidermal growth factor (1 nM) were added to the culture medium. Hepatocytes were isolated from 8-week-old male FVB/N or line AM3 mice by a two-step collagenase perfusion of the liver followed by isodensity centrifugation in Percoll (viability >95%) as previously described (Bottinger *et al.*, 1996).

Proliferation assays and fibronectin secretion assays

DNA synthesis in primary acinar preparations and primary hepatocytes was measured after incubation of cells with [^{3}H]thymidine between 48 and 72 h after plating, followed by trichloroacetic acid (TCA) precipitation of cell extracts as described (Bottinger *et al.*, 1996). [^{3}H]Thymidine incorporation was measured in a liquid scintillation counter and normalized for DNA content per plate. Recombinant human TGF-β1, porcine TGF-β2 and recombinant human TGF-β3 (all R&D Systems) and recombinant human activin (Genentech), were added between 24 and 72 h after cell plating as indicated.

Fibronectin production by hepatocytes was measured as described (Wrana *et al.*, 1992). Fibronectin band intensities were quantified by densitometry and relative amounts expressed as x-fold difference compared with fibronectin secreted by untreated cells.

Affinity labeling and immunoprecipitation

Porcine TGF-β1 was iodinated as described (Frolik *et al.*, 1984) and used for the labeling of cell surface binding proteins on freshly prepared primary hepatocytes and acinar preparations as described (Geiser *et al.*, 1992). Equal amounts of protein were incubated overnight at 4°C with 1.5 μg/ml of rabbit polyclonal antibodies in 500 μl of lysis buffer or rabbit IgG, respectively. Antigen–antibody complexes were precipitated with protein A–Sepharose and eluted by boiling in 2× SDS sample loading buffer (Novex) containing 5% β-mercaptoethanol. Samples were subjected to SDS–PAGE, and complexes of [^{125}I]TGF-β1 ligand and bound receptors demonstrated by autoradiography.

Histology and immunohistochemistry

Tissues were fixed in 10% formaldehyde or 70% ethanol, as indicated, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E), toluidine blue or reticulin by standard methods. The extent of fibrosis was quantified using the Leitz Quantimat 600 Image analysis system, in which the percentage area of positive stain was determined by gray scale analysis. Immunohistochemical staining was performed using an indirect immunoperoxidase detection protocol (Vectastain Elite kit, Vector Laboratories). DNR was detected in ethanol-fixed tissue sections after antigen retrieval with microwave treatment by polyclonal anti-human TβRII (residues 1–28) (Upstate Biotechnology). All the following staining was performed on 10% formaldehyde-fixed tissue sections. TGF-β1 was detected with the rabbit polyclonal antibody LC(1-30) (Flanders *et al.*, 1989). TGF-β2 and β3 were detected with rabbit polyclonal antibodies TGF-β2(50-75-2) (Flanders *et al.*, 1990) and TGF-β3(50-60-3) (Flanders *et al.*, 1991). PCNA was detected by mouse monoclonal antibody PC10 (Dako). Macrophage glycoprotein Mac-2 was detected by mononclonal anti-Mac-2 antibody (ATCC). AE1/AE3 antibody (BioGenex Laboratories) was used to detect cytokeratins 1–8, 10, 14, 15, 16 and 19. von Willebrand factor was detected with a polyclonal anti-factor VIII antibody (Dako). For cytokeratin and von Willebrand factor staining, antigen retrieval was performed by treating sections for 12 min with 0.1% trypsin and 0.1% calcium chloride in Tris-buffered saline (TBS).

In vivo proliferation and apoptosis

PCNA-positive acinar cells per high power field (HPF) (0.07 mm^2) were counted. The mean of 10 fields was calculated and the number of PCNA-positive acinar cells/mm^2 computed. In some animals, the ratio of PCNA-positive and total number of acinar cells was calculated. The percentage of proliferating ductular cells in line AM3 pancreas represents the average ratio of the number of PCNA-positive and total ductular cells counted in 10 HPFs (0.07 mm^2) per pancreas. Apoptotic cells were identified on H&E-stained slides by a combination of pyknotic nucleus and condensed eosinophilic cytoplasm. A collection of apoptotic debris phagocytosed by another cell was counted as a single apoptotic cell. Milder changes of apoptosis such as cytoplasmic condensation and clumped hyperchromatic chromatin were not counted. Apoptotic cells per HPF (0.2 mm^2) were counted in 20 fields. The mean of apoptotic cells in 20 HPFs was used to compute the number of apoptotic cells/mm^2. In addition, we assessed apoptosis in the liver using a TUNEL labeling method (Trevigen).

