Virchows Arch (1996) 427:595–606

ORIGINAL ARTICLE

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Development of colonic and pancreatic endocrine tumours in mice expressing a glucagon-SV40 T antigen transgene

Received: 28 August 1995 / Accepted: 11 December 1995

Abstract We report the histological, immunohistochemical and ultrastructural changes in mice containing a chimeric glucagon-simian virus 40 T antigen (SV40Tag) gene. Transgene expression was detected in endocrine cells of pancreas, small and large intestine. Hyperplasia of glucagon-containing cells developed in pancreas and large bowel by gestational day 19. In large bowel, hyperplastic cells increased in number postnatally and invasive carcinomas were identified at 4 weeks; several animals had lymph node metastases. In contrast, no pathology was detected in the small bowel in any of the transgenic mice. Colonic tumours expressed SV40Tag, proglucagon-derived peptides and peptide YY (PYY); scattered cells contained cholecystokinin or glycoprotein hormone α-subunit. Somatostatin or serotonin was also detected in some tumours. By electron microscopy, the colonic tumours retained features of endocrine differentiation, but secretory granules were smaller than those of non-tumorous intestinal glucagon-producing L cells. In postnatal pancreas, atypical cells containing SV40Tag and glucagon were initially clustered at the periphery of islets; this atypical hyperplasia progressed to neoplasia by 11–12 weeks. Some neoplastic pancreatic cells contained glucagon, PYY or vasoactive intestinal peptide immunopositivity, but most were negative for all peptides; they contained immunoreactivity for tyrosine hydroxylase and by electron microscopy, pancreatic tumour cells had neuronal features. Pancreatic polypeptide was not detected in the non-tumorous islets of transgenic animals. This line of transgenic mice provides a model for the analysis of endocrine tumour progression in the gut and pancreas.

Key words Transgenic mice · Glucagon · Colon carcinoma · Pancreatic endocrine carcinoma · Morphology

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Introduction

Transgenic mice permit the development of models of disease by the expression of specific genomic sequences under the regulatory control of different promoters. These animal models have provided insight into several aspects of human disease including cytogenesis of tumours, the control of tissue specific expression and oncogenesis [11, 24, 27]. Neuroendocrine tumours have arisen in transgenic mice which express oncogenes in specific neuroendocrine cells under the control of hormone gene regulatory sequences [2, 16, 17, 23, 33, 40–42]. These transgenic models have provided information about the anatomical origin, secretory properties and clinical behaviour of neuroendocrine tumours in vivo and have also been used for the establishment of cell lines in vitro.

Whereas several lines of transgenic mice that develop pancreatic endocrine tumours have been described [5, 16, 17, 23, 40–42], there are fewer models of intestinal endocrine tumorigenesis [22, 40, 42]. The glucagon gene is expressed in A cells of the pancreatic islets of Langerhans, in the L cells of the intestine and in the central nervous system [14, 15, 21, 43]. However, expression of the SV40 T antigen in glucagon cells directed by ≈1300 base pairs of the rat glucagon 5′-flanking region resulted only in transgene expression in brain and pancreas, but not in the gastrointestinal tract [17]. We therefore generated transgenic mice containing ≈2000 base pairs of the glu-

cagon gene 5′-flanking sequence upstream of the SV40 T antigen. As we have previously reported, these animals developed endocrine carcinomas in both pancreas and large bowel [30].

We now report the detailed morphological features of the lesions by histology, immunohistochemistry and electron microscopy. The results of these analyses shed light on the process of endocrine tumour progression in the gut and provide insight into the ontogeny and cytodifferentiation of gut and pancreatic endocrine cells.

Materials and methods

Generation of transgenic mice

For transgene (GLUTag) construction, a 2.0 kilobase rat glucagon gene *Eco*RI/*Acc*I fragment, which included the 5′-flanking sequences and 58 base pairs of exon 1, was ligated to a *Bgl*II/*Bam*HI fragment of SV40 T antigen coding sequences as described previously [30]. Transgenic mice were generated in the CD strain by the method of Hogan et al. [26]. To identify transgenic pups in a litter, tail DNA was analysed for the presence of the GLUTag transgene by Southern blot. Expression of the transgene led to elevated plasma levels of glucagon and its related peptides, glicentin and oxyntomodulin; the detailed results of biochemical analyses have been reported elsewhere [10].

Morphological methods

Transgenic mice and age- and sex-matched controls were sacrificed by cardiac puncture and autopsied. Animals were examined at fetal day 19 (n=10), postnatal days 3 (n=5) and 7 (n=7), and weekly up to 14 weeks of age (n=50).

For light microscopy, fresh tissue was fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections 5 μm thick were stained with haematoxylin-eosin and the periodic acid-Schiff (PAS) technique; sections of bowel were also stained with alcian blue (AB) for mucin detection.

Immunohistochemical stains to localize cytoplasmic antigens and endocrine peptides were performed with the avidin-biotin-peroxidase complex technique on paraffin sections. The duration of exposure to primary antiserum was 24 h at 4°C.

Primary antisera were directed against the following antigens and were used at the specified dilutions: neuron specific enolase (NSE) (Dako Corporation, Santa Barbara, Calif.) 1:400; insulin (Dako) 1:1000; glucagon (Diagnostic Products Corp., Los Angeles, Calif.) prediluted; glucagon-like peptide-1 (GLP-1) (prepared by D.J.D.) 1:1000; peptide YY (PYY) (Penninsula Laboratories Inc., Belmont, Calif.) 1:1000; somatostatin (donated by Dr. S. Reichlin, Tufts University, Boston, Mass.) 1:2000; pancreatic polypeptide (PP) (Dako) 1:2000; calcitonin (Dako) 1:2000; bombesin and cholecystokinin (Serotec, Oxon, England) 1:2000 and 1:5000 respectively; serotonin (Immunonuclear Corporation, Stillwater, Minn.) 1:4000; gastrin (Diagnostic Products Corp.) prediluted; vasoactive intestinal peptide (VIP) (Incstar Corporation, Stillwater, Minn.) 1:400; α-subunit of glycoprotein hormones (Biogenex Laboratories, San Ramon, Calif.) 1:1000; adrenocorticotropic hormone (ACTH) (donated by Dr. A.F. Parlow, Pituitary Hormone Distribution Program, NIADDK, Bethesda, Md.) 1:2000; β-endorphin (donated by Dr. P. Petrusz, University of North Carolina, Chapel Hill , N.C.) 1:1000; corticotropin-releasing hormone (CRH) (donated by Dr. W. Vale, The Salk Institute, La Jolla, Calif.) 1:100. Monoclonal antibodies were used to localize chromogranin A (Enzo Diagnostics Inc., New York, N.Y.) 0.48 mg/ml; synaptophysin (Dako) 43 μg/ml; growth hormone-releasing hormone (GRH) (donated by Dr. T. Sano, University of Tokushima Medical School, Toskushima, Japan) 1:30; neurofilaments (Sanbio, Uden, The Netherlands) 1:10; tyrosine hydroxylase (Boehringer-Mannheim, Mannheim, Germany) 1:80; and carcinoembryonic antigen (CEA) (Zymed Laboratories Inc., San Francisco, Calif.) 1:20.

