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GROWTH REGULATION BY INSULIN-LIKE GROWTH FACTORS IN LUNG CANCER

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Summary—Lung cancer is a major health problem, with over 38,000 new cases expected every year in West Germany. A more complete understanding of the biology of lung cancer will hopefully lead to therapeutic modalities. The possible autocrine growth regulation in small-cell lung cancer and non-small-cell lung cancer has been demonstrated for bombesin/GRP, vasopressin, neurotensin, EGF/TGFα, transferrin-related peptides and insulin-like growth factors. This contribution concentrates on recent data concerning binding sites, growth promoting effects and secretion of IGFs in lung cancer cell lines. The production of IGF-binding proteins which were also produced by lung cancer cell lines modifies the autocrine/paracrine model for IGFs since then proteins can either enhance or inhibit the effect of IGFs on tumor growth.

INTRODUCTION

Lung cancer is the most common type of cancer in men all over the world and is the leading cause of cancer mortality in males in more than 35 countries. For females, lung cancer is expected to become more common as the percentage of woman smokers continues to increase [1]. Human lung cancer can be subgrouped into two major categories, one being the small-cell carcinoma (SCLC) and the other the non-small-cell carcinoma (NSCLC); the latter are subdivided in squamous-, adeno- and large-cell carcinoma. SCLC represents about 25–30% of all lung cancer.

SCLC differs from NSCLC by its characteristic neuroendocrine properties, by its tendency to metastasize rapidly and by its responsiveness to chemotherapy and radiotherapy [2].

We and others have developed *in vitro* growth of permanent cell lines of SCLC and NSCLC from patients with this disease, in order to better understand the origin and biology of this tumor [3, 4]. SCLC cell lines showing properties of the neuroendocrine system, such as L-DOPA decarboxlase, bombesin-like immunoreactivity, neuron-specific enolase and the BB isoenzyme of creatine kinase, belong to the so-called classic subtype, and SCLC cells lacking these markers represent the variant subtype [5]. The lung cancer cell lines have provided material for studying the origin and growth requirements of these tumors, the regulation of secretory products and the mechanism of hormone synthesis.

It has been proposed that transformed cells may express, maintain or enhance their malignant phenotype by the secretion of autocrine growth factors, thus enabling a positive feedback loop, whereby a cell may both produce and respond to the same growth factor [6]. This autocrine regulatory system may lead to a growth advantage of tumor cells. Table 1 summarizes the possible autocrine growth factors in lung cancer [7]. In SCLC cell surface receptors, peptide hormone production and proliferative effects have been shown for bombesin/GRP, vasopressin and neurotensin, whereas these peptides are probably without importance in NSCLC. On the other hand EGF/TGFα receptors, a production of TGFα and a growth promotion by EGF/TGFα are only relevant in NSCLC. In addition, an autocrine loop has been suggested for transferrin-related peptides in all types of lung cancer. Recent data also show the family of insulin-like growth factors (IGFs) to be important regulatory peptides in lung cancer [8]. These IGFs have a similar structure to proinsulin and are the main growth

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Table 1. Possible autocrine growth factors in lung cancer

	SCLC			NSCLC		
	Receptor	Product.	Mitog.	Receptor	Product.	Mitog.
Bombesin/GRP, vasopressin, neurotensin	+	+	+	0	0	0
EGF/TGFα	(+)	+	0	+	+	+
Transferrin-related peptides	+	+	+	+	+	+
IGF	+	+	+	+	+	+

factors for mammalian cells [9]. IGF-II is predominantly important for embryonal development, whereas IGF-I is mainly active in postembryonal life.

In the following, this contribution will concentrate on recent data concerning binding sites, growth promoting effects as well as a secretion of IGFs in lung cancer cell lines.

IGF-BINDING SITES

Both IGFs bind to the IGF-I receptor and induce metabolic and proliferative effects [9]. The IGF-I receptor is a tyrosine kinase, while the mechanism of signal transduction via the IGF-II receptor is still not established. It could be demonstrated that the IGF-II receptor is a bifunctional protein, both binding IGF-II and mannose-6-phosphate.

