

Insulin-Like Growth Factor Family and Combined Antisense Approach in Therapy of Lung Carcinoma

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Abstract

Background: Perturbation in a level of any peptide from insulin-like growth factor (IGF) family (ligands, receptors, and binding proteins) seems to be implicated in lung cancer formation; IGF ligands and IGF-I receptor through their mitogenic and anti-apoptotic action, and the manose 6-phosphate/insulin-like growth factor II receptor (M6-P/IGF-IIR) possibly as a tumor suppressor.

Materials and Methods: To determine the identity, role, and mutual relationship of IGFs in lung cancer growth and maintenance, we examined IGF's gene (by RT-PCR) and protein (by immunohistochemistry) expression in 69 human lung carcinoma tissues. We also examined IGF-I receptor numbers (Scatchard analysis) and IGF-II production and release (by Western blot) in IGF-II/IGF-IR mRNA positive and negative lung carcinomas. Finally, the potential role of IGF-IR and IGF-II as growth promoting factors in lung cancer was studied using antisense oligodeoxynucleotides that specifically inhibit IGF-IR and IGF-II mRNA.

Results: Thirty-two tumors were positive for IGF-I, 39 for IGF-II, 48 for IGF-IR, and 35 for IGFBP-4 mRNA. Seventeen tumors were concomitantly positive for all four IGFs, whereas 34 were positive for IGF-II, IGF-IR, and IGFBP-4

mRNA. An elevated amount of IGF-II peptide was secreted into the growth medium of cell cultures established from five different IGF-II/IGF-IR mRNA positive lung cancer tissues. The cells also expressed elevated numbers of IGF-IR. Nine IGF-II-negative and 19 IGF-II-positive lung cancers of different stages were selected, and M6-P/IGF-II receptor was determined immunohistochemically. Most of the IGF-II-negative tumors were strongly positive for M6-P/IGF-IIR. IGF-II-positive tumors were mostly negative for M6-P/IGF-II receptors. Antisense oligodeoxynucleotides to IGF-II significantly inhibited, by 25–60%, the *in vitro* growth of all six lung cancer cell lines. However, the best results (growth inhibition of up to 80%) were achieved with concomitant antisense treatment (to IGF-IR and IGF-II).

Conclusion: Our data suggest that lung cancer cells produce IGF-IR and IGF-II, which in turn stimulates their proliferation by autocrine mechanism. Cancer cell proliferation can be abrogated or alleviated by blocking the mRNA activity of these genes indicating that an antisense approach may represent an effective and practical cancer gene therapy strategy.

Introduction

Numerous growth factors and receptors have been shown to be involved in the development and maintenance of transformed phenotype of lung cancer cell lines and *in vivo* growing lung tumors, including neuroendocrine small cell lung carcinoma. These growth factors are peptides with either autocrine or paracrine effects. Essential is the role of peptides from insulin-like growth factor (IGF) family of ligands, receptors, and binding proteins, which form highly regulated networks of interactions (1). They are insulin-like growth factors (IGF) -I and -II, whose growth promoting action is mediated mostly by IGF-I receptor

(IGF-IR) and at least six IGF binding proteins (IGFBP) that regulate IGF-I and IGF-II bioavailability. In turn IGFBP activity is regulated by IGFBP proteases among other factors.

IGF-I and IGF-II and the IGF-I receptor are critically important for normal growth and development of the organism. IGF-I receptors mediates IGF-I and IGF-II action on prenatal growth and IGF-I action on postnatal growth. Mice depleted of IGF-I receptors die at birth as a result of poor muscular development. However, IGF ligands are also implicated in numerous pathologic states, including malignancy, where they serve as endocrine, autocrine, and paracrine stimulators of mitogenesis, survival, and cellular transformation (2,3). These actions are mediated through the IGF-I receptor. They also act synergistically with other growth factors and steroids and antagonize the effect of antiproliferative molecules on cancer growth.

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IGFBP have opposing action (to IGF ligands/receptors) mediated either by IGF binding or by direct inhibitory effects on target cells, however, only when not under the strong activity of IGFBP proteases that enhance IGFs bioavailability by cleaving IGFBPs (4).