To localize SV40 large T antigen, fresh tissue was snap-frozen in liquid nitrogen; frozen sections 5 μm thick were mounted on glass slides and fixed in acetone. The indirect immunoperoxidase technique was performed with the monoclonal antibody MAB-419 (a generous gift of Dr. A. Levine, Princeton, N.Y.), at a dilution of 1:20, with incubation for 1 h at room temperature.

For both methods of immunolocalization, the reaction was visualized using 3,3′-diaminobenzidine and hydrogen peroxide. Appropriate positive and negative controls were performed in each case.

For transmission electron microscopy, small pieces of tissue were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded ethanols and propylene oxide and embedded in an Epon-Araldite mixture. Semithin sections were stained with toluidine blue; ultrathin sections of selected areas were stained with uranyl acetate and lead citrate and examined with a Philips 410LS electron microscope.

Results

Three different transgenic lines demonstrated identical tissue-specific patterns of GLUTag gene expression as assessed by Northern blot, PCR and immunohistochemical analyses. Transgene expression was detected in the brain, pancreas and intestine of all three lines as reported previously [30]. The detailed morphological studies reported herein were obtained from one line (GLUTag Y). Transgenic mice were significantly smaller than their wild-type littermates [30]. Their plasma levels of glucagon and glucagon-related peptides were markedly elevated [10]. The mice became wasted and died 4–12 weeks after birth from bowel obstruction.

Gross findings

Autopsies of transgenic mice revealed gross pathology only in the large intestine. By 4–8 weeks of age, there was marked caecal dilatation and the large bowel was thickened from the caecum to the rectum.

Histological and immunohistochemical findings

In fetuses at day 19 of gestation, no morphological alteration was detected in any tissue with haematoxylin and eosin. However, immunohistochemical stains revealed that the number of GLP-1 immunoreactive cells in large bowel, especially in the caecum, was increased over that in normal controls (Fig. 1a,b); the scattered individual cells and small clusters of cells containing GLP-1 were confined to the epithelium of mucosal crypts. SV40 T antigen was localized in the nuclei of scattered cells in the same areas. Glucagon-immunoreactive cells were also more prominent in the developing pancreases of transgenic mice at day 19 of gestation than in non-transgenic littermates (Fig. 1c,d); these cells also demonstrated nuclear reactivity for SV40 T antigen. The distribution and number of cells containing other pancreatic hor-

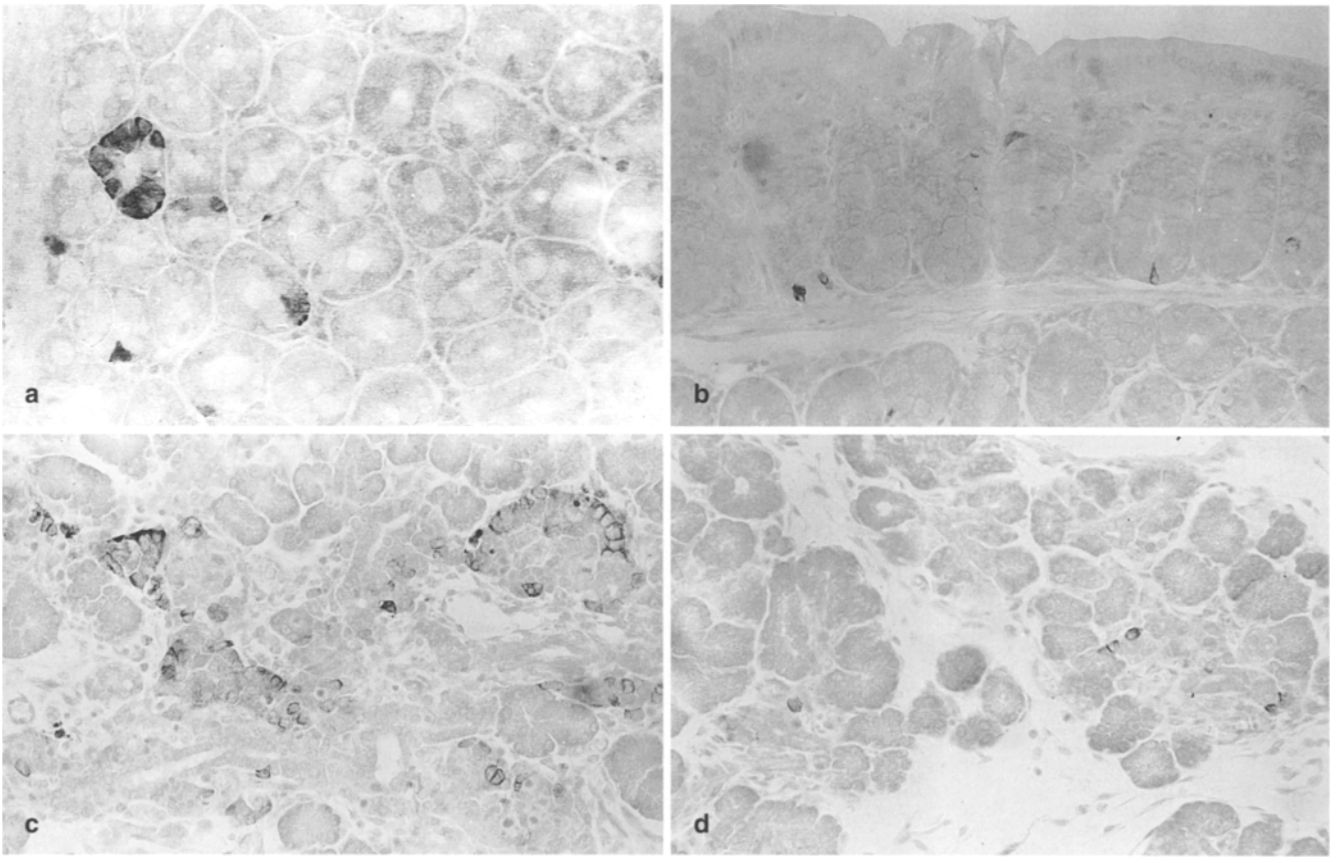

Fig. 1 In the large bowel of a glucagon-SV40 transgenic fetus at day 19 of gestation (**a**), the number of GLP-1 immunoreactive cells is greater than the number seen in a non-transgenic littermate (**b**). At the same stage of gestation, the pancreas of a transgenic animal (**c**) contains more numerous cells immunoreactive for glucagon than in the pancreas of a non-transgenic littermate (**d**). Avidin-biotin-peroxidase technique, ×200

mones was normal, with the exception of PP which was not consistently detected. The small bowel of transgenic mice at fetal day 19 had no immunohistochemical evidence of hyperplasia of glucagon- or GLP-1 containing cells.

