Thorax 1991;46:924–929

New perspectives in lung cancer · 2

Series editor: S G Spiro

Growth factors and lung cancer

Penella J Woll

Growth factors are a diverse group of signalling molecules taking part in the control of cell proliferation. Whether they act on postganglionic receptors (neurotransmitters), nearby cells (autocrine and paracrine hormones), or distant target organs (endocrine hormones), they require specific receptors and intracellular signal transduction pathways to stimulate cell division. Abnormal expression of growth factors, their receptors, or their signalling pathways may result in the unrestrained growth of cancer. The finding that many such changes are associated with oncogene activation has underpinned the hypothesis that cancer results from cumulative somatic mutations.

In addition to advancing our understanding of carcinogenesis, elucidation of the action of growth factors presents exciting opportunities for the development of new therapeutic strategies against cancer. This review considers the evidence for the action of growth factors in lung cancer and the implications for treatment.

Small cell lung cancer

About a quarter of lung cancers are small cell lung cancer. They are characterised by the presence of cytoplasmic secretory granules and their ability to synthesise a wide range of peptides and hormones (table 1). Among these, the bombesin related peptides, including gastrin releasing peptide, first attracted interest as putative autocrine growth factors, but many others are now known to act as mitogens in experimental systems,[1] and a more complex picture is emerging of growth stimulation by multiple autocrine and paracrine loops.

Cancer Research Campaign Department of Medical Oncology, Christie Hospital, Manchester M20 9BX
P J Woll

Correspondence to:
Dr Woll

Table 1 Factors synthesised by small cell lung cancer

Adrenocorticotrophin	Lipotropin
Atrial natriuretic peptide	Neurotensin
Bombesin/gastrin releasing peptide	Neuromedin B
Calcitonin	Oestradiol
Calcitonin gene related peptide	Opioid peptides
Cholecystokinin	Oxytocin
Chorionic gonadotrophin	Parathyroid hormone
Endothelin	Physalaemin
Follicle stimulating hormone	Prolactin
Gastrin	Serotonin
Glucagon	Somatostatin
Granulocyte colony stimulating factor	Substance K
Growth hormone	Substance P
Growth hormone releasing factor	Transferrin
Insulin like growth factor binding proteins	Vasoactive intestinal peptide
Insulin like growth factor I	Vasopressin

BOMBESIN/GASTRIN RELEASING PEPTIDE

Gastrin releasing peptide (27 amino acids) is the principal mammalian homologue of the amphibian peptide bombesin (14 amino acids). It is present in neurones of the gut and central nervous system. Although it is abundant in fetal lung, with maximal expression of messenger RNA (mRNA) at 16–30 weeks, reduced concentrations are found in infants with the respiratory distress syndrome and it is sparse in adult lung.[2,3] These observations led to the intriguing suggestion that bombesin/gastrin releasing peptide could act as a growth factor for fetal lung.

Bombesin/gastrin releasing peptide is found in specimens and cell lines of small cell lung cancer and the concentrations correlate well with those of gastrin releasing peptide mRNA.[4–6] The finding that bombesin/gastrin releasing peptide could stimulate the growth of murine Swiss 3T3 fibroblasts focussed attention on neuropeptides as possible tumour growth factors.[7] The binding of iodine-125 labelled gastrin releasing peptide to small cell lung cancer cells suggested that specific receptors are present on these cells.[8] Gastrin releasing peptide has since been shown to stimulate the growth of small cell lung cancers in vitro and in vivo.[9,10] Cuttitta *et al*[11] used a monoclonal antibody to bombesin to inhibit the growth of two small cell lung cancer cell lines in vitro and of one as a xenograft in nude mice. There is thus persuasive evidence that bombesin/gastrin releasing peptide can act as an autocrine growth factor in at least some small cell lung cancers.

Because of the complex interactions among growth factors in tissue preparations and in vivo, the characterisation of their individual effects has been possible only in homogeneous cell lines such as Swiss 3T3 cells. These cells attain quiescence in serum depleted medium, but resume DNA synthesis after the addition of fresh serum or purified growth factors. The mode of action of bombesin/gastrin releasing peptide has been studied in detail in these cells and serves as a paradigm of growth factor action. Swiss 3T3 cells have proved to be a robust model for small cell lung cancer because they have receptors for a range of mitogenic neuropeptides,[12] including bombesin/gastrin releasing peptide, bradykinin, endothelins, vasopressin, and vasoactive intestinal peptide (see below).