IGF-II is a commonly expressed growth factor in many tumors and may enhance tumor growth acting via the over-expressed IGF-I receptor. The *IGF-II* gene is transcribed from four different promoters, and promoters P3 and P4 have been implicated in most tumors studied (5). IGF-II protein could be expressed at various molecular weights; the most active form, with regard to binding to IGF-I receptors, is 7.5 kDa (6). Larger forms lack posttranslational modification and are implicated in tumor-associated hypoglycemia. The secretion of IGF-II by tumors and tumor-derived cell lines suggest that it may act as autocrine regulator of cell proliferation. The frequent expression of *IGF-II* gene in human and animal tumors additionally supports the belief that IGF-II plays an important role in tumor development. Its over-expression increases the probability of malignant transformation, and the mammary gland is at particularly high risk of tumor development (7).

The IGF-I receptor has been identified as a potential control point for transformed cells (8). It is also a well-defined target of IGF-II-modulated cell growth, both normal and neoplastic (9). In addition to being stimulated by IGF-II, IGF-I receptors may be overexpressed in some tumors, due to the mutations in tumor suppressor gene products such as p53 and WT-1 or growth factors such as β FGF and PDGF. Overexpression of receptors transforms cells to a phenotype of the anchorage-independent growth factor (10).

Contrary to IGF-IR, the mannose 6-phosphate/insulin-like growth factor II receptor (M6-P/IGF-IIR) mediates the activation of the growth inhibitor (TGF- β 1) and clearance of IGF-II. Binding of IGF-II to IGF-IIR results in degradation of the ligand, making it unable to activate IGF-IR. M6-P/IGF-IIR has also been shown to be mutated in a number of human tumors including squamous cell carcinoma of the lung (11). Both of these findings identify M6-P/IGF-IIR as a tumor suppressor.

The role of IGF-II in transformation depends on tumor type (5). In this respect, in human lung cancer cell lines the *IGF-II* gene is more widely expressed than the *IGF-I* gene (12). There may be also a close association between *IGF-II* and *IGFBP-2* gene expression, such that, when there is production of IGF-II, IGFBP-2 is the principal BP (12,13). Takanami's findings suggest that IGF-I and -II play an important role in tumor progression and that IGF-II is a useful prognostic marker for pulmonary adenocarcinomas (14-16). The idea that the IGF-II/IGF-IR autocrine loop is a common growth mechanism in epithelial cancer is drawing more and more attention (11,17). Therefore, these tumors would be excellent candidates for therapeutic strategies aimed at interference with this pathway. Disruption of the IGF-II pathway

can be accomplished through binding of growth factors, blockage of receptors, or inhibition of secondary targets. Here we examine the gene expression of IGF ligands and receptors in different lung cancers. Combined treatment with antisense oligodeoxynucleotides to IGF-IR and IGF-II significantly inhibited the growth of six lung cancer cell lines.

Materials and Methods

Tissue Specimens

A total of 69 lung carcinomas (38 squamous cell carcinomas, 20 adenocarcinomas, 6 adenosquamous cell carcinomas, and 5 large cell carcinomas; Table 1) obtained from human tumor bank (18) were analyzed. These were obtained from patients treated surgically (46 males, aged 38-77 years, mean 60 years, and 23 females, aged 47-73, mean 60 years). The tissues were snap frozen in liquid nitrogen shortly after surgical removal, brought immediately to the laboratory, and stored at -80°C . A part of each frozen tumor sample was also embedded in paraffin. Sections of each paraffin block were stained with hematoxylin and eosin to confirm the exact tissue analyzed. Frozen sections (4 μm) adjacent to those used for histopathologic analysis were used for RNA analysis and establishment of primary cell cultures. All persons gave their informed consent prior to inclusion in the study. Local ethics committees approved the study.

RNA Extraction and Reverse Transcription PCR

For RNA extraction, tissue samples were homogenized in 1 ml of solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 5g/l sarkosyl and 100 mM 2-mercaptoethanol (19). Reverse transcription (42°C for 1 hr) procedure for all IGF RNAs was performed exactly as it is described in Pavelić et al. (20,21). The cDNA fragments were of the following size: IGF-I 397 bp, IGF-II 541 bp, IGF-IR 720 bp, IGFBP-4 180 bp, β -actin 317 bp (Fig. 1).