Animals at 3 and 7 days following birth demonstrated progressive epithelial cell proliferation in the large bowel. No discrete lesion was discernable by conventional histology; however, mitotic figures were prominent in crypt epithelium. Immunohistochemistry demonstrated a large number of GLP-1 and SV40 T antigen immunoreactive cells within the mucosal crypts (Fig. 2a,b). In some crypts, they were the dominant cell type, and in a few, they entirely encircled the lumen; in other areas, they formed solid nests surrounded by basement membrane. In 2- and 3-week-old animals, there was invasion of the basement membrane of crypts by individual cells with pleomorphic nuclei and numerous mitoses; clusters of those cells were found within the lamina propria. These cells demonstrated strong nuclear staining for SV40 T antigen and cytoplasmic immunopositivity for GLP-1.

In the large bowel of animals 4 weeks of age or older, the lamina propria was thickened and filled with nests and solid sheets of epithelial cells (Fig. 2c,d), which infiltrated through the muscularis propria (Fig. 2e) and beyond the serosa. Twelve of 38 animals with colonic tumours had metastases in pericolonic and para-aortic lymph nodes (Fig. 2f). The tumour cells had pleomorphic nuclei with multiple nucleoli, occasional intranuclear inclusions, and strong positivity for SV40 T antigen (Fig. 2b,e). Tumour cells contained immunoreactivity for NSE and synaptophysin but not chromogranin. They stained intensely for GLP-1 and PYY (Fig. 2a,d) and were moderately positive for glucagon. In most animals, clusters of tumour cells stained for cholecystokinin and scattered cells contained the α-subunit of glycoprotein hormones. In three animals, the tumour had focal somatostatin positivity and in two, serotonin was localized in occasional tumour cells. Immunohistochemical stains for insulin, PP, VIP, gastrin, calcitonin, bombesin, ACTH, β-endorphin, CRH and GRH were negative in the tumours but were positive in the normal mouse control tissues. There was no evidence of exocrine differentiation; the tumours were entirely negative for mucin using the PAS and AB stains and did not contain immunoreactivity for CEA.

The pancreases of young animals 3, 7 and 14 days of age contained islets with occasional single pleomorphic cells at the periphery. These cells increased in number with age and in 4-week-old animals, many islets were large and displayed disrupted architecture. The large is-

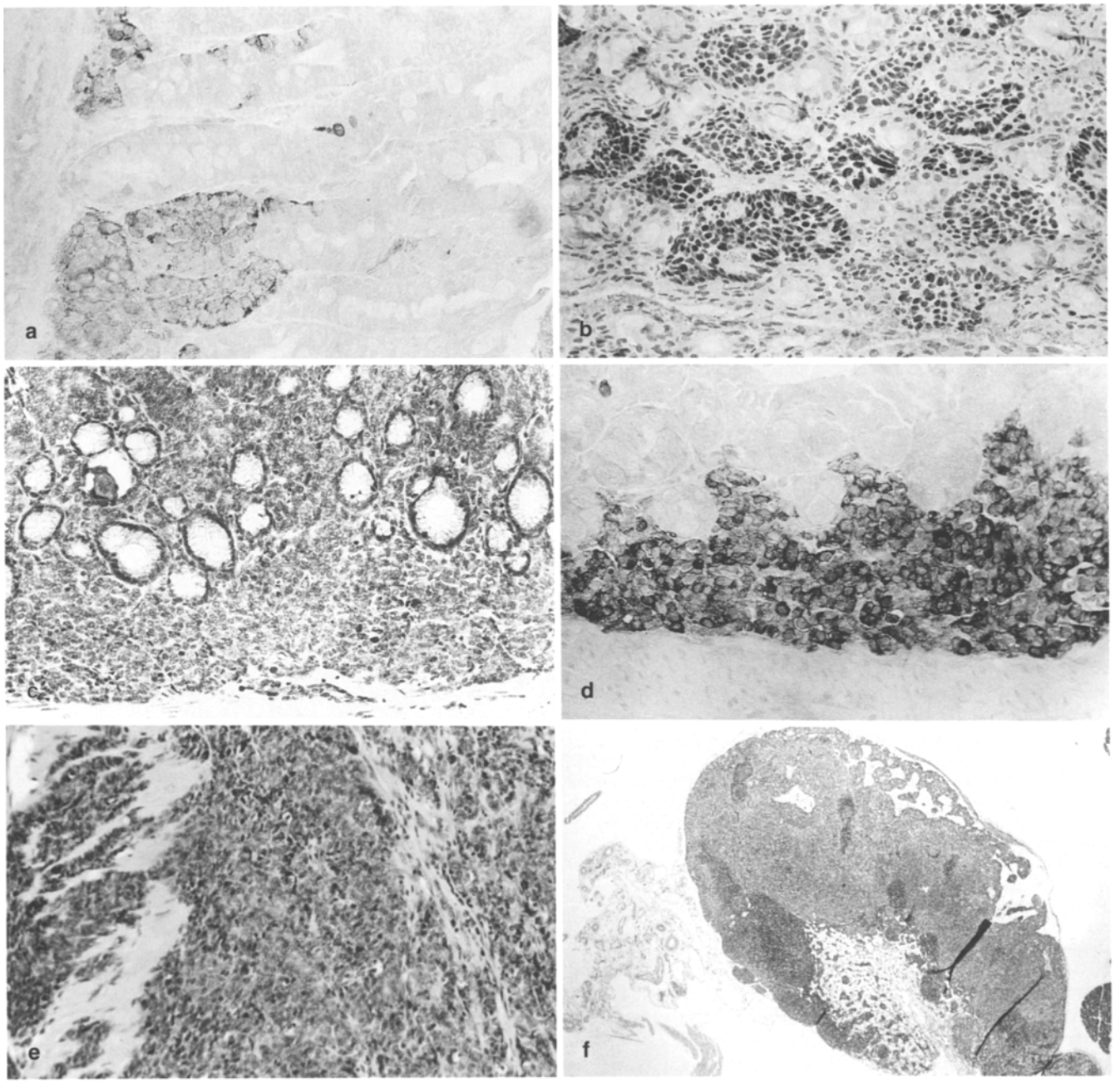

Fig. 2 **a** At 1 week of age, the large bowel of a glucagon-SV40 transgenic mouse has clusters of hyperplastic GLP-1-containing cells. Avidin-biotin-peroxidase technique, ×200. **b** The nuclei of the hyperplastic cells stain for SV40 T antigen. Indirect immunoperoxidase technique with hematoxylin counterstain, ×200. **c** At 4 weeks of age, transgenic animals develop marked proliferation of pleomorphic cells that fill the lamina propria of the large bowel; the morphological features are those of an endocrine carcinoma. Hematoxylin and eosin stain, ×200. **d** The cells filling the colonic lamina propria stain strongly for GLP-1. Avidin-biotin-peroxidase complex technique, ×200. **e** Some of the tumors progress to invasive carcinoma which penetrates through the muscular wall of the bowel. The nuclei of the invasive carcinoma are strongly positive for SV40 T antigen. Indirect immunoperoxidase technique, ×100. **f** Metastatic colonic carcinoma is found in a para-aortic lymph node. Hematoxylin and eosin stain, ×20