Bombesin/gastrin releasing peptide binds to a single class of high affinity receptors on the surface of Swiss 3T3 cells.[13] The receptors are glycoproteins with a relative molecular weight

(Mr) of 75 000–85 000, with a core of Mr 43 000.[14–16] They are coupled to one or more guanine nucleotide binding proteins (G proteins) as judged by the modulation of ligand binding in preparations of membranes and solubilised receptors.[12] The bombesin/gastrin releasing peptide receptor has recently been cloned and sequenced.[17 18] It belongs to the class of G protein coupled receptors with seven predicted transmembrane domains clustered to form a ligand binding pocket.[19 20] Other neuropeptide mitogens with receptors of this type are angiotensin, endothelin, serotonin, substance K, and substance P. Interestingly, angiotensin and serotonin receptors can behave like oncogenes.[21 22]

Binding of bombesin/gastrin releasing peptide to its receptor in Swiss 3T3 cells triggers a cascade of signals in the membrane, cytosol, and nucleus, culminating in DNA synthesis 10–15 hours later. One of the earliest events is a rapid mobilisation of calcium (Ca^{2+}) from intracellular stores, leading to a transient increase in the cytosolic Ca^{2+} concentration followed by Ca^{2+} efflux from the cells.[23] These changes are mediated by inositol 1,4,5-trisphosphate, which with 1,2-diacylglycerol is generated by hydrolysis of phosphatidyl inositol 4,5-bisphosphate by phospholipase C in the plasma membrane. 1,2-Diacylglycerol stimulates protein kinase C, causing phosphorylation of its major substrate, the 80 K protein.[24 25]

Other signalling pathways activated by bombesin/gastrin releasing peptide include arachidonic acid and prostaglandin release, cyclic AMP production, and the rapid exchange of sodium, potassium, and hydrogen ions across the cell membrane, leading to cytoplasmic alkalinisation and increased intracellular K^+ concentration.[26 27] In common with many other growth factors, bombesin/gastrin releasing peptide stimulates transient expression of the nuclear oncogenes c-fos and c-myc.[28] Thus bombesin/gastrin releasing peptide triggers a complex network of mitogenic signals incorporating an unusual degree of redundancy, which ensures amplification of the stimulus and suggests that it has an important role. Attempts to block growth factor action through any single pathway are unlikely to be successful, so therapeutic strategies must embrace this complexity.

Bombesin/gastrin releasing peptide signals in small cell lung cancer

Preliminary studies of the actions of bombesin/gastrin releasing peptide in small cell lung cancer suggest that the signals stimulated in these cells are similar to those seen in Swiss 3T3 cells. Rapid and transient mobilisation of intracellular Ca^{2+} occurs, with inositol phosphate turnover.[29 30] These observations confirm the value of Swiss 3T3 cells as a model system. We therefore tested the hypothesis that small cell lung cancer cells, like Swiss 3T3 cells, express receptors for numerous mitogenic neuropeptides.

We screened 32 peptides and hormones for their ability to mobilise intracellular Ca^{2+} in five small cell lung cancer cell lines.[31] The cells were incubated with the fluorescent Ca^{2+} indicator Fura-2, washed and resuspended in a cuvette in a continuously recording luminescence fluorimeter. All the cell lines responded to addition of peptide with rapid and transient mobilisation of intracellular Ca^{2+}, but the active peptides differed between the cell lines. Three cell lines responded to gastrin releasing peptide whereas all cell lines responded to vasopressin. Responses were also obtained with bradykinin, cholecystokinin, galanin, and neurotensin. The cells lost their responsiveness with repeated additions of the same peptide, but retained the ability to respond to unrelated peptides (homologous desensitisation). These effects were blocked by ligand specific antagonists. These observations indicate that the peptides act through distinct receptors and that small cell lung cancer cells have specific receptors for many peptides.

Additional factors have now been shown to bind to small cell lung cancer cells or stimulate intracellular signals (table 2). Many of these are neuropeptides and most can be synthesised by small cell lung cancer cells (table 1). All are known to act as growth promoting or inhibiting factors under appropriate conditions.[1] These findings have dramatically altered our view of growth control in small cell lung cancer. Only five years ago bombesin/gastrin releasing peptide was hailed as an autocrine growth factor for these cells. Now we envisage a complex network of autocrine and paracrine growth factors secreted by the tumours, interacting with tumour and host cells to support proliferation. Stimulation of tumour growth by these factors will be necessary to confirm this hypothesis.

OTHER NEUROPEPTIDE GROWTH FACTORS

So far only a few of the proposed growth factors for small cell lung cancer have been clearly shown to stimulate proliferation (table 3). Bombesin like peptides, including gastrin releasing peptide and neuromedin B, stimulate the growth of small cell lung cancer colonies in soft agar in vitro[9 32] but not in liquid culture, probably because maximal growth is already achieved in serum free HITES medium.[33] In nude mice bearing human small cell lung cancer xenografts and having thrice daily intraperitoneal injections of bombesin, tumour

Table 2 Factors that bind to small cell lung cancer cells or initiate signalling

Bombesin/gastrin releasing peptide
Bradykinin
Cholecystokinin
Galanin
Gastrin
Granulocyte colony stimulating factor
Granulocyte-macrophage colony stimulating factor
Insulin like growth factor I
Neurotensin
Opioid peptides
Physalaemin
Somatostatin
Tachykinins
Testosterone
Transferrin
Vasoactive intestinal peptide
Vasopressin

size and weight increased more than in a control group injected with saline.[10]

The opioid peptides, including enkephalins, dynorphins, and endorphins, are widely distributed in the mammalian central nervous system. Several receptor subtypes have been identified chemically and pharmacologically. Two small cell lung cancer cell lines have been shown to contain opioid peptides and receptors, but whether this is a common finding in fresh tumours samples is not clear.[34] Opioids have been reported to both stimulate and inhibit clonal small cell lung cancer growth in soft agar[35 36]; naloxone similarly elicits both responses.