Cultivation of Lung Cancer Cells

Tumor tissue, without necroses, was cut into small pieces and pressed through a nylon sieve. Cells (approximately $3-6 \times 10^5$) were put into T-75 flasks with RPMI 1640 medium supplemented with 15% fetal calf serum, 1% calf serum, 1% glutamine, and 20 mM HEPES. Tumor cell cultures, obtained from samples indicated in Table 1 in bold, were maintained as monolayers.

Cell number was determined by the MTT dye reduction assay. The cells (obtained from samples number 3, 32, 38, 48, 56, and 62; Table 1) seeded in 96-well plates (3×10^4 /well), were grown overnight at 37°C and 5% CO_2 . For an additional 72 hr, the cells were incubated in medium containing 0.1% bovine serum albumin, 5 $\mu\text{g}/\text{ml}$ transferrin, 5 ng/ml selenic acid, and antibiotics with or without addition of antisense or sense oligodeoxynucleotides.

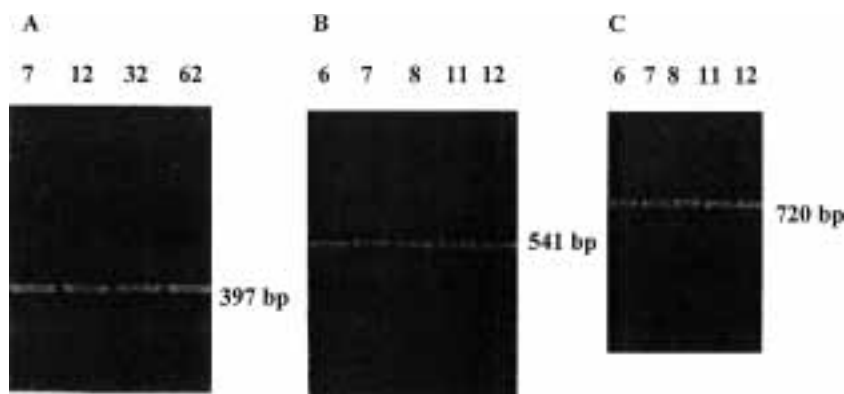


Fig. 1. IGF-I (A), IGF-II (B), and IGF receptor type I (C) mRNA (RT-PCR) in some of the specimens of lung cancer tissue. Numbers above the lines indicate the corresponding tumor sample (patient) listed in Table 1.

Absorbance was measured at 570 nm with ELISA plate reader (Labsystems, Multiscan MS). Data are expressed as the percentage of increase or decrease in cell growth compared to untreated control cells.

Immunohistochemistry

Immunohistochemical tests were performed on formalin-fixed, paraffin-embedded tissue using avidin-biotin-peroxidase method. Sections were cut

Table 1. Pathohistologic classification, TNM stage, and IGF's RNA positivity in lung cancer tissues

No	Patient Age	Patient Sex	Tumor Subtype	TNM Stage	IGF-I	IGF-II	IGF-IR	IGFBP-4
1	49	M	AC	I	+	-	-	-
2	56	M	AC	I	+	-	-	-
3	59	M	AC	I	-	+	+	+
4	57	M	AC	II	-	+	+	+
5	63	F	AC	II	-	-	-	-
6	51	F	AC	II	-	+	+	+
7	50	M	AC	II	+	+	+	+
8	61	M	AC	II	-	+	+	+
9	71	F	AC	II	-	-	-	-
10	65	M	AC	II	-	-	-	-
11	54	F	AC	II	+	+	+	+
12	58	M	AC	II	+	+	+	+
13	74	M	AC	II	-	+	+	+
14	65	M	AC	IIIa	+	+	+	+
15	70	F	AC	IIIa	-	+	+	+
16	62	M	AC	IIIa	-	+	+	+
17	52	M	AC	IIIa	+	-	+	-
18	52	M	AC	IIIa	-	-	+	-
19	68	F	AC	IIIa	+	-	+	-
20	66	F	AC	IIIa	+	-	+	-
21	77	M	AS	I	+	-	+	-
22	63	M	AS	I	-	-	-	-
23	65	M	AS	I	-	-	-	-
24	59	M	AS	I	+	+	+	+
25	67	M	AS	II	+	+	+	+
26	66	M	AS	II	+	-	-	-