lets were composed of two distinct cell populations (Fig. 3a). Centrally, there were compressed clusters of epithelial cells with vesicular nuclei and eosinophilic cytoplasm. At the periphery, there were numerous large cells with pleomorphic, hyperchromatic nuclei harbouring eosinophilic inclusions and abundant chromophobic cytoplasm with indistinct cell borders. The nuclei of these large cells stained for SV40 T antigen; their cytoplasm was positive for NSE and synaptophysin. Occasional large cells also contained cytoplasmic neurofilament, tyrosine hydroxylase, chromogranin, glucagon/GLP-1, PYY or VIP immunopositivity (Fig. 3b,c). The small clusters of morphologically normal islet cells contained NSE and synaptophysin, as well as insulin in the majority of cells and somatostatin in a few (Fig. 3d);

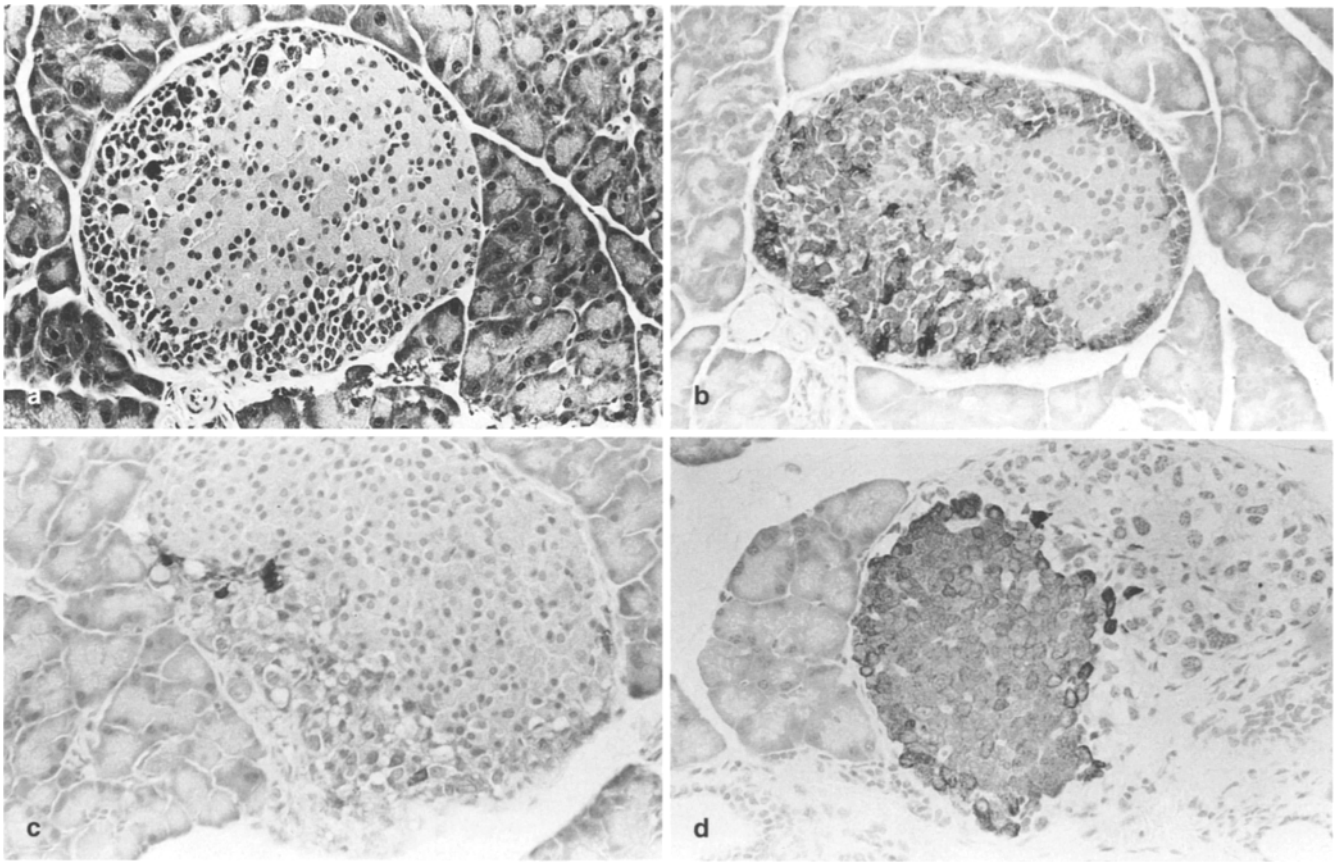

Fig. 3 **a** At 4 weeks of age, a transgenic mouse has large abnormal pancreatic islets with two cell populations; at the periphery crowded cells containing large pleomorphic nuclei surround normal islet cells. Hematoxylin & eosin stain, ×200. **b** The pleomorphic cells at the periphery of the islet contain strong immunoractivity for glucagon. Avidin-biotin-peroxidase technique, ×200. **c** Some large cells at the periphery of the islet contain focal immunoreactivity for tyrosine hydroxylase. Avidin-biotin-peroxidase technique, ×200. **d** The bland epithelial cells that comprise the majority of the islet contain insulin in the usual distribution. Avidin-biotin-peroxidase technique, ×200

no reactivity for chromogranin, SV40 T antigen, glucagon, or GLP-1 was found. PP immunoreactivity was not detected in pancreases of transgenic animals after birth.

In the 11- and 12-week-old mice, there were large nodules composed entirely of pleomorphic tumour cells; these resembled neoplasms; however, there was no evidence of infiltration into the surrounding exocrine pancreas or of metastatic spread. The tumour cells contained nuclear immunoreactivity for SV40 T antigen and intense cytoplasmic positivity for NSE, moderate staining for synaptophysin and focal neurofilament and tyrosine hydroxylase reactivity; however, they did not stain for chromogranin. Occasional cells were faintly positive for glucagon or PYY and less so for GLP-1; scattered cells had moderate VIP immunoreactivity. The majority of the cells were totally negative for all peptides examined.

Examination of other tissues obtained at complete autopsy revealed no other abnormalities. In particular, the stomach, small bowel, brain and liver showed no abnormality. Immunohistochemical stains for SV40 T antigen, glucagon and GLP-1 were performed on all stomach, bowel, brain and eye specimens. Occasional animals had evidence of an increased number of SV40 T antigen and GLP-1 immunoreactive cells in small bowel and there was a slight prominence of cells immunoreactive for both antigens in stomach, but these were individual cells and no solid nests were detected and therefore the diagnosis of hyperplasia could not be confirmed. Despite expression of the SV40 T antigen in brain, GLP-1 immunoreactive cells were difficult to visualize. There was no evidence of tumour proliferation in any of these tissues.