Neurotensin, a 13 amino acid neuropeptide, was first detected in small cell lung cancer 10 years ago.[6 37] It has recently been shown to mobilise intracellular Ca^{2+} in a subset of small cell lung cancer cells, apparently acting through its own receptors.[31 38 39] The finding that exogenous neurotensin can stimulate the clonal growth of small cell lung cancer in soft agar suggests that it too may be an autocrine or paracrine growth factor for small cell lung cancer.[35]

Galanin is a 29 amino acid peptide that occurs in neurones of the central and peripheral nervous system. It has been implicated in the control of pancreatic hormone release, smooth muscle contraction, and neuronal excitation. It has not so far been found in small cell lung cancer, nor has it been described as a mitogen. It was, however, found to mobilise intracellular Ca^{2+} in three of five small cell lung cancer cell lines tested.[31] Further studies have now confirmed its ability to induce Ca^{2+} mobilisation and inositol phosphate formation in some small cell lung cancer cells. Remarkably, it stimulates clonal growth in soft agar of small cell lung cancer cells expressing galanin receptors.[40] Other Ca^{2+} mobilising neuropeptides are under investigation as possible growth factors for small cell lung cancer.[41]

INSULIN LIKE GROWTH FACTOR I

Insulin like growth factor I (IGF-I) (somatomedin C) is a 70 amino acid peptide closely related to insulin and to IGF-II, with distinct high affinity receptors that have tyrosine kinase activity. Circulating concentrations of insulin like growth factor are some 1000 times higher than those of insulin, but most is complexed with specific binding proteins. Early studies of the nutritional requirements of small cell lung cancer in serum free medium established that insulin supplements were necessary,[33] but, as supraphysiological concentrations were needed for optimal growth, insulin seems likely to be acting with low affinity at the IGF-I receptor.

IGF-I is mitogenic for various cell types. It is secreted by small cell and non-small cell lung tumours and cell lines. Specific, high affinity IGF-I binding sites have been found on small cell lung cancer cells and exogenous IGF-I is mitogenic for these cells.[42–44] A monoclonal antibody to the IGF-I receptor (αIR-3) inhibited IGF-I and insulin stimulated growth of small cell lung cancer cell lines,[45] providing further evidence for an autocrine role for IGF-I. More recently, small cell lung cancer cells have been shown to secrete IGF binding proteins, which may also be important mediators of tumour growth.[46–48]

Table 3 Factors that stimulate cell proliferation in small cell lung cancer

Bombesin/gastrin releasing peptide/neuromedin B
Galanin
Insulin like growth factor I
Neurotensin
?β endorphin
?haemopoietic growth factors
?testosterone
?transferrin

OTHER GROWTH FACTORS

Transferrin, an 80 kDa β globulin, is synthesised in the liver and transports iron in the plasma. It is required for serum free culture of small cell lung cancer[33] and is secreted by some small cell lung cancer cell lines.[49 50] Whether transferrin has a simple nutritional role or acts as an autocrine growth factor in small cell lung cancer is not clear.

In many clinical studies of small cell lung cancer men have had a worse outcome than women. This observation led to the intriguing suggestion that androgens may promote the growth of small cell lung cancer. Maasberg *et al*[51] found specific androgen binding in eight of 13 small cell lung cancer cell lines and showed that testosterone and dehydrotestosterone stimulated their growth. These growth promoting effects were blocked by cyproterone acetate and flutamide. Clinical studies with antiandrogens are now in progress.

The recent use of haemopoietic growth factors to permit more intensive chemotherapy in patients with small cell lung cancer has led to an examination of the growth promoting activity of these factors in small cell lung cancer cells. Granulocyte colony stimulating factor (G-CSF) may be secreted by some small cell lung cancers.[52] G-CSF and granulocyte-macrophage CSF (GM-CSF) appear to bind to a minority of small cell lung cancer cell lines and can stimulate their growth in vitro.[53–55] These effects seem unlikely to prove important clinically, but this can be tested only in large randomised trials.