IGF-I-binding sites

These sites have been demonstrated by classical binding experiments, crosslinking of the receptor protein and by mRNA expression. Binding of radiolabeled IGF-I and displacement by increasing concentrations of unlabeled IGF-I is shown in Fig. 1. The Scatchard analysis shows a B_{max} of about 200 fmol/mg and a K_d of 1.8 nM. This suggests one class of binding sites and a receptor with high affinity. The high affinity binding of IGF-I is present in all lung cancer cell lines including the breast cancer cell line MCF-7 (Table 2). Since the binding can be displaced by a 100-fold higher concentration of insulin, which is typical for the IGF-I but not for the IGF-II receptor, it is concluded that the cell lines possess the IGF-I receptor. Crosslinking experiments show an IGF-I receptor of about 300 kDa. This protein is also precipitated with an IGF-I receptor antibody.

In addition, all lung cancer cell lines exhibit an mRNA transcript of 11 kb typical for the IGF-I receptor mRNA in northern blot analysis. The first 7 probes (lanes A–G) are prepared from SCLC and the next 9 probes (lanes H–P) from NSCLC lines (Fig. 2).

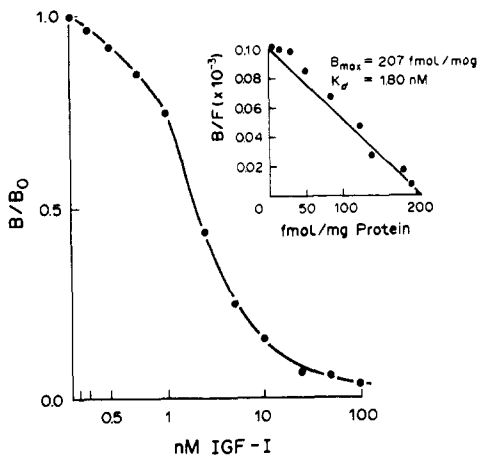

Fig. 1. Competition of labeled and unlabeled IGF-I for binding to SCLC cell line NCI-H69. Indicated amounts of unlabeled IGF-I and ^{125}I-labeled IGF-I were added simultaneously to the cells. *Ordinate*: bound IGF-I (B); bound IGF-I in the absence of unlabeled IGF-I (B_0). *Abscissa*: unlabeled IGF-I (nM). *Insert*: Scatchard plot of binding data.

Table 2. IGF-I-binding sites in human cancer cell lines

Cell line	Maximal binding (fmol/mg)	K_d
SCLC		
Classic		
SCLC-16HC	309	1.25
SCLC-22H	330	1.02
SCLC-24H	317	0.89
SCLC-86M1	186	1.33
NCI-H69	207	1.80
NCI-H146	164	0.61
NCI-N592	272	3.05
Variant		
SCLC-16HV	532	5.21
SCLC-21H	1121	3.19
NCI-H82	1230	3.08
NCI-N526	400	2.88
DMS-79	131	2.80
Squamous cell carcinoma		
EPLC-32M1	721	2.43
EPLC-65H	280	0.75
U-1752	361	4.64
Adenocarcinoma		
NCI-H23	247	0.74
NCI-H125	110	0.94
A549	304	1.67
LCLC		
LCLC-97TM1	59	1.10
LCLC-103H	345	1.53
U-1810	261	0.99
Mesothelioma		
MSTO-211H	127	1.48
Breast carcinoma		
MCF-7	483	2.72
Myeloid leukemia		
HL-60	213	2.24