Ultrastructural findings

Electron microscopy was performed on the colon and pancreas of two mice at 3 weeks of age and two mice at 11–12 weeks of age.

In the younger animals, numerous endocrine cells were found within the epithelium of crypts as well as on the surface of the large bowel mucosa; individual tumour cells and clusters of tumour cells were also found within the lamina propria of the bowel, lacking surrounding basement membrane. The cells contained large, irregular nuclei with convoluted nuclear membranes and prominent nucleoli (Fig. 4). Endocrine cells predominated in most crypts and surrounded residual goblet cells (Fig. 4a). They maintained a columnar configuration

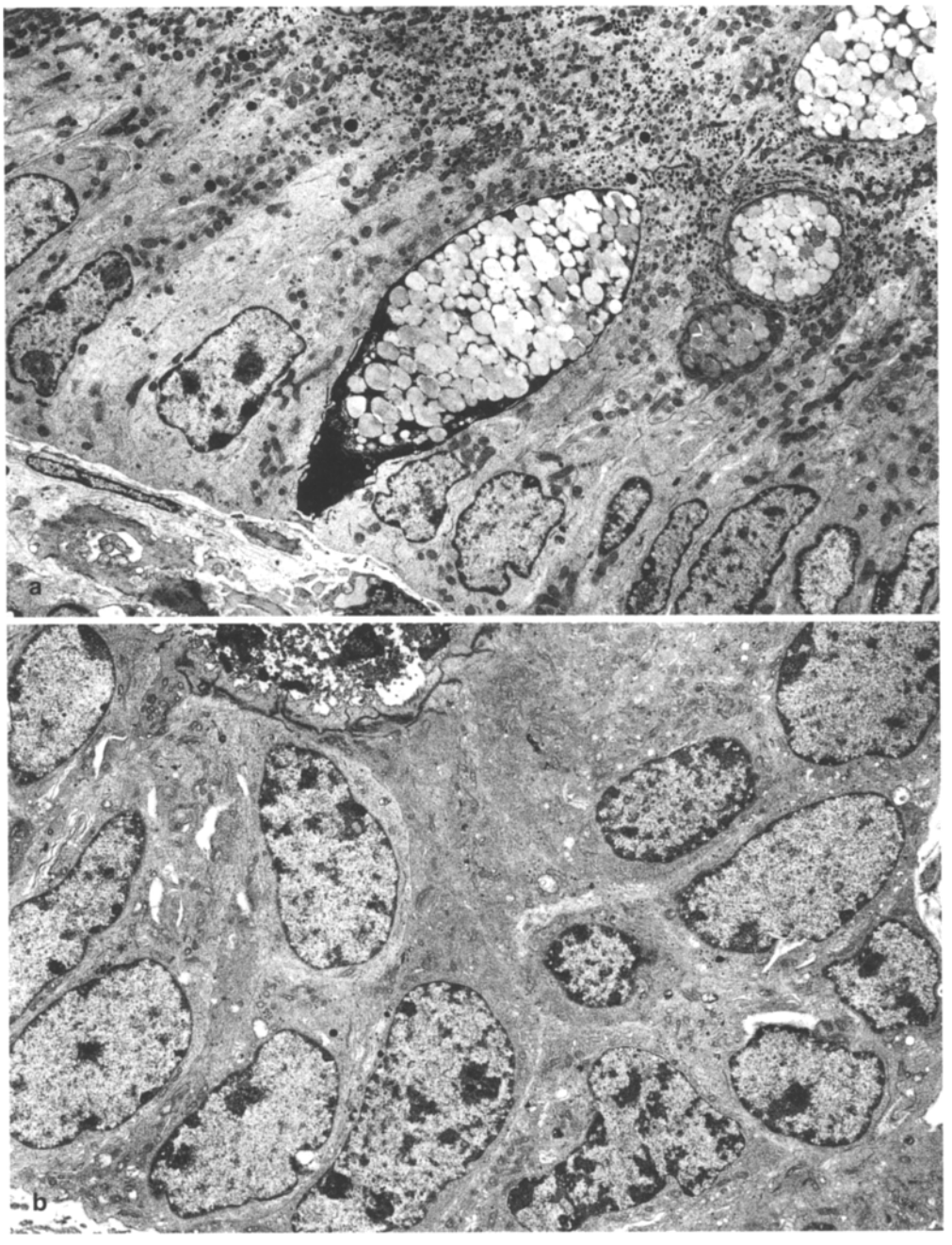

Fig. 4 **a** At 3 weeks of age, the colon contains numerous columnar endocrine cells surrounding a single residual goblet cell within a crypt. Nuclei are basal and secretory granules accumulate near the lumen. ×3700. **b** In another animal at 3 weeks of age the colonic crypt epithelium consists entirely of pleomorphic endocrine cells with sparse secretory granules and polygonal shape lacking polarity. ×3700

with basal nuclei. Their abundant cytoplasm contained many short profiles of rough endoplasmic reticulum and large Golgi complexes on the luminal side of the nucleus. Secretory granules were numerous and aggregated at the luminal pole of the cells; they were variable in morphology, measuring from 100 to 300 nm in diameter. Their contents were also of variable electron density and no discrete haloes were identified. Proliferation of endocrine cells led to the development of stratified epithelium in crypts with polygonal cells at the base and loss of polarity of the endocrine cells (Fig. 4b). The cytoplasmic features remained similar to those of more differentiated cells; however, the number of secretory granules was less in the polygonal cells. As the cells infiltrated through the basement membrane and into the stroma of the lamina propria, they became less well organized; they showed no distinct polarity and the polygonal cytoplasm was variable in amount. Nevertheless, they retained well-developed rough endoplasmic reticulum, juxtanuclear Golgi regions and moderate numbers of secretory granules of variable size, shape and electron density.