GROWTH INHIBITORY FACTORS

The identification of diverse growth promoting factors was soon followed by the finding that their actions were context dependent and that some could promote and inhibit growth under different conditions.[56] Some factors are predominantly growth inhibiting, and combinations of factors are likely to maintain homeostasis in normal tissues. This balance may be disturbed in wounds and tumours. The use of growth inhibitory factors to treat cancer is an attractive proposition. Interferons have been shown to have some growth retarding effects in small cell lung cancer and are undergoing clinical trials.[57]

Physalaemin, an amphibian tachykinin, has

been identified in small cell lung cancer cells.[58] Although specific physalaemin receptors have not been found on small cell lung cancer cells, physalaemin mobilises intracellular Ca^{2+} in some cell lines.[59] Exogenous physalaemin inhibits clonal and mass culture growth of small cell lung cancer in vitro at picomolar concentrations, and may therefore act as an autocrine or paracrine growth regulator in vivo.[60]

Physiological release of somatomedins, including IGF-I, is stimulated by human growth hormone and inhibited by somatostatin. Somatostatin also has direct antiproliferative effects in many tumour types. Receptors for somatostatin are present on perhaps half of primary small cell lung cancer tumours and present an interesting target for treatment.[61 62] Long acting somatostatin analogues are now available for clinical study. The finding that a drug of this type can inhibit the growth of small cell lung cancer in vitro and in xenografts in nude mice is encouraging.[63 64]

Non-small cell lung cancer

Non-small cell lung cancers form a heterogeneous group of tumours that include large cell and squamous cell carcinomas and adenocarcinomas. They show varying degrees of differentiation and can express a range of growth factors.

Epidermal growth factor and transforming growth factor α (TGF α) have been demonstrated in non-small cell lung cancer cell lines and tumours. Both bind to the epidermal growth factor receptor, which is present in some non-small cell lung cancers, and autocrine growth stimulation has been suggested.[65–68] $p185^{neu}$, the product of the HER2/neu oncogene, is a transmembrane protein having homology with the epidermal growth factor receptor and is also present in some non-small cell lung cancers.[69] Further growth factors and neuroendocrine markers expressed by non-small cell lung cancers include platelet derived growth factor, bombesin/gastrin releasing peptide, neurone specific enolase, and chromogranin A. The expression of growth factors and their receptors in some non-small cell lung cancers appears to be related to aggressive clinical behaviour and increased likelihood of response to chemotherapy.[70–72]

Therapeutic implications

The rapid progress achieved in identifying growth factors for lung cancer has raised hopes of more rational anticancer treatments. These may be thought of as breaking the loop of autocrine or paracrine growth stimulation at the level of growth factor, receptor, or intracellular signals. The increasing evidence that several growth factors stimulate proliferation of these tumours[41] suggests that no single antiproliferative agent is likely to be curative. On the other hand, the interdependence of diverse growth factors may mean that small changes in the hormonal milieu could have profound effects on growth. This approach has been exploited to good effect in the hormonal treatment of breast cancer.

The expression of growth factor receptors on cancer cells can be exploited by conjugating toxins or radioisotopes to the ligand. The epidermal growth factor receptor has been targeted in this way by conjugating *Pseudomonas* exotoxin subunits to transforming growth factor-α. The resulting compound is cytotoxic in vitro to cells carrying the epidermal growth factor receptor and cytostatic for xenografts of these tumours but dose limiting toxicity occurs in normal liver. Further developments are needed before clinical studies can start.

Antibodies to growth factors and their receptors can be used to block autocrine or paracrine growth stimulation. The monoclonal bombesin antibody 2A11[11] has entered clinical study in patients with small cell lung cancer in the United States, at the National Cancer Institute, Bethesda, Maryland. Radioisotope labelled antibody has shown some degree of localisation in the tumour and the gastrointestinal tract, but at the low doses used so far no antitumour effects have been seen. The potential problems of anti-idiotype reactions have not been seen. To reduce this risk newer approaches, using Fab fragments and humanised antibodies (rodent antibody variable regions linked to human constant regions), are being developed.

The large size of antibodies might restrict their penetration of tumours. Small peptides are an attractive alternative approach. Like agonists or antibodies, antagonists could be linked to toxins or radioisotopes if desired. The development of peptide antagonists to neuropeptide growth factors for small cell lung cancer is an area of active research.[73] Swiss 3T3 cells have again proved useful as a model system for testing new compounds. Ligand specific antagonists, such as the pseudopeptide analogues of bombesin, have been shown to inhibit bombesin stimulated mitogenesis in these cells, and these antagonists have been tested in small cell lung cancer in vitro and in vivo.[74 75] Because bombesin/gastrin releasing peptide is not mitogenic for all small cell lung cancers, specific antagonists will be useful for only a minority of patients. In contrast, broad spectrum antagonists, with activity against many mitogenic neuropeptides, will have wider application.[76] New peptide antagonists with enhanced potency and reduced toxicity are being developed, but much interest has also centred on the benzodiazepine like non-peptide antagonists.[73 77] These compounds are orally bioavailable and have a longer plasma half life than the peptide antagonists.