(Continued)

Table 1. (Continued)

No	Patient Age	Patient Sex	Tumor Subtype	TNM Stage	IGF-I	IGF-II	IGF-IR	IGFBP-4
27	61	F	LCA	I	+	-	+	-
28	57	F	LCA	I	-	-	-	-
29	68	M	LCA	IIIa	-	+	+	-
30	60	M	LCA	IIIa	-	+	+	-
31	65	M	LCA	IIIa	+	-	-	-
32	65	M	SCC	I	+	+	+	+
33	41	M	SCC	I	+	-	-	-
34	57	F	SCC	I	+	+	+	+
35	73	F	SCC	I	+	+	+	+
36	57	F	SCC	I	+	-	-	-
37	73	M	SCC	I	-	-	+	+
38	38	M	SCC	I	-	+	+	+
39	44	M	SCC	I	-	+	+	+
40	57	M	SCC	I	+	-	-	-
41	50	M	SCC	I	-	-	+	-
42	60	F	SCC	I	-	+	+	+
43	51	M	SCC	I	-	-	-	-
44	53	F	SCC	I	+	+	+	+
45	51	M	SCC	II	+	+	+	+
46	47	F	SCC	II	+	-	-	-
47	59	F	SCC	II	+	+	+	+
48	77	M	SCC	II	-	+	+	-
49	47	M	SCC	II	-	-	-	-
50	59	M	SCC	II	-	-	-	-
51	63	M	SCC	II	+	-	-	-
52	62	M	SCC	II	-	+	+	+
53	60	F	SCC	II	-	+	+	+
54	58	F	SCC	II	+	+	+	-
55	73	F	SCC	II	+	+	+	+
56	49	M	SCC	II	-	+	+	-
57	65	M	SCC	II	-	-	-	-
58	64	M	SCC	II	+	+	+	+
59	48	M	SCC	IIIa	-	+	+	+
60	55	F	SCC	IIIa	-	+	+	+
61	64	F	SCC	IIIa	-	+	+	+
62	77	M	SCC	IIIa	+	+	+	+
63	49	M	SCC	IIIa	-	+	+	+
64	59	M	SCC	IIIa	+	+	+	+
65	70	F	SCC	IIIa	+	+	+	+
66	71	M	SCC	IIIa	-	+	+	+
67	76	M	SCC	IIIa	-	-	+	-
68	53	M	SCC	IIIa	-	-	-	-
69	68	F	SCC	IIIa	-	-	-	-

Abbreviations: AC, adenocarcinoma; AS, adenosquamous cell carcinoma; LSC, large cell carcinoma; SCC, squamous cell carcinoma; M, male; F, female; (**bold**) = tumor cell cultures maintained in monolayers.

Table 2. Number of IGF mRNA positive lung cancer tissues

		+IGF-IR	+IGFBP-4	+IGF-IR +IGFBP-4	+IGF-IR +IGFBP-4 +IGF-II
IGF-I	32 46.4%	23 33.3%	17 24.6%	17 24.6%	17 24.6%
IGF-II	39 56.5%	39 56.5%	34 49.2%	34 49.2%	—
IGF-IR	48 69.6%	—	35 50.7%	—	—
IGFBP-4	35 50.7%	35 50.7%	—	—	—
None IGF	12 17.4%	—	—	—	—
Total	69 100%	—	—	—	—

