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GROWTH FACTORS AND CARCINOID TUMOURS

OLA NILSSON, BO WÄNGBERG, AMANDA MCRAE, ANNICA DAHLSTRÖM and HÅKAN AHLMAN

The presence of growth factors and their receptors in human midgut carcinoids and in gastric carcinoids of Mastomys have been investigated. Human midgut carcinoid tumours produce IGF-I as demonstrated by immunocytochemistry and radioimmunoassay. IGF-I receptors were detectable in half of the tumours and stimulation of cultured tumour cells with IGF-I enhanced DNA synthesis. IGF-I may therefore act as an autocrine stimulator of carcinoid tumour growth. Expression of TGF-α and EGF-receptors could also be demonstrated in midgut carcinoids by immunocytochemistry and Northern analysis, suggesting that TGF-α participates in the autocrine modulation of carcinoid growth. Co-culture of human midgut carcinoid tumours and rat fetal cholinergic neurons demonstrated secretion of a potent neuronotrophic factor by cultured tumour cells. IGF-I and TGF-α may account for these neuronotrophic effects, but carcinoid tumours may also secrete an as yet unidentified growth factor. Gastric (ECL cell) carcinoids developed rapidly in Mastomys during hypergastrinemia due to histamine$_2$-receptor blockade, suggesting that gastrin is an essential growth factor for these carcinoids.

Tumour cells in culture have the capacity of proliferation even when grown under serum-free conditions and are thus presumed to utilize autonomous activator pathways, e.g. intrinsic production of growth factors, synthesis of altered receptors for growth factors and activation of postreceptor pathways (1, 2). Several malignant tumours and cell lines have been shown to produce growth factors (1–11). Growth factors demonstrated in malignant cells include platelet-derived growth factor (PDGF) (4), insulin-like growth factors (IGF I and II) (5–8), basic fibroblast growth factor (bFGF) (9) and transforming growth factors (TGF-α and TGF-β) (10, 11). Since receptors for these growth factors have been demonstrated in the tumour tissue as well, a paracrine or autocrine regulation of tumour cell growth has been suggested (1, 2, 4, 8, 10).

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From Departments of Surgery and Pathology, and Institute of Neurobiology, University of Göteborg, Göteborg, Sweden.
Correspondence to: Dr Ola Nilsson, Department of Pathology, Sahlgrenska Hospital, University of Göteborg, S-413 45 Göteborg, Sweden.
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Growth factors may influence not only cell proliferation but also the cell differentiation, e.g. tumour cells may synthesize and secrete nerve growth factor (NGF), or subunits thereof (12, 13), promoting outgrowth of neurite-like processes from the tumour cells. Human endocrine tumour cells from midgut carcinoid tumours and pheochromocytomas may thus secrete neuronotrophic factors in culture causing transformation of tumour cells into a neuronal phenotype (14). In co-culture experiments between such tumour cells and rat fetal cholinergic neurons it has been shown that the tumour produced neuronotropic factor is transferable with specific actions on cholinergic neurons (15).

To study mechanisms responsible for the development of gastrointestinal endocrine tumours (carcinoids), experimental models utilizing the African rodent Mastomys natalensis have been employed (16–19). These animals may develop spontaneous gastric carcinoids originating from the histamine-containing enterochromaffin-like (ECL) cells of the oxyntic mucosa. Treatment of Mastomys with potent inhibitors of gastric acid secretion has also been shown to induce rapid formation of these tumours during the hypergastrinemic state (20, 21). In this model gastrin may exert its action as a growth factor for ECL cells. High affinity binding sites for gastrin have been demonstrated

on ECL cells and ECLoma cells (22, 23), even though the presence of authentic gastrin/cholecystokinin receptors on ECLomas has not yet been proven.

At present time, relatively little is known about the production of growth factors by carcinoid tumours. In this review we will present data from our own investigations on growth factors and carcinoid tumours: IGF-I and TGF-α and their receptors on human midgut carcinoid tumours as well as gastrin and its receptor in the Mastomys tumour model.

IGF-I and its receptor

The IGFs have structural homology with proinsulin and have biological effects similar to insulin. IGF-I and IGF-II have each been isolated from human serum and sequenced (24, 25). IGF-I (somatomedin C) (26) is a basic 70 amino acid polypeptide mainly produced in the liver but also in kidneys, lungs, testes and salivary glands (27). The expression of IGF-I mRNA and production of IGF-I protein is to a large extent controlled by growth hormone (27, 28). IGF-I plays an important role in the physiological regulation of cell growth and differentiation (29). Previously IGF-I has been demonstrated in certain tumour cell lines, e.g. breast cancer, small cell lung cancer, primitive neuroectodermal tumours and pancreatic cancer (5, 7, 8, 10).

The mitogenic effects of IGFs are mediated via specific receptors: type I and type II. The type I receptor is composed of 4 subunits and exhibits tyrosine kinase activity (30), while the type II receptor is a single polypeptide chain (31). Type I and type II receptors have previously been demonstrated on several tumour types, e.g. Wilms' tumour, neuroblastoma, pheochromocytoma, small cell lung cancer, breast cancer and thyroid adenomas (8, 32).