Acknowledgements

We thank Drs Miriam Anver (NCI-FCRDC, Frederick, MD), Dale Bockman (Medical College of Georgia, GA) and Nora Sarvetnick (Scripps Institute, La Jolla, CA) for useful discussions. We thank Dr Craig Logsdon (University of Michigan, Ann Arbor, MI) for advice in establishing pancreatic acinar cell cultures. We thank Drs Murray Korc (University of California, Irvine, CA) and Anita Roberts (NCI, Bethesda, MD) for critical reading of the manuscript. We are grateful to Genentech, Inc. (South San Francisco, CA) for the gift of recombinant human activin, to Dr Richard Palmiter (University of Washington, Seattle, WA) for the pMT-LCR plasmids and to Heather Winbrow for technical assistance.

References

Baldwin,R.L., Friess,H., Yokohama,M., Lopez,M.E., Kobrin,M.S., Buchler,M.W. and Korc,M. (1996) Attenuated ALK5 receptor expression in human pancreatic cancer: correlation with resistance to growth inhibition. *Int. J. Cancer*, **67**, 283–288.

Bascom,C.C., Wolfshohl,J.R., Coffey,R.J.,Jr, Madisen,L., Webb,N.R., Purchio,A.R., Derynck,R. and Moses,H.L. (1989) Complex regulation of transforming growth factor beta 1, beta 2 and beta 3 mRNA expression in mouse fibroblasts and keratinocytes by transforming growth factors beta 1 and beta 2. *Mol. Cell. Biol.*, **9**, 5508–5515.

Bockman,D.E. (1981) Cells of origin of pancreatic cancer: experimental animal tumors related to human pancreas. *Cancer*, **47**, 1528–1534.

Bockman,D.E. and Merlino,G. (1992) Cytological changes in the pancreas of transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology*, **103**, 1883–1892.

Bonham,M.J. and Danielpour,D. (1996) Improved purification and yields by RNeasy. *BioTechniques*, **21**, 57–60.

Brand,T., MacLellan,W.R. and Schneider,M.D. (1993) A dominant-negative receptor for type beta transforming growth factors created by deletion of the kinase domain. *J. Biol. Chem.*, **268**, 11500–11503.

Bottinger,E.P. *et al*. (1996) The recombinant proregion of transforming growth factor β1 (latency-associated peptide) inhibits active transforming growth factor β1 in transgenic mice. *Proc. Natl Acad. Sci. USA*, **93**, 5877–5882.

Chen,R.H., Ebner,R. and Derynck,R. (1993) Inactivation of the type II receptor reveals two receptor pathways for the diverse TGF-beta activities. *Science*, **260**, 1335–1338.

Cui,W., Fowlis,D.J., Cousins,F.M., Duffie,E., Bryson,S., Balmain,A. and Akhurst,R.J. (1995) Concerted action of TGF-beta 1 and its type II receptor in control of epidermal homeostasis in transgenic mice. *Genes Dev.*, **9**, 945–955.

De Lisle,R.C. and Logsdon,C.D. (1990) Pancreatic acinar cells in culture: expression of acinar and ductal antigens in a growth-related manner. *Eur. J. Cell Biol.*, **51**, 64–75.

Eustis,S.L., Boorman,G.A. and Hayashi,Y. (1990) Exocrine pancreas. In Boorman,G.A., Eustis,S.L., Elwell,M.R., Montgomery,C.A. and MacKenzie,W.F. (eds), *Pathology of the Fischer Rat.* Academic Press, Inc., San Diego, pp. 95–108.

Evan,G.I., Wyllie,A.H., Gilbert,C.S., Littlewood,T.D., Land,H., Brooks,M., Waters,C.M., Penn,L.Z. and Hancock,D.C. (1992) Induction of apoptosis in fibroblasts by c-myc protein. *Cell*, **69**, 119–128.

Flaks,B. (1984) Histogenesis of pancreatic carcinogenesis in the hamster: ultrastructural evidence. *Environ. Health Perspect.*, **56**, 187–203.

Flanders,K.C., Thompson,N.L., Cissel,D.S., Van Obberghen-Schilling,E., Baker,C.C., Kass,M.E., Ellingsworth,L.R., Roberts,A.B. and Sporn,M.B. (1989) Transforming growth factor-beta 1: histochemical localization with antibodies to different epitopes. *J. Cell Biol.*, **108**, 653–660.

Flanders,K.C., Cissel,D.S., Mullen,L.T., Danielpour,D., Sporn,M.B. and Roberts,A.B. (1990) Antibodies to transforming growth factor-beta 2 peptides: specific detection of TGF-beta 2 in immunoassays. *Growth Factors*, **3**, 45–52.