at 4 μm , deparaffinized, and rehydrated. After blocking endogenous peroxidase activity by 3% hydrogen peroxide, the slides were subjected to a heat-induced epitope retrieval technique in 10 mM citrate buffer (pH 6.0) in an 850 W microwave for 10 min. Anti-IGF-II (Upstate Biotechnologies, Lake Placid, NY, USA) is an mouse monoclonal antibody, diluted 1:50, and incubated for 15–18 hr. Anti-IGF-IR (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) is a rabbit polyclonal antibody, diluted 1:100, and incubated also for 15–18 hr. Anti M6-P/IGF-IIR rabbit polyclonal antibody was generous gift from Zeneco (URL: <http://www.geneimprint.com>). Detection was achieved using the DAKO (Carpinteria, CA, USA) LSAB 2 kit, according to manufacturer’s instructions. Negative controls were stained by substitution of the primary antibodies with nonimmune mouse or rabbit immunoglobulins at same concentrations as the corresponding antibodies. Appropriate positive controls (normal thyroid gland for IGF-IR, Wilms’ tumor for IGF-II and normal colon for M6-P/IGF-IIR) were stained positively. The tumor cells showed, in both cases, strong diffuse

cytoplasmic immunopositivity for IGF-II and M6-P/IGF-IIR, and cytoplasmic and focal membranous reactivity for IGF-IR (Table 2).

IGF-II Studies in Cell Growth Medium

Tumor cell cultures, established from samples number 7, 62 (IGF-II/IGF-IR mRNA positive), and 33 (IGF-II/IGF-IR mRNA negative, control cells) (Table 1) were grown in serum-containing medium in 1 \times 24 well culture plates. Immunoenzymatic detection of IGF-II was done in conditioned medium collected on days 5 and 25 of in vitro cell growth by using a mouse monoclonal antibody directed against native rat IGF-II and a secondary goat anti-mouse IgG serum conjugated to horseradish peroxidase. Semiquantitative measurements of immunoreactive IGF-II were performed by chemiluminescence on serial dilutions of conditioned media spotted on BA85 nitrocellulose filters. Signals were analyzed using Pharmacia (Newark, NJ, USA) LKB system. Values for IGF-II were interpolated from standard curves using serial dilutions of human IGF-II. IGF-II concentrations are expressed as $\text{ng}/2 \times 10^6$ cells (Table 3).

Table 3. The level of IGF-II peptide in cell growth medium and number of IGF-I receptors on cultured lung cancer cells

Cell Type	IGF-II ($\text{ng}/2 \times 10^6$ Cells)*		IGF-IR B_{max} ($\text{fmo}1/2 \times 10^6$ Cells)*	
	Day 5	Day 25	Day 5	Day 25
AC, case # 7	323.3 \pm 37.9	34.7 \pm 11.6	210.7 \pm 18.8	24.3 \pm 17.2
SCC, case # 62	231.3 \pm 33.6	214.7 \pm 19.6	180.0 \pm 26.9	66.0 \pm 16.5
SCC, case # 33 [†]	7.0 \pm 2.0	4.0 \pm 3.6	60.0 \pm 18.7	13.7 \pm 2.0

*Values are the mean \pm SD of three independent experiments (in all cases statistically different from control values obtained with cells #33).

[†]IGF-II/IGF-IR mRNA negative cells.

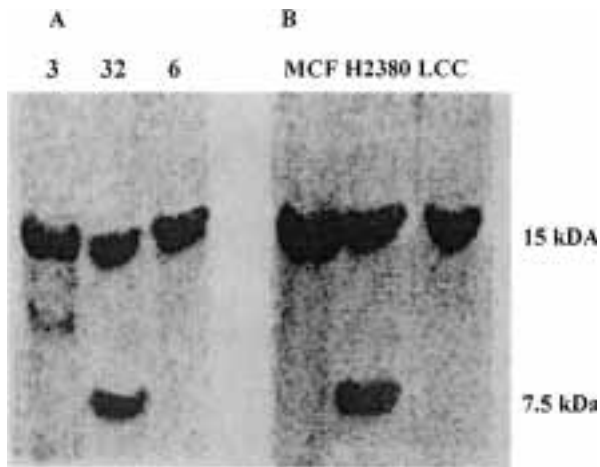


Fig. 2. Western blot analysis of IGF-II secreted into the growth medium (day 10) of (A) cell cultures established from three lung cancer tissues and (B) in three established cell lines (two breast adenocarcinomas, MCF-7 and H2380, and primary lung carcinoid cell line, LCC). Numbers above the lines in A indicate the corresponding tumor sample (patient) listed in Table 1.