By immunocytochemistry, IGF-I-positive tumour cells were demonstrated in 11 consecutive cases of midgut carcinoid tumours (Fig. 1A and C). Reverse phase HPLC of tumour extracts demonstrated a major IGF-I immunoreactive component, eluting in the position of recombinant human IGF-I, but more hydrophobic forms also occurred (Fig. 1E–F). These components may represent pre- or proforms of IGF-I, or proteolytically processed fragments of the peptide. Conditioned serum-free media from primary cultures of these tumours contained detectable amounts of IGF-I (20–25 ng/ml) as measured by radioimmunoassay, indicating secretion of IGF-I from tumour cells into the culture medium (33).

IGF-I receptors were detected immunocytochemically in 4 out of 10 tumours investigated (Fig. 1B). Cultured tumour cells with immunoreactive IGF-I receptors could be stimulated to enhanced growth, measured as an increase in DNA content, by exogenous administration of IGF-I (1 000 ng/ml) every 3–4 days during 2 weeks. Tumour cell cultures without immunocytochemical expression of IGF-I receptors did not respond to IGF-I, not even at high concentrations (1 000–10 000 ng/ml) (33). A role for IGF-I in the autonomous growth of carcinoid tumours is still only speculative. However, IGF-I and the proliferating cell nuclear antigen (PCNA)/cyclin were co-distributed in the same regions of primary tumours, suggesting a high production of IGF-I in carcinoid tumour cells undergoing DNA synthesis (Fig. 1C–D).

The even distribution of IGF-I over the entire tumour cell cytoplasm suggests a lack of storage capacity and may indicate a constitutive hypersecretion of IGF-I (Fig. 2A). When IGF-I was determined in peripheral plasma from these patients all had normal levels, which also indicates a local paracrine or autocrine action of this growth factor.

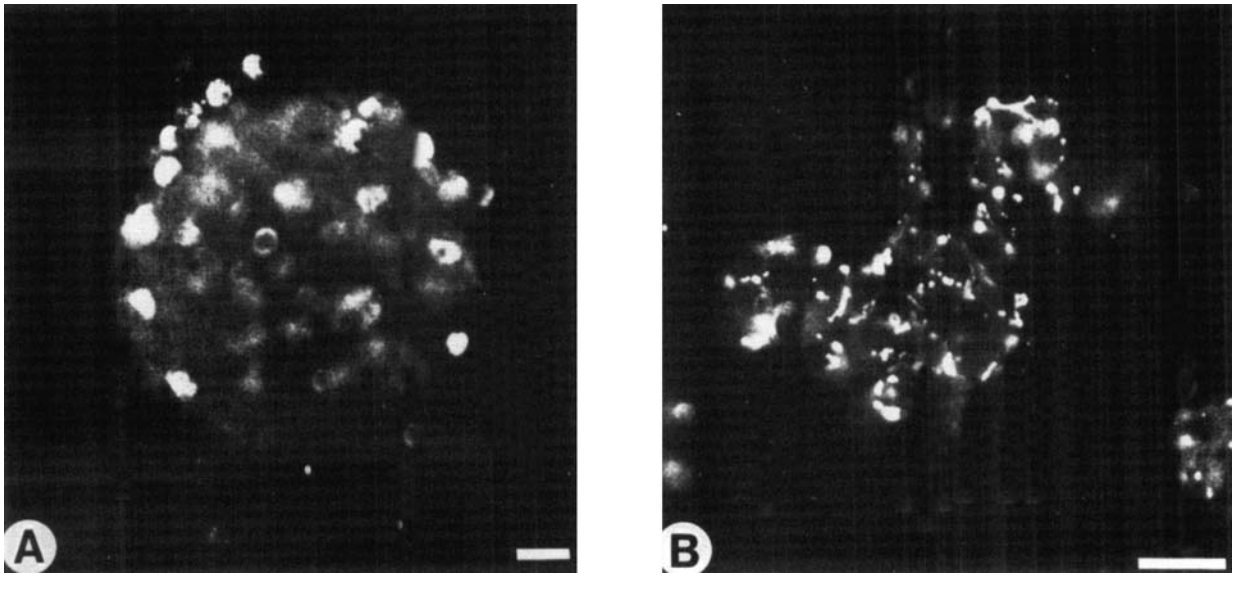


Fig. 1. A and B. For legend, see p. 117.