The use of growth inhibitory factors (see above) as therapeutic agents is a direct development from growth factor research. Clinical studies with somatostatin analogues are now in progress. The role of cytokines, such as interferon, in altering the expression of growth factors and their receptors on tumour cells is also under investigation. Preliminary results using α interferon as maintenance treatment in patients with small cell lung cancer in complete remission were encouraging, but need confir-

mation in a larger study; the mechanism underlying these effects remains to be determined.[57]

Increasing knowledge of the signal transduction pathways for growth factors has led to speculation that they could offer novel targets for anticancer treatment. The protein kinase C activator bryostatin is already under clinical study in Britain. G proteins and oncogenes are attractive targets, as they are often overexpressed in malignant cells. The problems of delivering chemotherapeutic agents, such as antisense oligonucleotides, into the intact cell offer further challenges to ingenuity. These approaches have the advantage of blocking signals from many mitogens. Attacking such ubiquitous targets might be expected to have toxic effects throughout the body, but this should not be assumed. The calcium channel blockers, including nifedipine and verapamil, show that selective pharmacological effects can be obtained with such apparently blunt instruments.

Studies of the expression of growth factor receptors have shown that some receptors become down regulated after prolonged exposure to ligand (homologous desensitisation), and others are down regulated by exposure of the cell to other ligands (heterologous desensitisation). The down regulation of the bombesin/gastrin releasing peptide receptor after exposure of Swiss 3T3 cells to vasopressin is a good example of this.[27] This phenomenon might be exploited therapeutically by the use of partial agonists to desensitise tumour cells to growth factors.

Conclusion

Small cell lung cancer is exquisitely chemosensitive and drug treatment can reduce tumour mass dramatically but relapse due to development of resistance is almost invariable. Despite intensive research into cytotoxic chemotherapy, the survival of patients with lung cancer has not improved in the past 10 years. Recent discoveries in lung tumour biology have raised hopes of new treatments and improved survival for patients with both small cell and non-small cell lung cancer in the future.

As knowledge of the action of growth factors in lung cancer has increased, an initially simple picture has become more complex. The number of factors known to be implicated in the proliferation of small cell lung cancer is still rising, but it is clear that all the tumours secrete and respond to various growth factors. Understanding how these factors act opens up exciting new therapeutic strategies. Innovative compounds are already reaching patients, and close collaboration between laboratory and clinic will be needed to exploit their promise to the full.