Flanders,K.C., Ludecke,G., Engels,S., Cissel,D.S., Roberts,A.B., Kondaiah,P., Lafyatis,R., Sporn,M.B. and Unsicker,K. (1991) Localization and actions of transforming growth factor-betas in the embryonic nervous system. *Development*, **113**, 183–191.

Friess,H., Yamanaka,Y., Buchler,M., Ebert,M., Beger,H.G., Gold,L.I. and Korc,M. (1993) Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology*, **105**, 1846–1856.

Frolik,C.A., Wakefield,L.M., Smith,D.M. and Sporn,M.B. (1984) Characterization of a membrane receptor for transforming growth factor-beta in normal rat kidney fibroblasts. *J. Biol. Chem.*, **259**, 10995–11000.

Geiser,A.G., Burmester,J.K., Webbink,R., Roberts,A.B. and Sporn,M.B. (1992) Inhibition of growth by transforming growth factor-beta following fusion of two nonresponsive human carcinoma cell lines. Implication of the type II receptor in growth inhibitory responses. *J. Biol. Chem.*, **267**, 2588–2593.

Githens,S. (1993) Differentiation and development of the pancreas in animals. In *The Pancreas: Biology, Pathobiology and Disease*. Go,V.L.W., DiMagno,E.P., Gardner,J.D., Lebenthal,E., Reber,H.A. and Scheele,G.A. (eds), Raven Press, New York, pp. 21–55.

Gold,L.I. and Korc,M. (1994) Expression of transforming growth factor-β1, 2 and 3 mRNA and protein in human cancers. *Digest. Surg.*, **11**, 150–156.

Gold,L.I., Saxena,B., Mittal,K.R., Marmor,M., Goswami,S., Nactigal,L., Korc,M. and Demopoulos,R.I. (1994) Increased expression of transforming growth factor beta isoforms and basic fibroblast growth factor in complex hyperplasia and adenocarcinoma of the endometrium: evidence for paracrine and autocrine action. *Cancer Res.*, **54**, 2347–2358.

Gorsch,S.M., Memoli,V.A., Stukel,T.A., Gold,L.I. and Arrick,B.A. (1992) Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. *Cancer Res.*, **52**, 6949–6952.

Hahn,S.A. *et al.* (1996) *DPC4*, a candidate tumor supressor gene at chromosome 18q21.1. *Science*, **271**, 350–353.

Howes,K.A., Ransom,N., Papermaster,D.S., Lasudry,J.G.H., Albert,D.M. and Windle,J.J. (1994) Apoptosis or retinoblastoma: alternative fates of photoreceptors expressing the HPV-16 E7 gene in the presence or absence of p53. *Genes Dev.*, **8**, 1300–1310.

Hsing,A.Y., Kadomatsu,K., Bonham,M.J. and Danielpour,D. (1996) Regulation of apoptosis induced by transforming growth factor-β1 in nontumorigenic and tumorigenic rat prostatic epithelial cell lines. *Cancer Res.*, **56**, 5146–5149.

Jacks,T., Fazeli,A., Schmitt,E.M., Bronson,R.T., Goodell,M.A. and Weinberg,R.A. (1992) Effects of an Rb mutation in the mouse. *Nature*, **359**, 295–300.

Jhappan,C., Stahle,C., Harkins,R.N., Fausto,N., Smith,G.H. and Merlino,G.T. (1990) TGF alpha overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell*, **61**, 1137–1146.

Kim,S.J., Angel,P., Lafyatis,R., Hattori,K., Kim,K.Y., Sporn,M.B., Karin,M. and Roberts,A.B. (1990) Autoinduction of transforming growth factor beta 1 is mediated by the AP-1 complex. *Mol. Cell. Biol.*, **10**, 1492–1497.

Kulkarni,A.B. *et al.* (1993) Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl Acad. Sci. USA*, **90**, 770–774.

Lagna,G., Hata,A., Hemmati-Brivanlou,A. and Massague,J. (1996) Partnership between DPC4 and SMAD proteins in TGF-β signalling pathways. *Nature*, **383**, 832–836.

Lee,M.S. *et al.* (1995) Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor-beta 1. *Am. J. Pathol.*, **147**, 42–52.

Logsdon,C.D. (1995) Pancreatic duct cell cultures: there is more to ducts than salty water. *Gastroenterology*, **109**, 1005–1009.

Logsdon,C.D. and Williams,J.A. (1983) Pancreatic acini in short-term culture: regulation by EGF, carbachol, insulin and corticosterone. *Am. J. Physiol.*, **244**, G675–G682.

Logsdon,C.D., Keyes,L. and Beauchamp,R.D. (1992) Transforming growth factor-beta (TGF-beta 1) inhibits pancreatic acinar cell growth. *Am. J. Physiol.*, **262**, G364–G368.