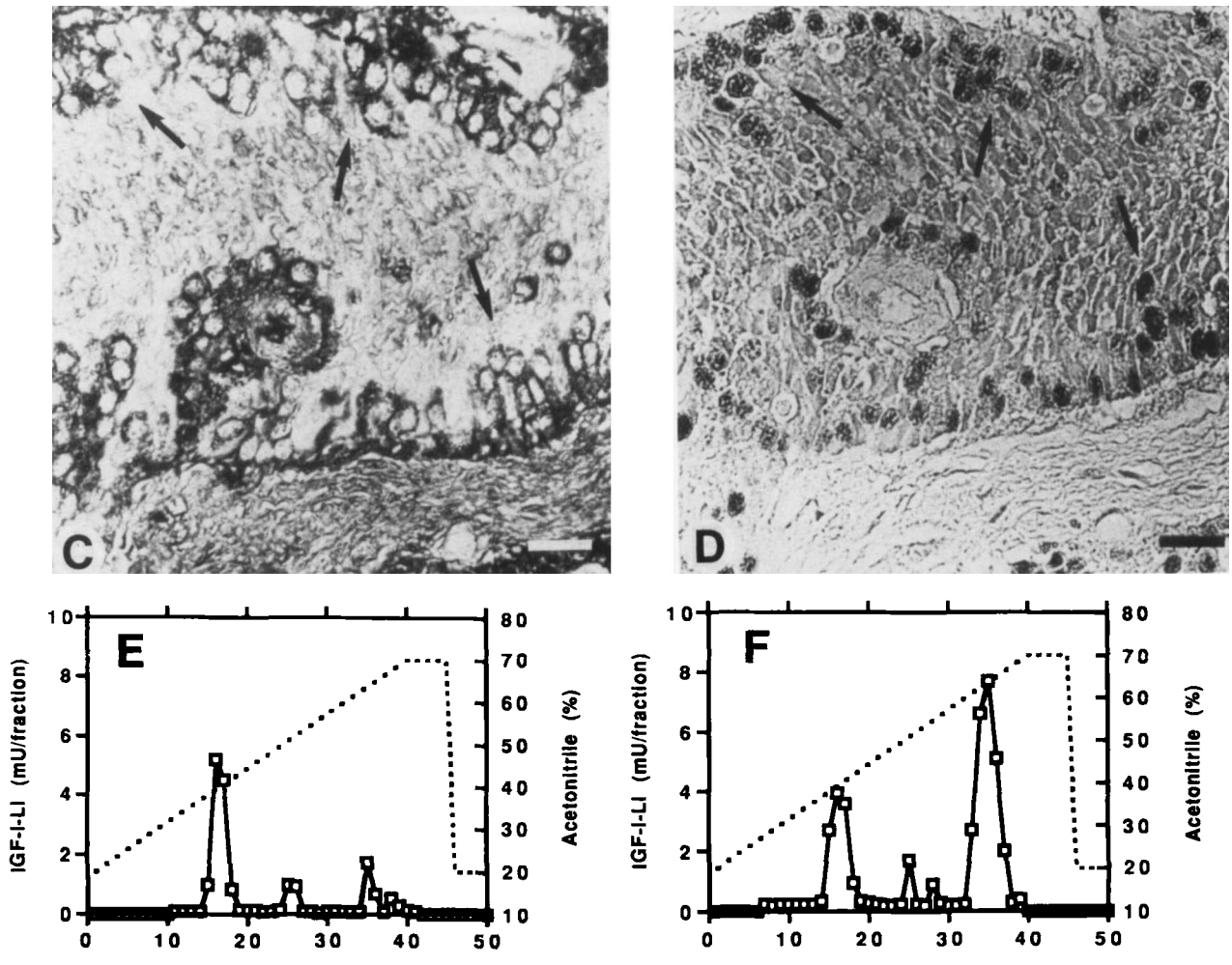


Fig. 1. Immunocytochemical localization of IGF-I (A) and IGF-I receptors (B) in cultured tumour cells from a human midgut carcinoid. The even distribution of IGF-I in tumour cells is evident, while IGF-I receptor labelling is localized over plasma membranes. In this surgical specimen from another midgut carcinoid (C) immunolabelling with IGF-I antiserum is confined to the cytoplasm of tumour cells with no labelling of cell nuclei. In the adjacent section tumour cells with nuclei immunoreactive to PCNA/cyclin antibodies show a similar distribution as that obtained with the IGF-I antiserum (D). HPLC elution profiles of recombinant human IGF-I (E) and tissue extract from one midgut carcinoid (F) show that rhIGF-I eluted as one major peak and two smaller late peaks. The tumour extract eluted in 3 peaks at the same positions. Bars indicate 20 μm.

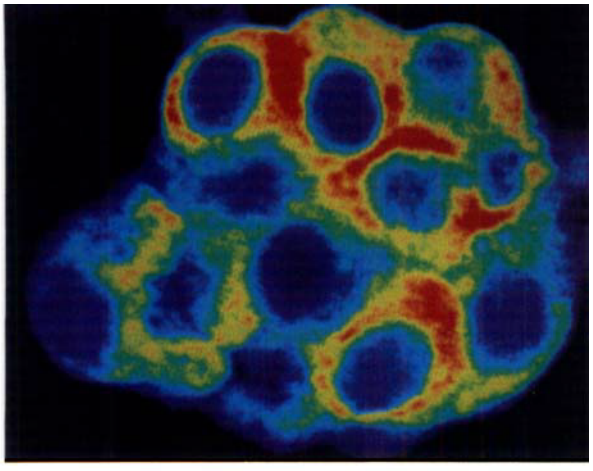
From previous studies there seems to be an inverse relationship between IGF-I and IGF-II in neuroendocrine tumours: Small cell lung carcinomas and primitive neuroectodermal tumours primarily produce IGF-I, while pheochromocytomas almost exclusively produce IGF-II (8).