In the large bowels of the older animals, normal architecture was difficult to discern and the entire bowel wall was filled with nests and sheets of tumour cells showing markedly variable degrees of endocrine differentiation. In some areas, tumour cells had scanty cytoplasm with

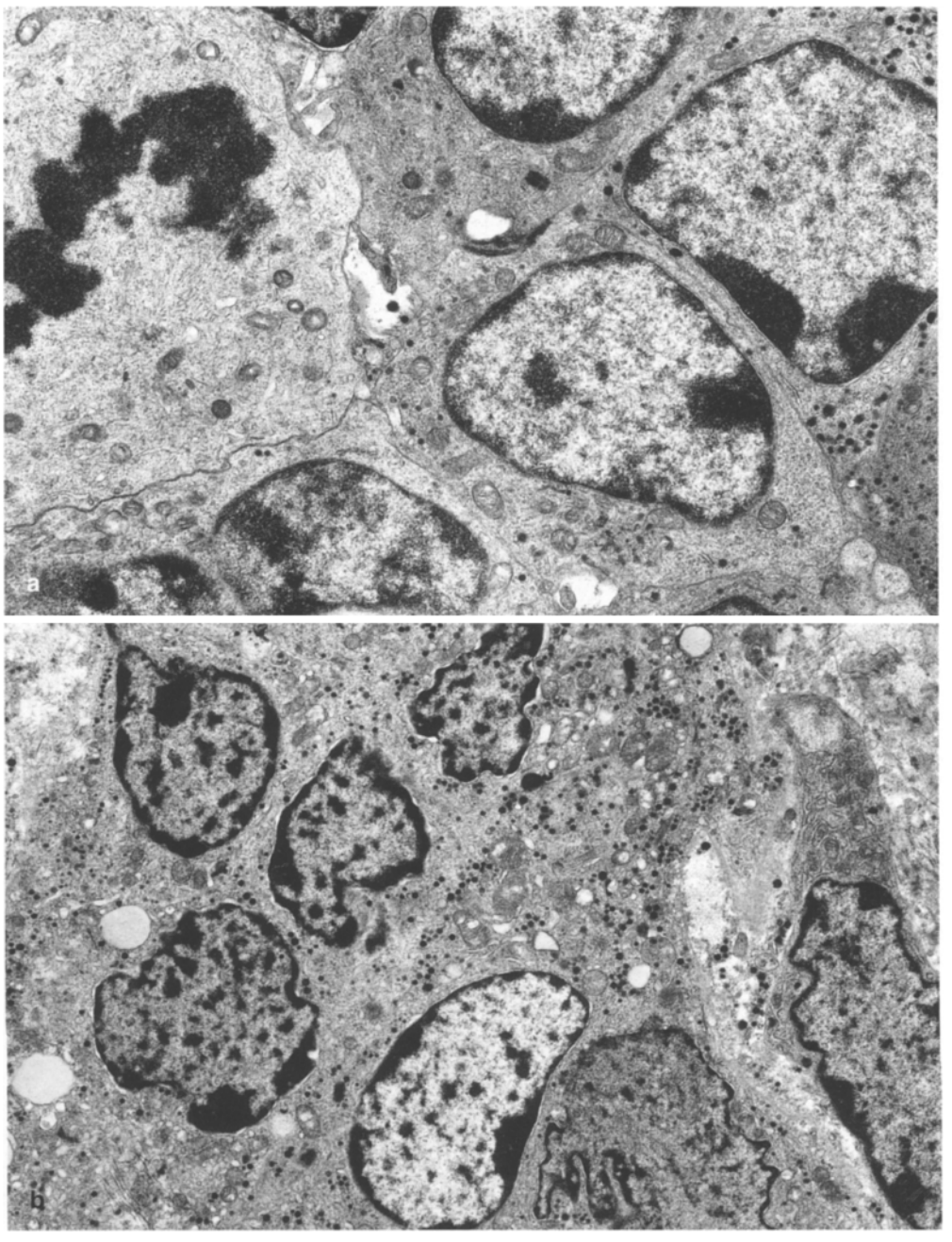

Fig. 5 **a** The colon of a 12-week-old transgenic mouse is infiltrated by pleomorphic cells with prominent mitoses, scanty cytoplasm and poorly developed organelles. Only the few secretory granules attest to the endocrine differentiation of these tumour cells. ×10 100. **b** In the same animal, other areas of the colonic tumour are comprised of differentiated endocrine cells with numerous small electron-dense secretory granules. ×7200

poorly developed organelles and only occasional scattered secretory granules (Fig. 5a). In other areas, more differentiated cells contained numerous secretory granules, well-developed profiles of rough endoplasmic reticulum and Golgi complexes (Fig. 5b).

At 3 weeks, the pancreas of transgenic animals contained discrete islets in which B cells predominated. At the periphery of the islets, large cells were identified which corresponded to the atypical cells seen by light microscopy (Fig. 6a). In the older animals, the large pleomorphic cells were numerous and formed large nodules (Fig. 6b). These cells had markedly convoluted nuclei with prominent intranuclear cytoplasmic inclusions as well as multiple nucleoli. They resembled neurons with abundant polygonal cytoplasm and prominent cytoplasmic processes that interdigitated in a stroma resembling neuropil (Fig. 7). The cytoplasm of the tumour cells contained parallel arrays of rough endoplasmic reticulum and very large juxtanuclear Golgi regions harbouring forming secretory granules. Mature secretory granules were restricted to cell processes; the secretory granules were small, measuring approximately 100 nm in diameter, and had a variable electron density. Interspersed among these large cells were smaller cells with pleomorphic nuclei and scant, poorly developed cytoplasm.