1 Woll PJ. Neuropeptide growth factors and cancer. *Br J Cancer* 1991;63:469–75.
2 Ghatei MA, Sheppard MN, Henzen-Logman S, Blank MA, Polak JM, Bloom SR. Bombesin and vasoactive intestinal peptide in the developing lung: marked changes in the respiratory distress syndrome. *J Clin Endocrinol Metab* 1983;57:1226–32.
3 Spindel ER, Sunday ME, Hofler H, Wolfe HJ, Habener JF, Chin WW. Transient elevation of messenger RNA encoding gastrin-releasing peptide, a putative pulmonary growth factor in human fetal lung. *J Clin Invest* 1987; 80:1172–9.
4 Moody TW, Pert CB, Gazdar AF, Carney DN, Minna JD. High levels of intracellular bombesin characterize human small-cell lung carcinoma. *Science* 1981;214:1246–8.
5 Suzuki M, Yamaguchi K, Abe K, *et al*. Detection of gastrin-releasing peptide mRNA in small cell lung carcinomas and medullary thyroid carcinomas using synthetic oligodeoxyribo-nucleotide probes. *Jpn J Clin Oncol* 1987; 17:157–63.
6 Wood SM, Wood JR, Ghatei MA, Lee YC, O'Shaughnessy D, Bloom SR. Bombesin, somatostatin and neurotensin-like immunoreactivity in bronchial carcinoma. *J Clin Endocrinol Metab* 1981;53:1310–2.
7 Rozengurt E, Sinnett-Smith J. Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells. *Proc Natl Acad Sci USA* 1983;80:2936–40.
8 Moody TW, Carney DN, Cuttitta F, Quattrocchi K, Minna JD. High affinity receptors for bombesin/GRP-like peptides on human small cell lung cancer. *Life Sci* 1985; 37:105–13.
9 Carney DN, Cuttitta F, Moody TW, Minna JD. Selective stimulation of small cell lung cancer clonal growth by bombesin and gastrin-releasing peptide. *Cancer Res* 1987; 47:821–5.
10 Alexander RW, Upp JR, Poston GJ, Gupta V, Townsend CM, Thompson JC. Effects of bombesin on growth of human small cell lung carcinoma *in vivo*. *Cancer Res* 1988; 48:1439–41.
11 Cuttitta F, Carney DN, Mulshine J, *et al*. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. *Nature* 1985;316:823–6.
12 Rozengurt E, Fabregat I, Coffer A, Gil J, Sinnett-Smith J. Mitogenic signalling through the bombesin receptor: role of a guanine nucleotide regulatory protein. *J Cell Sci* 1990;(suppl 13):43–56.
13 Zachary I, Rozengurt E. High-affinity receptors for peptides of the bombesin family in Swiss 3T3 cells. *Proc Natl Acad Sci USA* 1985;82:7616–20.
14 Kris RM, Hazan R, Villines J, Moody TW, Schlessinger J. Identification of the bombesin receptor on murine and human cells by cross-linking experiments. *J Biol Chem* 1987;262:11215–20.
15 Zachary I, Rozengurt E. Identification of a receptor for peptides of the bombesin family in Swiss 3T3 cells by affinity cross-linking. *J Biol Chem* 1987;262:3947–50.
16 Sinnett-Smith J, Zachary I, Rozengurt E. Characterization of a bombesin receptor on Swiss mouse 3T3 cells by affinity cross-linking. *J Cell Biochem* 1988;38:237–49.
17 Spindel ER, Giladi E, Brehm P, Goodman RH, Segerson TP. Cloning and functional characterization of a complementary DNA encoding the murine fibroblast bombesin/gastrin-releasing peptide receptor. *Mol Endocrinol* 1990;4:1956–63.
18 Battey JF, Way JM, Corjay MH, *et al*. Molecular cloning of the bombesin/gastrin-releasing peptide receptor from Swiss 3T3 cells. *Proc Natl Acad Sci USA* 1991;88:395–9.
19 Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ. Model systems for the study of seven transmembrane segment receptors. *Annu Rev Biochem* 1991;60:653–88.
20 O'Dowa BE, Lefkowitz RJ, Caron MG. Structure of the adrenergic and related receptors. *Annu Rev Neurosci* 1989;12:67–83.
21 Jackson TR, Blair LAC, Marshall J, Goedert M, Hanley MR. The *mas* oncogene encodes an angiotensin receptor. *Nature* 1988;335:437–40.
22 Julius D, Livelli TJ, Jessell TM, Axel R. Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. *Science* 1989;244:1057–62.
23 Mendoza SA, Schneider JA, Lopez-Rivas A, Sinnett-Smith JW, Rozengurt E. Early events elicited by bombesin and structurally-related peptides in quiescent Swiss 3T3 cells. II. Changes in Na^+ and Ca^{2+} fluxes, Na^+/K^+ pump activity, and intracellular pH. *J Cell Biol* 1986;102: 2223–33.
24 Zachary I, Sinnett-Smith JW, Rozengurt E. Early events elicited by peptides of the bombesin family in Swiss 3T3 cells. I. Activation of protein kinase C and inhibition of epidermal growth factor binding. *J Cell Biol* 1986;102: 2211–22.
25 Erusalimsky JD, Brooks SF, Herget T, Morris C, Rozengurt E. Molecular cloning and characterization of the acidic 80kDa protein kinase C substrate from rat brain. *J Biol Chem* 1991;266:7073–80.
26 Rozengurt E. Early signals in the mitogenic response. *Science* 1986;234:161–6.
27 Millar JBA, Rozengurt E. Arachidonic acid release by bombesin: a novel post-receptor target for heterologous mitogenic desensitization. *J Biol Chem* 1990;265:19973–9.
28 Rozengurt E, Sinnett-Smith J. Early signals underlying the induction of the c-*fos* and c-*myc* genes in quiescent fibroblasts: studies with bombesin and other growth factors. *Progr Nucl Acid Res Mol Biol* 1988;35:261–95.
29 Heikkila R, Trepel JB, Cuttitta F, Neckers LM, Sausville EA. Bombesin-related peptides induce calcium mobiliza-