The genetic changes underlying IGF overexpression in neuroendocrine tumours are still poorly understood. IGF-I has been mapped to the human chromosome 12, which also carries the gene for the proto-oncogene C-Ki-ras2. IGF-II on the other hand has been localized to the short arm of chromosome 11, which carries the gene for the proto-oncogene c-Ha-ras1 (34, 35). Overexpression of IGF-I, but not of IGF-II, has been correlated to the t (11; 22) chromosome translocation in neuroendocrine tumours (36).

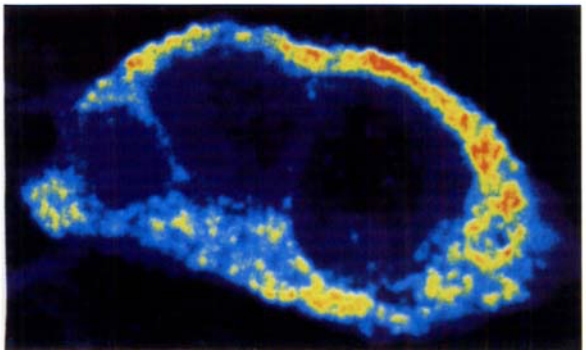
Of potential therapeutic interest is the fact that addition of a somatostatin analogue (octreotide 10^{-8} M) to carcinoid cell cultures for 4 days significantly reduced the levels of IGF-I in the medium (33). The effects of different somatostatin analogues on carcinoid tumour cell proliferation have not yet been fully evaluated.

TGF-α and the EGF receptor

Epidermal growth factor (EGF) is a 53 amino acid polypeptide, which can stimulate or inhibit proliferation and differentiation in a variety of tissues both during development and in the adult (37, 38). EGF interacts with the cells via cell surface receptors. Blockade of these receptors by specific antibodies has enabled the study of cells depleted of the EGF signal.



(A)



(B)

Fig. 2. Immunocytochemical demonstration of TGF-α (A) and EGF-receptors (B) in cultured human midgut carcinoid tumour cells using confocal laser scanning. Note the diffuse cytoplasmic distribution of TGF-α in tumour cells with no labelling over cell nuclei. EGF-receptor labelling is confined to tumour cell membranes.

The EGF receptor is a 170 kD transmembrane glycoprotein with tyrosine kinase activity. Modulation of the receptor activity may include autophosphorylation of receptor regulatory sites, receptor degradation subsequent to agonist binding or intracellular sequestration (39). The EGF receptor interaction has been widely discussed in carcinogenesis since EGF induces transformation-associated phenotypes in target cells. These effects are not fully reversible after withdrawal of EGF (40–42). EGF also promotes viral and chemical carcinogenesis in several model systems (40–42, 44, 45).

Many tumour cells produce TGF-α, which is a 50 amino acid peptide structurally related to EGF, and binds to the same receptor. TGF-α also occurs in normal cells, but is produced in abnormal quantities in tumour cells (38, 46, 47). Endogenous production of TGF-α may explain the loss of EGF-binding capacity after malignant transformation (46, 48). A second family of transforming growth factors, TGF-βs, which is neither structurally nor functionally related to TGF-α, is under certain circumstances required in addition to TGF-α for promotion of cell growth. The combination of these factors was earlier referred to as the sarcoma growth factor (49).

The EGF receptor is related to certain transforming proteins, which have the capacity to phosphorylate proteins on tyrosine residues, a capacity also shared by the receptors of PDGF, insulin and IGF (50). The cytoplasmic part of the receptor has a 95% homology with the v-erb B-transforming protein of the avian erythroblastosis virus (51, 52). The EGF receptor itself can be converted into an oncogene during amplification of the EGF-receptor gene or at retroviral infection (53). Gliomas and many carcinoma cell lines, e.g. colorectal carcinomas, demonstrate a high degree of EGF receptor expression frequently in combination with EGF receptor gene amplification (54, 55). These findings indicate that the EGF receptor is important in the growth regulation of these tumour types.

We have investigated the presence of TGF-α and its receptor, the EGF receptor, in human chromaffin tumours. Surgical specimens as well as primary cultures of midgut carcinoid tumours (n = 18), phechromocytomas (n = 4) and paragangliomas (n = 2) were investigated by immunocytochemistry and Northern blot analysis (56). All tumours were positively labelled by the TGF-α antibodies. Confocal laser scanning of cultured tumour cells demonstrated that immunoreactive TGF-α was localized over cytoplasm and cell membranes of tumour cells (Fig. 2A). EGF-receptor immunoreactivity was mainly located over tumour cell membranes (Fig. 2B). Northern blot analysis of the carcinoid tumours revealed TGF-α mRNA transcripts of 4.5–4.8 kb size and EGF-receptor transcripts of 10 kb size, thus demonstrating that TGF-α and EGF-receptors were co-expressed in these tumours (56).