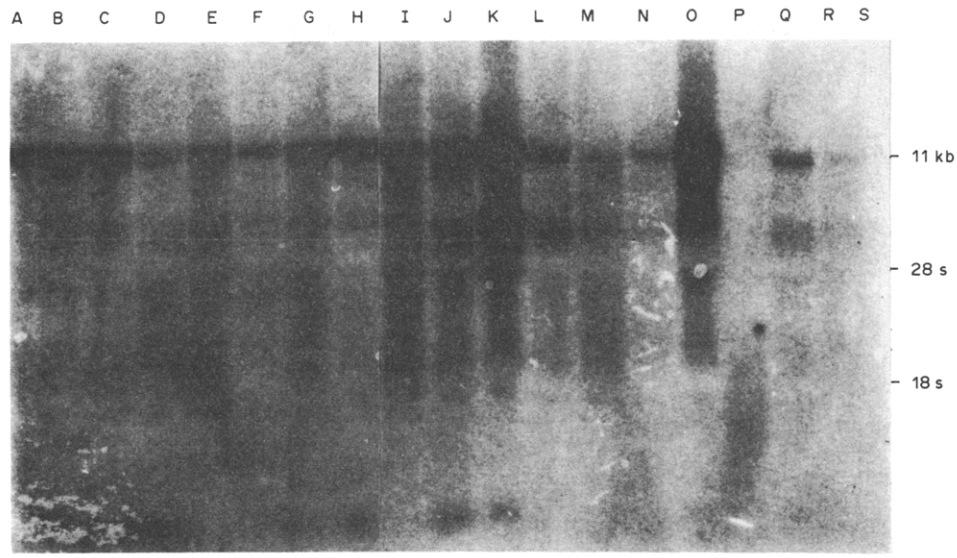

Fig. 2. Northern blot analysis of IGF-I receptor mRNA of SCLC and NSCLC cell lines. A 730 bp Eco RI fragment of pIGF-I-R.8 was used. An IGF-I receptor specific band of 11 kb was detected. The mobilities of 28 S (5.0 kb) and 18 S (2.1 kb) ribosomal RNAs are indicated on the right. A, SCLC-16HV; B, SCLC-22H; C, SCLC-24H; D, NCI-H69; E, NCI-H82; F, SCLC-16HC; G, SCLC-86M1; H, NCI-H23; I, EPLC-65H; J, DMS-79; K, EPLC-32M1; L, LCLC-103H; M, NCI-H125; N, LCLC-97TM1; O, A549; P, U-1810; Q, HL-60; R, A431; S, Daudy.