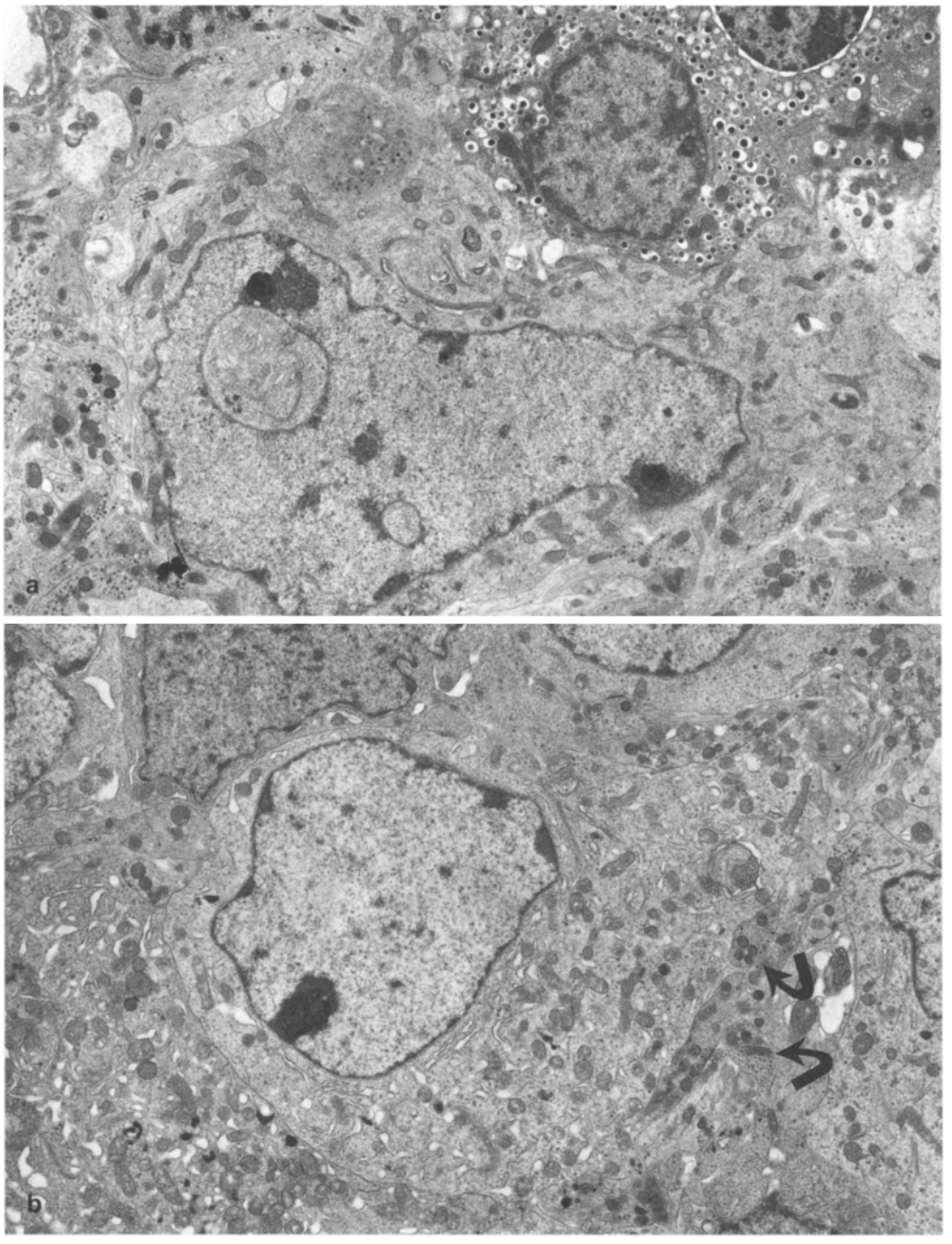

Fig. 6 **a** In the pancreas of a 3-week-old transgenic mouse, an occasional large pleomorphic cell is found adjacent to typical B cells that comprise most of the islet. ×5800. **b** In an 11-week-old transgenic mouse, the majority of the enlarged islet consists of atypical cells that resemble neurons with polygonal cytoplasm and interdigitating processes. The cells have well-formed rough endoplasmic reticulum, prominent Golgi complexes and sparse, small secretory granules that accumulate preferentially in processes (*arrows*). ×6000

In young animals, the islets were composed mainly of unremarkable B cells; only occasional small cells with secretory granules resembling those of D cells were seen and no cells with the morphology of PP cells were identified. In older animals, tumour cells with features of neurons predominated; small clusters of B cells with normal ultrastructural morphology were identified in some islets.

Discussion

In this report, we describe the morphological features of intestinal and pancreatic endocrine tumours arising in mice bearing a glucagon-SV40 T antigen transgene. These transgenic mice not only provide information concerning the structure and function of the glucagon gene [30] but also permit characterization of the cells that produce glucagon, providing insight into their ontogeny and molecular and cellular function in vivo.

The location of the gut-endocrine tumours was somewhat unexpected. In most mammalian species including mice, glucagon-producing L cells are found throughout the small bowel, where they are present in greater numbers than in the large bowel [14, 21, 43]. Nevertheless, glucagon is found in moderate quantities in the colon [14, 43] and tumours producing glucagon and its related peptides have been described in the rectum [18, 19]. In

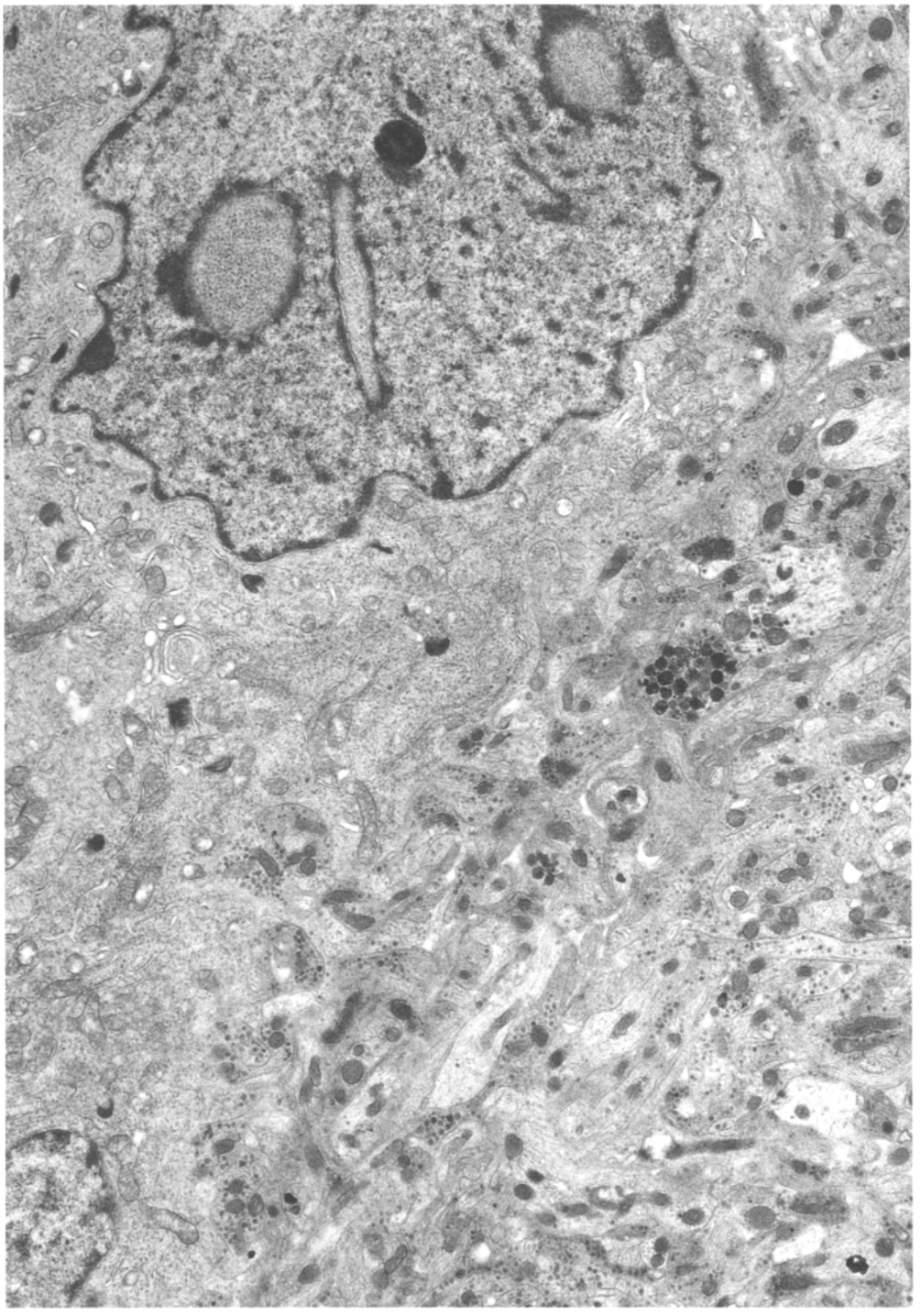

Fig. 7 The atypical cells comprising the bulk of islets in 11- to 12-week-old transgenic mice have convoluted nuclei with cytoplasmic and punctate inclusions. Their interdigitating processes form a stroma that resembles neuropil (*bottom right*). ×8700

our transgenic mice, glucagon was identified in enteroendocrine cells of the small bowel and stomach, and the same cells expressed SV40 T antigen; however, there was no evidence of hyperplasia or neoplasia in these areas. Differing levels of transgene expression cannot account for this observation, since the relative degree of transgene expression did not correlate with the development of tumours in the various tissues of these animals [30]. It is possible that endocrine cells of the large bowel may be more susceptible to neoplastic transformation, perhaps due to the environment; this is consistent with the known predominance of neoplasms in that location of the gut. Transgene expression may be insufficient to confer a transformed phenotype in endocrine cells of the small intestine. It may be that expression of other proteins, such as p53 or pRB [34, 49], is different in the colon and small bowel, modifying the transforming ability of SV 40 T antigen in vivo.