The neuronotrophic effect mediated by midgut carcinoid tumour cells

During long-term culture, cells from individual midgut carcinoid tumours gradually lost their normal phenotype and developed long cytoplasmic processes with varicose-like enlargements and growth cones. These cells were characterized immunocytochemically and shown to contain tachykinin- and serotonin-like immunoreactivity. In addition they were clearly labelled by antisera against the neurofilament triplet and were weakly labelled with monoclonal antibodies against SV2, a synaptic vesicle membrane component. At the ultrastructural level they contained typical electron-dense granules as well as neurofilaments (14).

In order to investigate whether cultured carcinoid tumour cells produce an endogenous factor responsible for this neuronal transformation, these tumour cell cultures were incubated with NGF-antisera, which gave a strong positive immunoreaction. We thereafter developed a co-culture system between human midgut carcinoid tumour cells and rat fetal CNS neurons known to possess NGF-

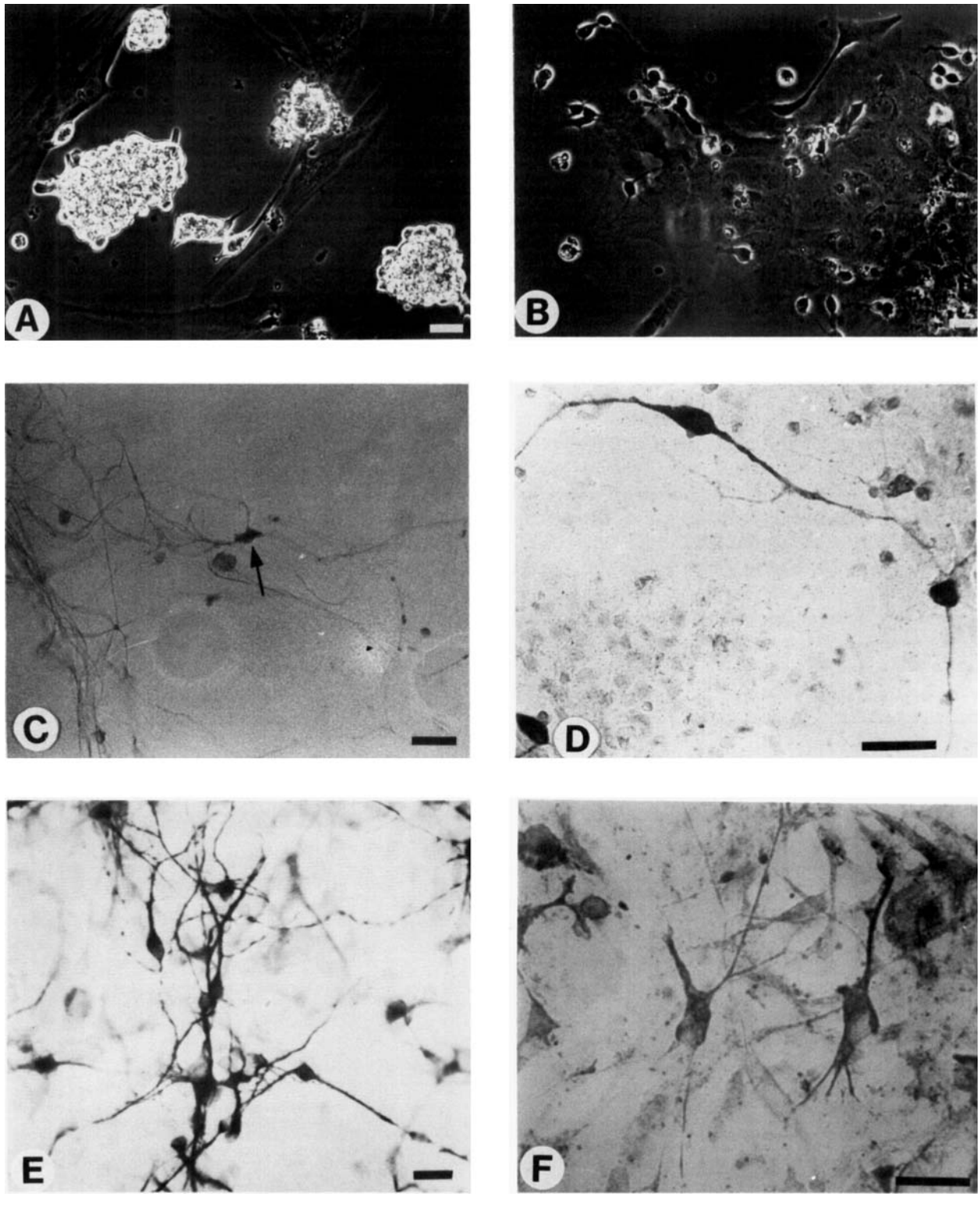


Fig. 3. Phase contrast microscopy of monocultures of human midgut carcinoid tumour cells (A) and rat septal neurons (gestational day E 17) (B) grown serum-free for 4 days. Tumour cells tolerated these conditions, while most neurons had deteriorated and detached from the matrix. Monocultures of rat septal neurons grown for 8 days in conditioned serum-free medium from a midgut carcinoid (C) demonstrate large neurite networks emanating from acetylcholinesterase-positive cell bodies (→). In co-cultures between human tumour cells and rat septal neurons under serum-free conditions for 8 days demonstrated acetylcholinesterase-positive neurons with long neurites and multiple branching points. Immunocytochemical demonstration of IGF-I receptors (E) and EGF receptors (F) on rat septal neurons in monocultures. Bars indicate 50 μm.