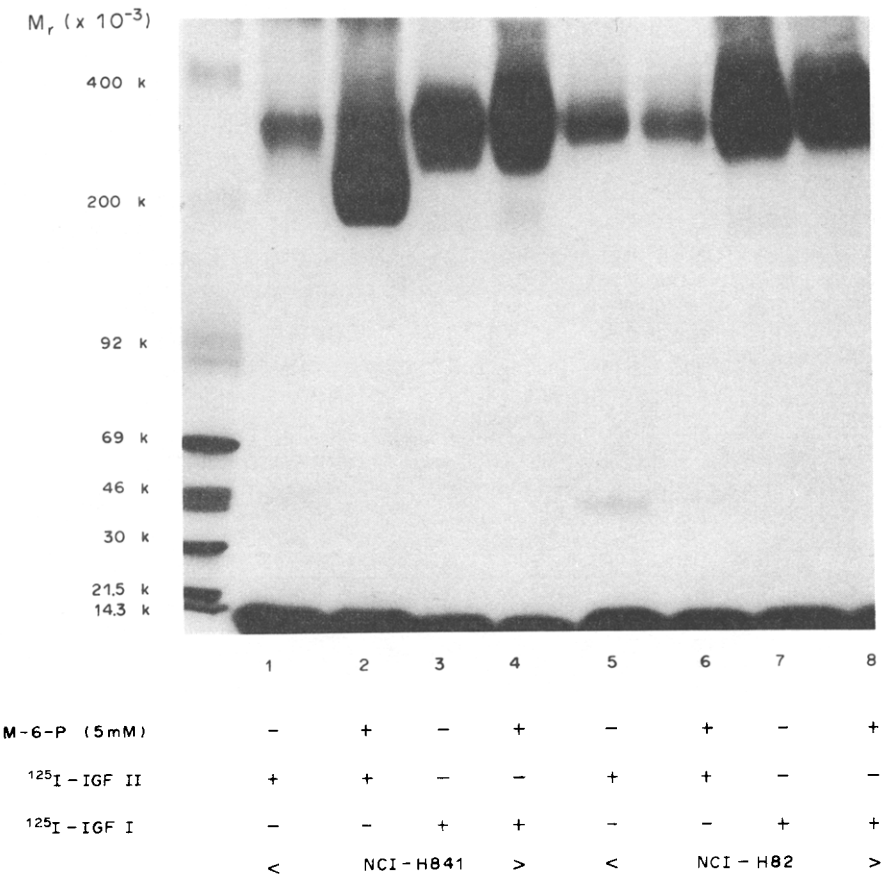

Fig. 3. Autoradiogram showing the size and specificity of ^{125}I-labeled IGF-I and ^{125}I-labeled IGF-II crosslinked complexes on the SCLC cell lines NCI-H841 and NCI-H82. The IGF-I receptor protein is presented with approx. 300 kDa, the IGF-II receptor with 220 kDa. The presence of cold IGF-I, IGF-II and mannose-6-phosphate is indicated at the bottom.

IGF-II-binding sites

These sites are more difficult to demonstrate. We only succeeded in crosslinking the receptor on subcellular fractions in the presence of mannose-6-phosphate. A crosslinking analysis (Fig. 3) shows the IGF-II receptor protein with approx. 220 kDa (lane 2) in comparison with the IGF-I receptor with 300 kDa (lane 3) on the SCLC cell line NCI-H841. The other cell line (NCI-H82) only exhibits the IGF-I receptor.

GROWTH PROMOTING EFFECTS

One important action of the IGFs, apart from the anabolic effect on cartilage cell and insulin-like effects on fat cells, is their ability to stimulate DNA-synthesis and cell proliferation in fibroblasts and other cell types. The growth promoting effects of exogenously added IGF-I and IGF-II were shown by clonogenic assay and by [^{3}H]thymidine uptake in SCLC and NSCLC cell lines. The example (Fig. 4) shows a mitogenic response to IGF-II in four different SCLC lines between 10^{-8} and 10^{-10} M. Similar effects could be demonstrated with IGF-II in the individual cell lines. This finding, together with a comparable stimulation by a 100-fold higher concentration of insulin, indicates that the mitogenicity of both IGF-I and IGF-II is mediated via the IGF-I receptor.

PRODUCTION OF IGFs

mRNA expression, characteristic of IGF-II, was shown in many of the cell lines of SCLC and NSCLC origin. In the corresponding cell lines, however, no IGF-I-related mRNA could be demonstrated. On the other hand, IGF-I immunoreactivity was detected in cell pellets and the conditioned media of most of the SCLC

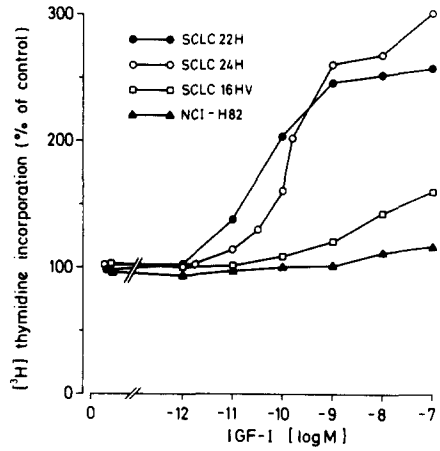

Fig. 4. Effect of IGF-I in different concentrations on DNA-synthesis in four SCLC cell lines.

lines (Table 3). The immunoreactive IGF-I in conditioned media increased as a function of time and the production could be inhibited by the addition of cycloheximide, suggesting protein synthesis. These results indicate that an IGF-I-related material is produced at least in SCLC lines, a protein, which still has to be characterized. It probably belongs to the insulin family but it is not identical with IGF-II or relaxin.