tion in a subset of human small cell lung cancer cell lines. *J Biol Chem* 1987;**262**:16456–60.
30 Trepel JB, Moyer JD, Heikkila R, Sausville EA. Modulation of bombesin-induced phosphatidylinositol hydrolysis in a small-cell lung-cancer cell line. *Biochem J* 1988;**255**: 403–10.
31 Woll PJ, Rozengurt E. Multiple neuropeptides mobilise calcium in small cell lung cancer: effects of vasopressin, bradykinin, cholecystokinin, galanin and neurotensin. *Biochem Biophys Res Commun* 1989;**164**:66–73.
32 Bepler G, Rotsch M, Jaques G, *et al.* Peptides and growth factors in small cell lung cancer: production, binding sites, and growth effects. *J Cancer Res Clin Oncol* 1988;**114**: 235–44.
33 Simms E, Gazdar AF, Abrams PG, Minna JD. Growth of human small cell (oat cell) carcinoma of the lung in serum-free growth factor-supplemented medium. *Cancer Res* 1980;**40**:4356–63.
34 Roth KA, Barchas JD. Small cell carcinoma cell lines contain opioid peptides and receptors. *Cancer* 1986;**57**:769–73.
35 Davis TP, Burgess HS, Crowell S, Moody TW, Culling-Berglund A, Liu RH. β-endorphin and neurotensin stimulate in vitro clonal growth of human SCLC cells. *Eur J Pharmacol* 1989;**161**:283–5.
36 Maneckjee R, Minna JD. Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. *Proc Natl Acad Sci USA* 1990;**87**:3294–8.
37 Goedert M, Reeve JG, Emson PC, Bleehen NM. Neurotensin in human small cell lung carcinoma. *Br J Cancer* 1984;**50**:179–83.
38 Staley J, Fiskum G, Davis TP, Moody TW. Neurotensin elevates cytosolic calcium in small cell lung cancer cells. *Peptides* 1989;**10**:1217–21.
39 Bunn PA, Dienhart DG, Chan D, *et al.* Neuropeptide stimulation of calcium flux in human lung cancer cells: delineation of alternate pathways. *Proc Natl Acad Sci USA* 1990;**87**:2162–6.
40 Sethi T, Rozengurt E. Galanin stimulates Ca^{2+} mobilization, inositol phosphate accumulation and clonal growth in small cell lung cancer cells. *Cancer Res* 1991;**51**:1674–9.
41 Sethi T, Rozengurt E. Multiple neuropeptides stimulate clonal growth of small cell lung cancer: effects of bradykinin, vasopressin, cholecystokinin, galanin and neurotensin. *Cancer Res* 1991;**51**:3621–3.
42 Jaques G, Rotsch M, Wegmann C, Worsch U, Maasberg M, Havemann K. Production of immunoreactive insulin-like growth factor I and response to exogenous IGF-I in small cell lung cancer cell lines. *Exp Cell Res* 1988;**176**:336–43.
43 Macaulay VM, Teale JD, Everard MJ, Joshi GP, Smith IE, Millar JL. Somatomedin-C/insulin-like growth factor-I is a mitogen for human small cell lung cancer. *Br J Cancer* 1988;**57**:91–3.
44 Macaulay VM, Everard MJ, Teale JD, *et al.* Autocrine function for insulin-like growth factor I in human small cell lung cancer cell lines and fresh tumor cells. *Cancer Res* 1990;**50**:2511–7.
45 Nakanishi Y, Mulshine JL, Kasprzyk PG, *et al.* Insulin-like growth factor-I can mediate autocrine proliferation of human small cell lung cancer cell lines in vitro. *J Clin Invest* 1988;**82**:354–9.
46 Jaques G, Kiefer P, Rotsch M, *et al.* Production of insulin-like growth factor binding proteins by small-cell lung cancer cell lines. *Exp Cell Res* 1989;**184**:396–406.
47 Reeve JG, Payne JA, Bleehen NM. Production of immunoreactive insulin-like growth factor-I (IGF-I) and IGF-I binding proteins by human lung tumours. *Br J Cancer* 1990;**61**:727–31.
48 Kiefer P, Jaques G, Schöneberger J, Heinrich G, Havemann K. Insulin-like growth factor binding protein expression in human small cell lung cancer cell lines. *Exp Cell Res* 1991;**192**:414–7.
49 Nakanishi Y, Cuttitta F, Kasprzyk PG, *et al.* Growth factor effects on small cell lung cancer cells using a colorimetric assay: can a transferrin-like factor mediate autocrine growth? *Expl Cell Biol* 1988;**56**:74–85.
50 Vostrejs M, Moran PL, Seligman PA. Transferrin synthesis by small cell lung cancer cells acts as an autocrine regulator of cellular proliferation. *J Clin Invest* 1988;**82**:331–9.
51 Maasberg M, Rotsch M, Jaques G, Enderle-Schmidt U, Weehle R, Havemann K. Androgen receptors, androgen-dependent proliferation, and 5α-reductase activity of small-cell lung cancer cell lines. *Int J Cancer* 1989;**43**: 685–91.
52 Abe K, Kameya T, Yamaguchi K, *et al.* Hormone-producing lung cancers. Endocrinologic and morphologic studies. In: Becker KL, Gazdar AF, eds. *The endocrine lung in health and disease*. London: Saunders, 1984: 549–95.