Using this model, we have analysed the stepwise progression of tumorigenesis from early pre-neoplastic

changes to frank malignancy. We found the classical sequence of hyperplasia proceeding to epithelial infiltration through basement membrane and subsequent proliferation in the lamina propria. Endocrine cell hyperplasia has been described in the human gut [31] and can be induced by experimental manipulation with drugs and hormonal alteration [4]; it has been suggested that hyperplasia may precede neoplasia in the human [31]; however, this theory remains controversial.

There is only one previous model of endocrine tumour formation in the gastrointestinal tract of transgenic mice [22, 40]. These tumours arose in small bowel of mice doubly transgenic for both the SV40 large T antigen and the polyoma small t antigen; they expressed secretin predominantly and several other gut endocrine peptides as minor products [40]. The tumours developed rapidly in young animals and had a high proliferative rate with a marked degree of anaplasia, and they showed significant loss of morphological endocrine markers [42]. The mechanisms responsible for targeting transgene expression to the endocrine cells of small intestine in these mice remain unclear but are likely attributable to chromosomal integration site effects.

In contrast, the intestinal endocrine tumours that developed in our model were observed in all founder animals and hence did not arise as a consequence of one or more integration site-specific events. The reproducible biology of endocrine cell proliferation progressing to tumour development and metastasis in these animals is probably attributable to the consequences of SV40 T antigen expression in gastrointestinal endocrine cells.

Our animal tumours exhibit the early reproducible invasiveness characteristic of human gut endocrine tumours. This model is therefore appropriate to study the sequence of mutational events that may also occur in human intestinal endocrine neoplasms. It will be of great interest to analyse these colonic tumours for the expression of oncogenes that are known to be activated in intestinal adenocarcinomas [3].

The plurihormonal nature of the tumours that developed in these animals provides yet another example of the heterogeneity of endocrine cells, increasing with tumour progression. The colocalization of PYY in these tumours is not surprising, since PYY has been colocalized with glucagon in pancreatic A cells [1] and is recognized as a product of L cells, predominantly in the distal ileum and colon [1, 7, 8, 35]; in vitro studies have confirmed cosecretion of the two hormones [9]. However, subpopulations of cells expressing other peptides inappropriate to the glucagon-producing L cell phenotype were also detected in these tumours. It may be that transformation of mature endocrine cells results in derepression of hormone gene expression; alternatively, the plurihormonal nature of these neoplasms is consistent with transformation of a pluripotent stem cell.

It has been proposed that a common stem cell gives rise to the four pancreatic endocrine cell types, as well as other cell types which develop and regress in the pancreas during ontogeny [2, 29, 37, 45, 48]; the presence of cell lines capable of producing multiple pancreatic peptides also supports the concept of multipotential progenitor cells [39]. Glucagon is expressed early in gestation [2, 25, 45]; therefore, if transgene expression was initiated in a common stem cell, it may have affected cells producing all four hormones. In our transgenic animals, B cells and D cells appear to have developed normally. The lack of detectable PP may indicate that its cell of origin is more closely related to A cells; this is consistent with the report of colocalization of PP and glucagon in a significant proportion of normal mouse islet cells [43] and in the developing mouse pancreas [25, 48]. Some investigators, however, have refuted these data and have suggested that PP is not detected until after birth in the mouse pancreas [47]. It is possible that expression of the oncogene in a common precursor prevented the cytodifferentiation of PP cells in our animals.

Less is known about the ontogeny of gut endocrine cells [13, 29]. Glucagon-immunoreactive cells are present in the gut early in fetal life, but their relationship to stem cells and other gut endocrine cells is unclear; the reason for the localization of cholecystokinin, α-subunit, occasionally somatostatin and serotonin in these gut endocrine tumours is unknown. Although still controversial, it is thought that the glandular and exocrine cell populations of the bowel derive from a common embryological source [12, 44], explaining the occurrence of tumours with mixed differentiation [6, 28, 50]; there were no exocrine features in the gut tumours of these transgenic animals.

The morphology of the atypical cells and tumours arising in the pancreas of these transgenic animals is unusual. The proliferation of a mantle of atypical cells at the periphery of the islets is similar to that reported in an unusual model of pancreatic endocrine tumorigenesis in mice bearing an elastase 1–SV40 T antigen fusion gene [5]. However, in our model, the tumour cells differ markedly from A cells or the components of glucagon-producing pancreatic tumours and display a number of morphological features of neurons, including neurofilament and tyrosine hydroxylase immunoreactivity and elongated cell processes with preferential storage of secretory granules in those processes. Pancreatic islet A cells are known to have a unique association with peripheral nerve ganglia and form what have been called "neuroinsular complexes" [32]; the interrelationships between neuronal bodies in the ganglia and islet cells is a topic of considerable interest.

There is good evidence that pancreatic endocrine cells do not originate in the neural crest [38], but there is no doubt that the cells have many similarities to neurons, which has led to their inclusion in the APUD (amine precursor uptake and decarboxylation) system [36] and some prefer to classify them as "paraneurons" [20]. Interestingly, tyrosine hydroxylase and other catecholamine-synthesizing enzymes have been localized in embryonic mouse pancreas in cells that are thought to be the precursors of islets; tyrosine hydroxylase is subsequently colocalized with glucagon and with insulin at the

onset of the detection of those hormones in the developing islet [2, 46, 47]. These findings are consistent with the postulate that insulin and glucagon presursors share features in common with dopaminergic neurons [37] and may explain the unusual neuronal differentiation of the pancreatic tumour cells which lacked features of A cells and contained very little glucagon or its related peptides.

Acknowledgements The authors gratefully acknowledge the invaluable technical assistance of Mark Moreland, Nahid Nelson, Lily Ramyar and Vera Szewchuk at St. Michael's Hospital, Toronto, and the secretarial help of Colette Drvodelic. This work was supported in part by operating grants of the Medical Research Council of Canada. D.J.D. is a career scientist of the Ontario Ministry of Health.

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