receptors. Immunocytochemically, NGF-receptor-like immunoreactivity was observed on the surface of both tumour cells and neurons. In serum-free monocultures tumour cells survived, while neurons rapidly died (Fig. 3A–B). In contrast to this control situation cholinergic neurons displayed a pronounced outgrowth of neuritic processes in serum-free co-culture with tumour cells. Cholinergic neurons of serum-free monocultures also thrived and showed outgrowth of neurites, when supplemented with serum-free conditioned tumour cell media (Fig. 3C–D). These observations strongly indicate that the tumour cells secrete transferable growth factor(s) with potent neuronotrophic actions (15).

However, bioassays for the biological activity of NGF, utilizing the chick embryo sympathetic ganglion, were negative for tumour media (15). Preliminary results from Northern blot analysis were also negative for the presence of authentic NGF in the tumour media. These findings have directed our interest toward IGF-I and TGF-α, two growth factors secreted from midgut carcinoid tumour cells (cf. above) with potential neuronotropic effects. Immunocytochemical studies of our target cells for the neuronotrophic action, the rat fetal cholinergic neurons, have demonstrated the presence of both IGF-I and EGF-receptors (Fig. 3E–F). Studies on exogenous application of these factors, alone or in combination, or inactivation of tumour-produced growth factors by neutralizing antibodies, need to be performed in serum-free monocultures of cholinergic neurons to further analyse the nature of the carcinoid neuronotrophic actions.

The Mastomys model

Mastomys natalensis is one of the most prevalent wild rodents in Africa and is a vector for several zoonoses. Gastric tumours and mucosal hypertrophy was observed in 40% of Mastomys dying from natural causes (16). These tumours were initially characterized as adenocarcinomas. Their endocrine features were later recognized and they were accordingly reclassified as gastric carcinoids (17). Their malignant potential was evident after transplantation to other Mastomys or nude mice (17, 57). After transplantation of tumours for several generations different tumour substrains have developed with main secretion of serotonin, histamine or both (58, 59). Inbreeding of Mastomys led to development of the Y and Z strains. The Z strain is prone to develop antral adenocarcinomas and fundic carcinoids, while the Y strain, like the wild type, developed only fundic carcinoids (60–62).

The gastric carcinoids are located in the acid-producing oxyntic mucosa. The histogenesis of spontaneous carcinoids was described by Soga et al. (63). During stage I (hyperplasia) proliferation of endocrine cells (argyrophilic with either the Sevier-Munger or Grimelius' silver reaction) takes place in the basal parts of the crypts. During stage II (preneoplasia) larger nodules within the gastric glands are formed. In stage III (neoplasia) the endocrine cells grow outside the glands in the lamina propria and may form micro- or macrocarcinoids. The lesions are multifocal and distributed all over the fundic mucosa.

The speed by which these endocrine lesions develop can be enhanced by blockade of histamine$_2$-receptors. After