Western Blot

IGF-II was analyzed by Western immunoblot using specific anti-IGF-II monoclonal antibody (Amano Pharmaceutical, Japan) (Fig. 2). The cell growth media collected on day 10 of cell growth (cultures established from samples 3, 32, and 56; Table 1) was electrophoresed on 16% SDS polyacrylamide gel under nonreducing conditions. The fractionated proteins were electroblotted on the nitro-cellulose sheet, blocked with 5% w/v skim milk, and then immunoblotted with anti-IGF-II monoclonal antibody for 16 hr. After washing, the sheet was incubated with anti-mouse IgG. IGF-II anti-IGF-II antibody complexes were detected with enhanced chemiluminescence system (22).

IGF-I Receptor Assay

IGF-I receptor detection was done on the cell cultures obtained from samples 7, 62, and 33 (Table 1). Cell receptor number was estimated by whole cell binding assay/Scatchard analysis (23). The cells were incubated, for 18 hr, with 125 I-labeled IGF-I and with or without increasing concentrations of unlabeled IGF-I. Finally, the cells were lysed in PBS containing Triton X-100 and 10% glycerol, and the radioactivity was counted in gamma counter (Pharmacia). Nonspecific binding represented about 5% of total 125 I-IGF-I binding.

Antisense and Sense Oligodeoxynucleotides

All oligodeoxynucleotides were synthesized as mixed phosphorothiodates. The IGF-IR: antisense 5'TCCTCCGAGCCAGACTT 3' (complementary to codons 21–29 of the signal sequence of the human

IGF-IR) and sense 5'AAGTCTGGCTCGGAGGA 3'; IFG-II: antisense 5'TTCCCCATTGGGATTCCCAT 3' (corresponding to the IGF-II mRNA initiation site) and sense 5'ATGGGAATCCCAATGGGGAA 3'.

Statistical Analysis

Statistically significant differences were determined by using Student *t*-test. The level of significance was set at $p < 0.01$.

Discussion

In our study, 82.6% of lung cancers expressed mRNA for one of the IGF members. The majority of tumors expressed IGF-II, IGF-IR, and IGFBP-4, indicating their important role in lung carcinogenesis. The growth of lung cancers that produce IGF-II was regulated by autocrine IGF-II/IGF-IR-mediated stimulation, as suggested by the observation that abrogation of receptor and/or ligand function by antisense oligodeoxynucleotides inhibited lung cancer cell proliferation in vitro. IGF-II-/IGF-IR-positive tumors, mostly those of advanced stage, fail to express M6-P/IGF-II receptors. Therefore, our results clearly demonstrate the importance of IGF-II, IGF-IR, and possibly M6-P/IGF-IIR in the pathogenesis of lung cancer (Table 4, Fig. 3).

Perturbation in a level of each IGF has been implicated in cancer formation in various cell types. Several studies have demonstrated an overexpression of IGF receptors and other members of the family by tumor cells, for example, of thyroid, breast, and colon cancer, hepatoma, and endometrial carcinoma (24–26). There is little doubt that many neoplastic cells express IGFs and show growth enhancement in response to IGFs.

There are also findings showing that IGF-IR is an absolute requirement for the establishment and maintenance of transformed phenotype in some tumors (27). In concordance with this are also our recent data showing undoubtedly that IGF-IR is important for development and progression of human hemangiopericytomas (20,21).

On the other hand, receptor function can be abrogated by utilizing monoclonal antibodies against receptors such as α IR3 (28), the polyanionic compound suramine (29), phosphorylation-defective analogs of the IGF-I ligand (28), or antisense oligodeoxynucleotides directed against the IGF-IR mRNA. A series of experiments based on antisense strategies against the IGF-IR (30–32) have unequivocally shown that a decrease in the number of IGF-IR causes a reversal of the transformed phenotype.