IGF-BINDING PROTEINS

In contrast to insulin, which circulates in plasma unbound, IGFs are transported by IGF-binding proteins. Three different binding proteins are described in the human system of which the genes are cloned:

1. IBP-53, to which 99% of the IGFs are attached in the serum [10].
2. IBP-1, identical with the placental protein BP-12 [11].
3. IBP-2, an IGF-binding protein mainly expressed in the neuronal system [12].

Table 3. IGF-I immunoreactivity in cells and conditioned media of SCLC cell lines

Cell line	Type	IGF-I immunoreactivity in cells (mIU/mg)	IGF-I immunoreactivity in conditioned media (mIU/mg)
SCLC-16HC	c[a]	30 ± 19	15 ± 1
SCLC-22H	c	17 ± 4	13 ± 2
SCLC-24H	c	23 ± 8	5 ± 1
SCLC-86M1	c	<0.5	<0.5
NCI-H69	c	39 ± 22	35 ± 7
NCI-H146	c	22 ± 4	18 ± 2
NCI-N592	c	35 ± 9	50 ± 4
SW-201 5	c	76 ± 3	ND[b]
SCLC-16HV	v	47 ± 17	118 ± 10
SCLC-21H	v	20 ± 9	ND
NCI-H82	v	<0.5	<0.5
NCI-N417	v	12 ± 5	ND
NCI-H526	v	<0.5	<0.5
DMS-79	v	43 ± 13	ND

[a]c = classic; v = variant.
[b]ND = not determined.

These binding proteins prolong the half-life of the IGFs, protect the organism from their metabolic effects and modulate the proliferative actions of IGFs in either inhibitory or stimulatory ways [13].

IGF-binding proteins could be demonstrated by applying an affinity-labeling technique. Six to nine conditioned media from SCLC cell lines show these binding proteins (Fig. 5). Similar binding proteins of about 30 kDa could be demonstrated in supernatants of NSCLC lines. The addition of unlabeled IGF-I or IGF-II, but not of insulin, inhibited this reaction. This is due to the fact that insulin crossreacts with IGF-I or IGF-II in its binding to the IGF-I receptor, but does not interact with the IGF-binding proteins. Therefore, these binding proteins in lung cancer cell lines are not identical with the IGF-I receptor.

The nucleotide sequence of the three aforementioned binding proteins has been published previously [10–12]. In using the corresponding cDNAs or specific oligonucleotides we demonstrated that SCLC lines regularly express the IBP-2 mRNA with 1.6 kb, without showing a signal for IBP-1 or IBP-53. It is of interest, that this gene is mainly expressed in brain, which may underline the neuroendocrine origin of SCLC.

In NSCLC the IBP-2 mRNA was not expressed. However, a number of the NSCLC lines expressed the 1.5 kb mRNA for IBP-1 and the 2.5 kb for IBP-53.

We can demonstrate [8, 14–17] (1) binding sites for IGFs, (2) a growth promoting effect