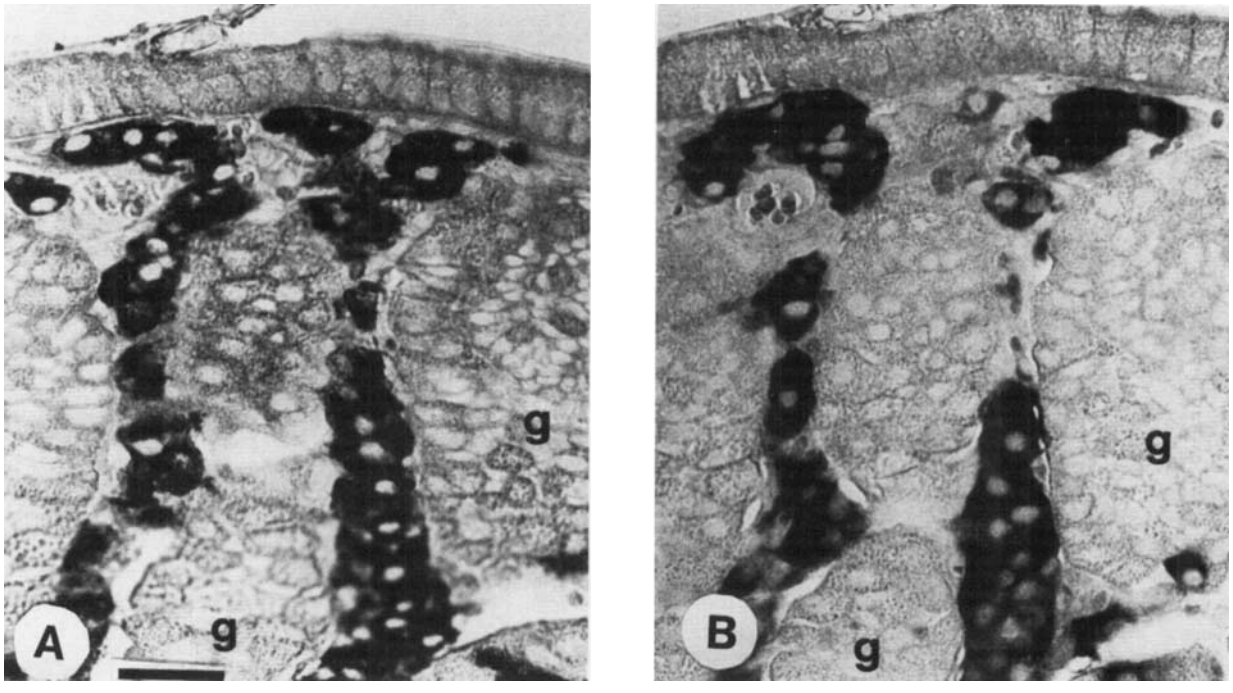
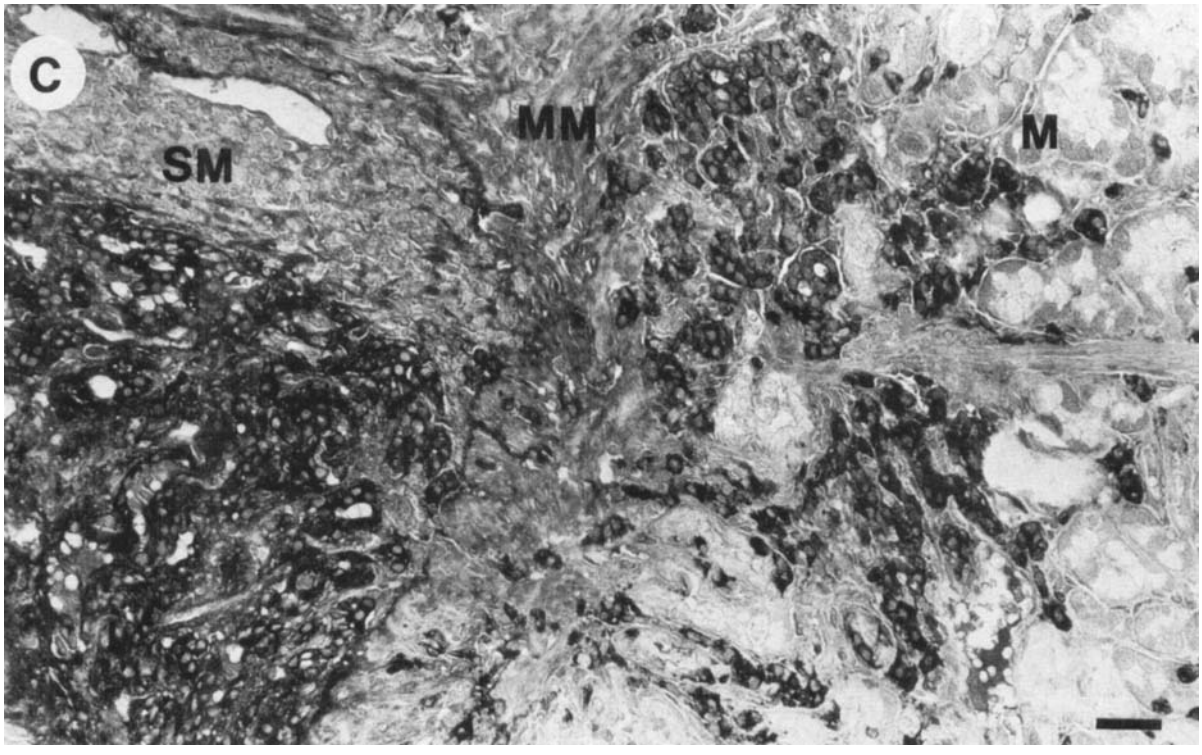


Fig. 4. A and B. For legend, see p. 121.