The role of IGF-IR and IGF-II in the proliferation of lung cancer cells remains poorly understood. However, IGF-II autocrine stimulation through IGF-IR found in some human tumors of epithelial origin (33,34) implies the possibility of the same mechanism of action in lung cancer. The results

Table 4. M6-P/IGF-IIR protein immunohistochemical test in IGF-II negative and IGF-II positive tumors

Patient	Tumor Subtype	TNM Stage	IGF-IR*	IGF-IR mRNA*	M6-P/IGF-IIR**
IGF-II negative tumors					
1	AC	I	0	-	+++
2	AC	I	0	-	++
5	AC	II	0	-	++
9	AC	II	0	-	+++
10	AC	II	0	-	+
31	LCA	IIIa	0	-	+++
67	SCC	IIIa	++	+	++
68	SCC	IIIa	0	-	+++
69	SCC	IIIa	0	-	+++
IGF-II positive tumors					
3	AC	I	++	+	0
4	AC	II	++	+	0
6	AC	II	+++	+	++
7	AC	II	++	+	++
8	AC	II	+++	+	+
11	AC	II	++	+	++
12	AC	II	+	+	+++
13	AC	II	+++	+	+
14	AC	IIIa	+++	+	+
15	AC	IIIa	++	+	0
16	AC	IIIa	+++	+	0
30	LCA	IIIa	+++	+	+
39	SCC	IIIa	+++	+	0
60	SCC	IIIa	+++	+	0
61	SCC	IIIa	++	+	++
62	SCC	IIIa	+++	+	+
63	SCC	IIIa	++	+	+
64	SCC	IIIa	+++	+	0
65	SCC	IIIa	+++	+	+

*mRNA positive (+) and negative (-) samples

**0 negative tumors; +, ++, +++ are weakly positive ++, moderately positive, +++ strongly positive tumors.

presented in this paper prove all prerequisites that IGF's autocrine loop operates in lung cancer. These are concomitant expression of IGF-I, IGF-II, and IGF-IR mRNA and corresponding proteins, as well as tumor cell growth inhibition by IGF-II/IGF-IR antisense therapy. Recently, we showed a similar pattern of IGF's action in hemangiopericytomas. Almost all tumors expressed IGF-IR and/or IGF-II mRNA, and inhibition of ligand and receptor activity abrogated tumor proliferation in vitro (20,21). These and the results of other authors suggest that IGF-II may function as important paracrine or autocrine growth factor for some tumors. In the lung carcinomas tested in this study, the autocrine IGF's action seems to be more plausible. This is corroborated especially by the fact that in vitro growing tumor cells, free of any non-lung cancer cells paracrine influence, express IGF-IR and secrete IGF-II. We strongly believe that some members of IGF family play a role in tumor self-incitement (35,36).

Contrary to the oncogenic activity of IGF-I, IGF-II, and IGF-IR, mannose 6-phosphate/IGF-II receptor functions in humans as a tumor suppressor; a significant part of this activity comes from the control of level of IGF-II. However, when mutated, most commonly by LOH in one allele and point mutation in another (11,37), it loses its antioncogenic activity and neoplastic transformation, caused by IGF-II overaccumulation, may occur. Our results prove this notion: IGF-II-/IGF-IR-negative tumors express strongly M6-P/IGF-IIR protein irrespective of tumor subtype and stage. On a contrary note, IGF-II-/IGF-IR-positive tumors were mostly negative or slightly positive for type 2 receptors. They fail, almost completely, to express type 2 receptors as they progress to more aggressive TNM stage (stage III). Loss of receptor function and associated accumulation of IGF-II, which binds to and activates IGF-IR, increases intracellular growth signals and promotes cell proliferation. This indicates that