Markowitz,S.D. and Roberts,A.B. (1996) Tumor suppressor activity of the TGF-β pathway in human cancers. *Cytokine Growth Factor Rev.*, **1**, 93–102.

Millan,F.A., Denhez,F., Kondaiah,P. and Akhurst,R.J. (1991) Embryonic gene expression patterns of TGF beta 1, beta 2 and beta 3 suggest different developmental functions *in vivo*. *Development*, **111**, 131–143.

Naik,P., Karrim,J. and Hanahan,D. (1996) The rise and fall of apoptosis during multistage tumorigenesis: down-modulation contributes to tumor progression from angiogenic progenitors. *Genes Dev.*, **10**, 2105–2116.

Nathan,C. and Sporn,M. (1991) Cytokines in context. *J. Cell Biol.*, **113**, 981–986.

Oberhammer,F.A., Pavelka,M., Sharma,S., Tiefenbacher,R., Purchio,A.F., Bursch,W. and Schulte-Hermann,R. (1992) Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. *Proc. Natl Acad. Sci. USA*, **89**, 5408–5412.

Ornitz,D.M., Hammer,R.E., Messing,A., Palmiter,R.D. and Brinster,R.L. (1987) Pancreatic neoplasia induced by SV40 T-antigen expression in acinar cells of transgenic mice. *Science*, **238**, 188–193.

Palmiter,R.D., Sandgren,E.P., Koeller,D.M. and Brinster,R.L. (1993) Distal regulatory elements from the mouse metallothionein locus stimulate gene expression in transgenic mice. *Mol. Cell. Biol.*, **13**, 5266–5275.

Parsa,I., Longnecker,D.S., Scarpelli,D.G., Pour,P., Reddy,J.K. and Lefkowitz,M. (1985) Ductal metaplasia of human exocrine pancreas and its association with carcinoma. *Cancer Res.*, **45**, 1285–1290.

Pierce,D.F.,Jr, Gorska,A.E., Chytil,A., Meise,K.S., Page,D.L., Coffey,R.J.,Jr and Moses,H.L. (1995) Mammary tumor suppression by transforming growth factor beta 1 transgene expression. *Proc. Natl Acad. Sci. USA*, **92**, 4254–4258.

Pierce,G.F., Mustoe,T.A., Lingelbach,J., Masakowski,V.R., Griffin,G.L., Senior,R.M. and Deuel,T.F. (1989) Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. *J. Cell Biol.*, **109**, 429–440.

Roberts,A.B. *et al.* (1986) Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc. Natl Acad. Sci. USA*, **83**, 4167–4171.

Roberts,A.B. and Sporn,M.B. (1990) The transforming growth factors-β. In Sporn,M.B. and Roberts,A.B. (eds), *Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors*. Springer-Verlag, Berlin, pp. 419–472.

Sandgren,E.P., Luetteke,N.C., Palmiter,R.D., Brinster,R.L. and Lee,D.C. (1990) Overexpression of TGF-alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia and carcinoma of the breast. *Cell*, **61**, 1121–1135.

Sandgren,E.P., Quaife,C.J., Paulovich,A.G., Palmiter,R.D. and Brinster,R.L. (1991) Pancreatic tumor pathogenesis reflects the causative genetic lesion. *Proc. Natl Acad. Sci. USA*, **88**, 93–97.

Scarpelli,D.G., Rao,M.S. and Reddy,J.K. (1984) Studies of pancreatic carcinogenesis in different animal models. *Environ. Health Perspect.*, **56**, 219–227.

Seifert,G. (1984) Lipomatous atrophy and other forms. In Kloppel,G. and Heitz,P.U. (eds), *Pancreatic Pathology.* Churchill Livingstone, Edinburgh, pp. 27–31.

White,E. (1996) Life, death and the pursuit of apoptosis. *Genes Dev.*, **10**, 1–15.

Wrana,J.L., Attisano,L., C:arcamo,J., Zentella,A., Doody,J., Laiho,M., Wang,X.F. and Massague,J. (1992) TGF beta signals through a heteromeric protein kinase receptor complex. *Cell*, **71**, 1003–1014.

Wrana,J.L., Attisano,L., Wieser,R., Ventura,F. and Massague,J. (1994) Mechanism of activation of the TGF-beta receptor. *Nature*, **370**, 341–347.

Zhang,Y., Feng,X.H., Wu,R.Y. and Derynck,R. (1996) Receptor-associated Mad homologues synergize as effectors of the TGF-β response. *Nature*, **383**, 168–172.

Received on November 19, 1996; revised on February 11, 1997