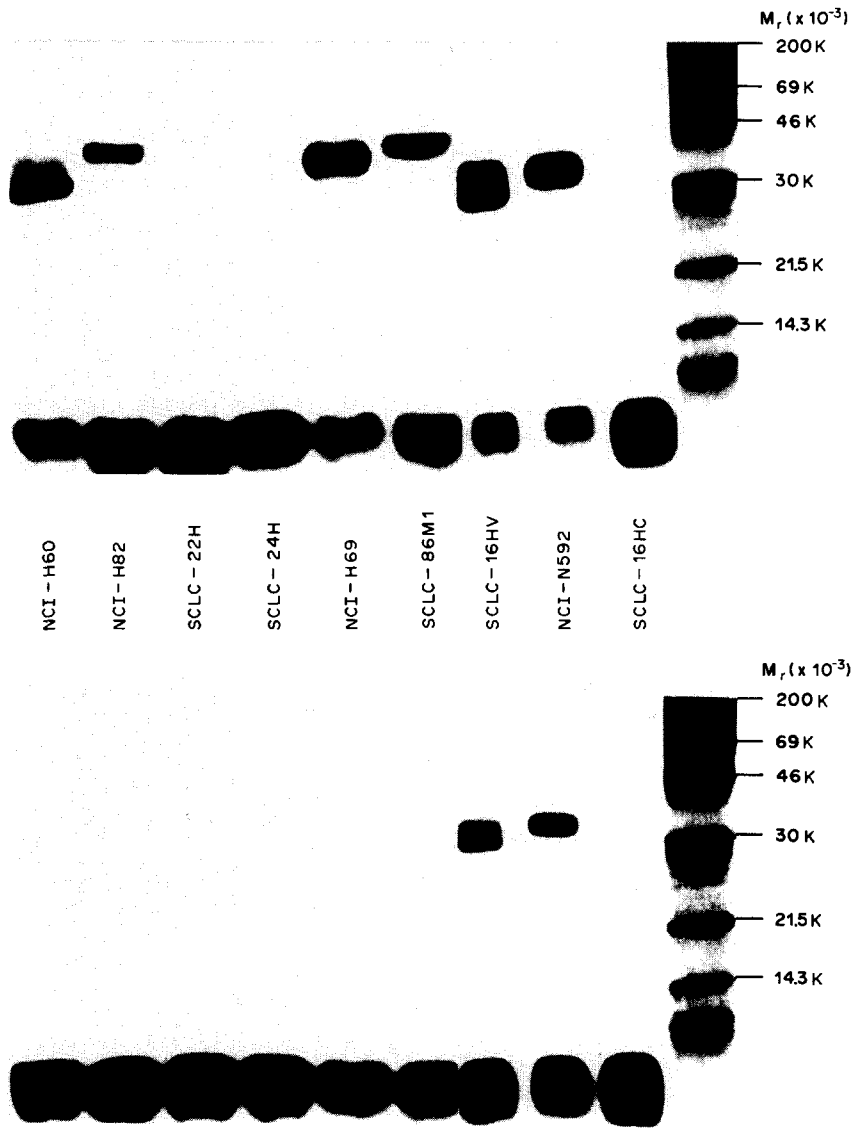

Fig. 5. Electrophoretic analysis (SDS–PAGE) and autoradiography of ^{125}I-labeled IGF-I-binding protein complexes in conditioned media of SCLC cell lines.

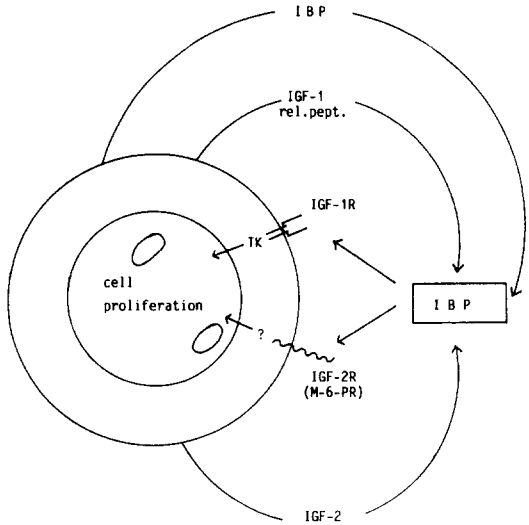

Fig. 6. Possible autocrine model for IGFs and IGF-binding proteins in lung cancer cell lines.

of these peptides, (3) the production of IGF-related proteins and (4) binding proteins for IGFs. These results on permanent cell lines suggest an autocrine growth stimulation of IGF-I and IGF-2 in both SCLC and NSCLC (Fig. 6).

Because of the IGF-binding proteins also produced by the tumor cells this autocrine/paracrine model for IGFs has to be modified. However, at this time it is not known whether the IGF-binding proteins enhance or inhibit the effect of IGFs on tumor growth.

In contrast to the IGF system, these binding proteins seem to have tissue specificity since they are expressed differently in SCLC and NSCLC. Manipulation of the binding proteins by interfering with their structure or their production make them future candidates for influencing lung cancer growth *in vivo*.

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