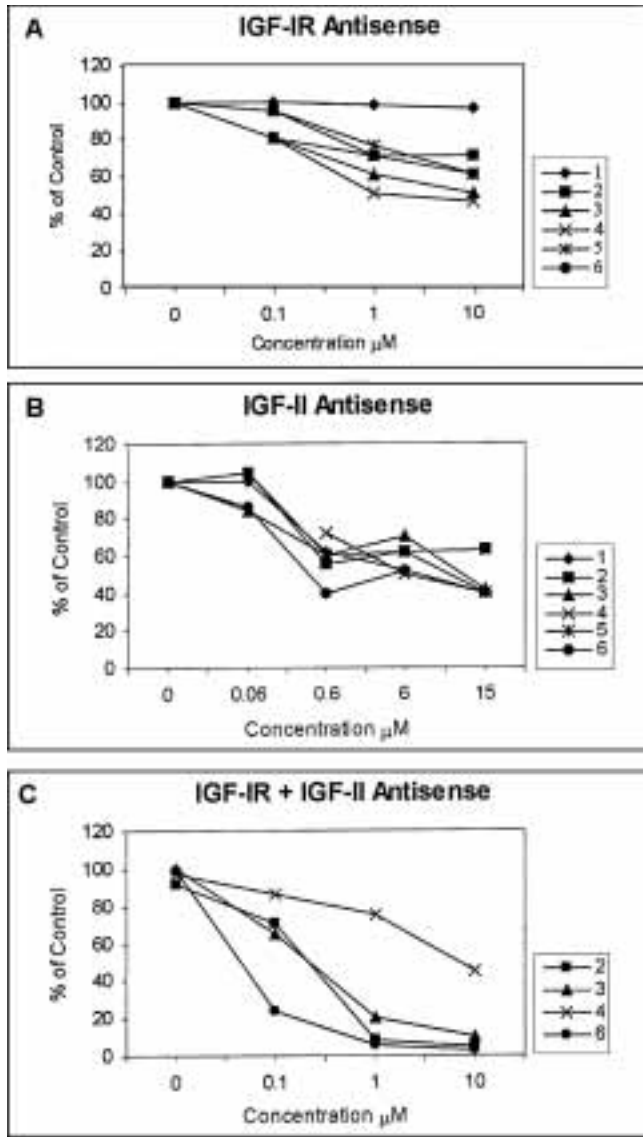


Fig. 3. Inhibition of lung cancer cell proliferation by an antisense oligodeoxynucleotides to IGF-IR mRNA (A) and IGF-II mRNA (B). Inhibition of lung cancer cell lines (line 2, sample 32; line 3, sample 38; line 4, sample 48; line 6, sample 62 from Table 1) by simultaneous treatment with antisense oligodeoxynucleotides to IGF-IR and IGF-II mRNAs (C). Each point represents the mean value of four replicate wells. The experiments were repeated three times.

IGF-IIR is also a part of IGF's autocrine loop, where operating as IGF-II antagonist, has tumor suppressor properties.

Considering the role of IGFs in neoplastic control, modulation of IGF physiology seems to be a reasonable choice in treatment of IGF-dependent cancers. There have been some successful attempts at IGF-targeted therapy of lung cancer or its brain metastasis by using either antisense approach, monoclonal antibodies, or suramin (38–41). Our in vitro results show that antisense therapy to IGF-II and

IGF-IR mRNA abrogates cell proliferation by preventing synthesis of specific proteins and disrupting intracellular signaling. However, despite the encouraging results from in vitro and in vivo studies using multiple cell lines, the limited clinical studies performed have been disappointing.

IGFBPs are small proteins that modulate IGF's biological effects by altering ligand–receptor interactions. Therefore, the concomitant expression of IGFBP-4 and IGF-I and IGF-II in 17 and 34 tumor samples, respectively, do not fit, at the moment, in the proposed autocrine IGF loop of action. However, the finding of gene expression at the mRNA level does not mean necessarily that the functional protein is synthesized; even if it is, there is the possibility that it is degraded rapidly by specific proteases. And finally, elevated expression of not only IGFBP-4, but also other binding proteins, together with elevated expression of IGF-I and IGF-II, was also observed by other authors, in both lung and other tumor models (42,43). In a healthy organism, IGFBP-4 is produced locally by several cell types. It can also bind to the cell membrane, but the regulatory mechanisms of both actions are still unknown. Therefore, the question on the role of IGFBP-4 in carcinogenesis has to be elucidated further.

In conclusion, our results support the hypothesis that production of IGF-I, IGF-II, IGF-IR, and M6-P/IGF-IIR, different from that in normal tissue, influence lung cancer growth by autocrine mechanism. Targeting of IGF-I, IGF-II, and/or IGF-IR by “antisense” approach may represent an effective and practical cancer gene therapy strategy.

Acknowledgments

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