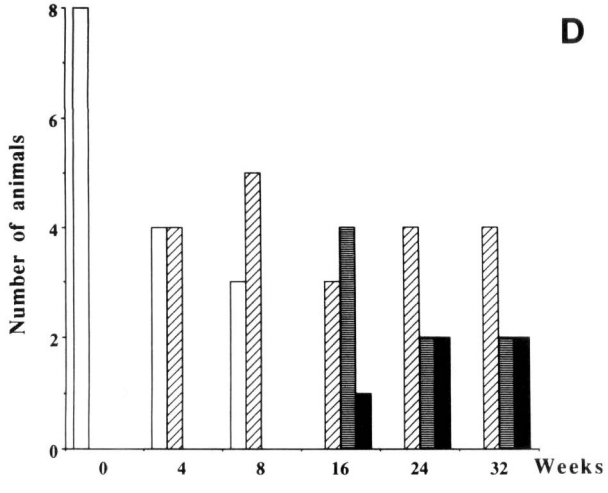


Fig. 4. Micrographs showing proliferation of endocrine cells in the oxyntic mucosa of Mastomys during loxtidine treatment (10 g/l in drinking water 24 weeks). Dysplastic growth of endocrine cells in the lamina propria surrounding the gastric glands (g) using chromogranin A + B (A) and histamine (B) immunostaining on adjacent sections. From this micrograph it is evident that the endocrine dysplasia is mainly due to proliferation of the histamine-producing ECL cells. Neoplastic growth (C) of endocrine cells with the mucosa (M) with penetration of the lamina muscularis mucosa (MM) and the submucosa (SM). The histopathological changes (D) of ECL cell growth in the oxyntic mucosa during loxtidine treatment show a gradual change from normal (□), via linear and nodular (▨) hyperplasia, dysplasia (▤) into true neoplasia (■). Bars indicate 20 μm.

8 weeks of such blockade (loxtidine in drinking water, 10 g/l) a marked hyperplasia of ECL cells was evident with doubling of the ECL cell density (21). At this stage linear or nodular hyperplasia according to the classification by Solcia et al. (64) was rare. After 16 weeks of treatment, hyperplasia with numerous chain formations and micronodules was evident and dysplastic lesions with microinvasion of the lamina propria started to appear (Fig. 4A–B). After 24 and 32 weeks the dysplasia was extensive with micro- and macrocarcinoids in one-third of the animals (Fig. 4C) (21). Histamine concentration and histidine decarboxylase activity in the fundic mucosa increased gradually during the long-term hypergastrinemia in parallel with the described morphological changes (21).

The genetic defect in Mastomys that makes this species susceptible to tumour formation has not yet been identified. The rapid induction of gastric carcinoids during hypergastrinemia suggests that gastrin may act as a promoter of tumour growth, directly or indirectly, activating protooncogenes and local production of growth factors. This assumption is also supported by the fact that gastrin/CCK receptors (Fig. 5) are abundant on tumour cell membranes from the transplantable E-line tumour (22). It will be of particular importance to perform reversibility studies in Mastomys after chronic acid inhibition to study if reversal of hyperplasia, or even of dysplasia/neoplasia, may occur.

In the rat it has been shown that induced hypergastrinemia (inhibition of gastric acid secretion or infusion of

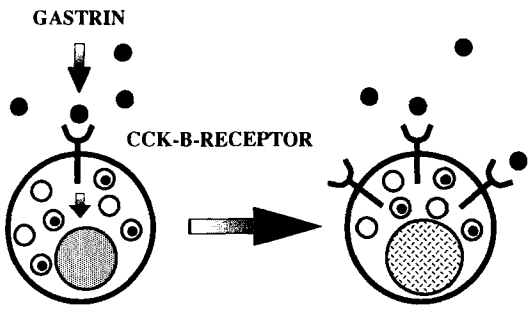


Fig. 5. Schematic illustration of the gastrin hypothesis for the transformation of ECL cells to gastric carcinoids of ECLoma type in Mastomys. During the hypergastrinemic state the cells start to overexpress the gastrin/CCK B receptor leading to an enhanced gastrin-receptor interaction with possible activation of proto-oncogenes and synthesis of growth factors.

gastrin) stimulates the proliferation of ECL cells in a reversible manner after short-term treatment (65). Life-long inhibition of acid secretion in the rat may also lead to formation of gastric carcinoids (66). The exact role of gastrin in the transformation of ECL cells remains to be clarified, since additional mechanisms may be involved as well. In the rat the number of antral G cells increases during acid inhibition, while somatostatin-containing D cells decrease (increased G/D cell ratio) (67). This may indicate simultaneous loss or downregulation of inhibitory mechanisms for ECL cell growth.

Concluding remarks

The identification of oncogenes, or tumour suppressor genes, and their products will increase our understanding of the role of hormones in tumour development. Of particular interest is the homology between the EGF receptor and v-erbB and between PDGF and the transforming protein $p28^{env\text{-}sis}$ as well as functional similarities between oncogene products and cellular regulators with tyrosine kinase activity. In hormone-producing tumours, like the carcinoids, overproduction of certain hormones may be a key event for the activation of proto-oncogenes, e.g. the potential role of hypergastrinemia for development of ECLomas in the Mastomys model (Fig. 5). Hormones or growth factors may also substitute for, or enhance, the activity of certain oncogenes in a similar way that EGF promotes the viral transformation of the endocrine granulosa cells. Further studies on tumours with specific hormone activity, manipulation of this activity by pharmacological tools, identification of characteristic growth factors, growth factor receptors and oncogene amplification will hopefully clarify the hormonal interaction with cells during carcinogenesis.

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