53 Avalos BR, Gasson JC, Hedvat C, *et al.* Human granulocyte colony-stimulating factor: biologic activities and receptor characterization on hematopoietic cells and small cell lung cancer cell lines. *Blood* 1990;**75**:851–7.
54 Foulke RS, Marshall MH, Trotta PP, von Hoff DD. *In vitro* assessment of the effects of granulocyte-macrophage colony-stimulating factor on primary human tumors and derived lines. *Cancer Res* 1990;**50**:6264–7.
55 Vellenga E, Biesma B, Meyer C, Wagteveld L, Esselink M, de Vries EGE. The effects of five hematopoietic growth factors on human small cell lung carcinoma cell lines: interleukin 3 enhances the proliferation in one of the eleven cell lines. *Cancer Res* 1991;**51**:73–6.
56 Sporn MB, Roberts AB. Peptide growth factors are multifunctional. *Nature* 1988;**332**:217–9.
57 Jett JR. Is there a role for interferon in the treatment of small cell lung cancer? *Lung Cancer* 1989;**5**:281–6.
58 Lazarus LH, DiAugustine RP, Jahnke GD, Hernandez O. Physalaemin: an amphibian tachykinin in human lung small-cell carcinoma. *Science* 1983;**219**:79–81.
59 Takuwa N, Takuwa Y, Ohue Y, *et al.* Stimulation of calcium mobilization but not proliferation by bombesin and tachykinin neuropeptides in human small cell lung cancer cells. *Cancer Res* 1990;**50**:240–4.
60 Bepler G, Carney DN, Gazdar AF, Minna JD. *In vitro* growth inhibition of human small cell lung cancer by physalaemin. *Cancer Res* 1987;**47**:2371–5.
61 Reubi JC, Waser B, Sheppard M, Macaulay V. Somatostatin receptors are present in small-cell but not in non-small-cell lung carcinomas: relationship to EGF-receptors. *Int J Cancer* 1990;**45**:269–74.
62 Sagman U, Mullen JB, Kovacs K, Kerbel R, Ginsberg R, Reubi J-C. Identification of somatostatin receptors in human small cell lung carcinoma. *Cancer* 1990;**66**: 2129–33.
63 Taylor JE, Bogden AE, Moreau J-P, Coy DH. *In vitro* and *in vivo* inhibition of human small cell lung carcinoma (NCI-H69) growth by a somatostatin analogue. *Biochem Biophys Res Commun* 1988;**153**:81–6.
64 Bogden AE, Taylor JE, Moreau J-P, Coy DH, LePage DJ. Response of human lung tumor xenografts to treatment with a somatostatin analogue (somatuline). *Cancer Res* 1990;**50**:4360–5.
65 Haeder M, Rotsch M, Bepler G, *et al.* Epidermal growth factor receptor expression in human lung cancer cell lines. *Cancer Res* 1988;**48**:1132–6.
66 Söderdahl G, Betsholtz C, Johansson A, Nilsson K, Bergh J. Differential expression of platelet-derived growth factor and transforming growth factor genes in small- and non-small-cell human lung carcinoma lines. *Int J Cancer* 1988;**41**:636–41.
67 Veale D, Kerr N, Gibson GJ, Harris AL. Characterization of epidermal growth factor receptor in primary human non-small cell lung cancer. *Cancer Res* 1989;**49**:1313–7.
68 Tateishi M, Ishida T, Mitsudomi T, Kaneko S, Sugimachi K. Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. *Cancer Res* 1990;**50**:7077–80.
69 Kern JA, Schwartz DA, Nordberg JE, *et al.* p185^{neu} expression in human lung adenocarcinomas predicts shortened survival. *Cancer Res* 1990;**50**:5184–91.
70 Mooi WJ, Dewar A, Springall D, Polak JM, Addis BJ. Non-small cell lung carcinomas with neuroendocrine features. A light microscopic, immunohistochemical and ultrastructural study of 11 cases. *Histopathology* 1988;**13**: 329–37.
71 Graziano SL, Mazid R, Newman N, *et al.* The use of neuroendocrine immunoperoxidase markers to predict chemotherapy response in patients with non-small-cell lung cancer. *J Clin Oncol* 1989;**7**:1398–406.
72 Hamid QA, Corrin B, Dewar A, Hoefler H, Sheppard MN. Expression of gastrin-releasing peptide (human bombesin) gene in large cell undifferentiated carcinoma of the lung. *J Pathol* 1990;**161**:145–51.
73 Woll PJ, Sethi T, Rozengurt E. Neuropeptide growth factors and antagonists. In: Tritton TR, Hickman JA, eds. *Cancer chemotherapy*. Oxford: Blackwell (in press).
74 Woll PJ, Rozengurt E. [Leu^{13}-*psi* (CH_2NH)Leu^{14}]bombesin is a specific bombesin receptor antagonist in Swiss 3T3 cells. *Biochem Biophys Res Commun* 1988;**155**:359–65.
75 Mahmoud S, Staley J, Taylor J, *et al.* [Psi^{13,14}] bombesin analogues inhibit growth of small cell lung cancer *in vitro* and *in vivo*. *Cancer Res* 1991;**51**:1798–802.
76 Woll PJ, Rozengurt E. A neuropeptide antagonist that inhibits the growth of small cell lung cancer *in vitro*. *Cancer Res* 1990;**50**:3968–73.
77 Chang RSL, Lotti VJ. Biochemical and pharmacological characterisation of an extremely potent and selective non-peptide cholecystokinin antagonist. *Proc Natl Acad Sci USA* 1986;**83**:4923–6.