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Molecular Cloning of IGFBP-5 from SCLC Cell Lines and Expression of IGFBP-4, IGFBP-5 and IGFBP-6 in Lung Cancer Cell Lines and Primary Tumours

Barbara R. Wegmann, Hans J. Schöneberger, Paul E. Kiefer, Gabriele Jaques, Detlef Brandscheid and Klaus Havemann

We showed recently that insulin-like growth factor binding protein (IGFBP)-1, -2 and -3 are differentially expressed in lung cancer and permanent lung cancer cell lines. Elevated levels of IGF binding capacity in serum of lung cancer patients were also reported. The function and tissue specificity of IGFBP are still obscure but they are probably local regulators of IGF action. Here we show the expression of IGFBP-4 transcripts in 11/11 small cell lung cancer (SCLC) cell lines, in nine out of 11 non-small cell lung cancer (NSCLC) cell lines, in 11/11 lung tumour specimens (10 derived from patients with NSCLC and one from SCLC origin) and in normal lung. In addition we isolated IGFBP-5 cDNA from λgt10 libraries of SCLC cell lines. With this IGFBP-5 cDNA we detected transcripts of different lengths in seven out of 11 SCLC cell lines, in 11/11 lung cancer specimens but only in one out of 11 NSCLC cell lines and in normal lung. IGFBP-6 was not detected in northern analysis of any tested SCLC cell line but it was expressed in nine out of 11 NSCLC cell lines and in nine out of 11 human lung cancer specimens and in normal lung.
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INTRODUCTION

LUNG CANCER is the most common type of cancer in humans worldwide. With respect to clinical, histological and biochemical features, lung cancer is divided into two major groups: the small cell lung cancer (SCLC) and the heterogenous group of non-small cell lung cancer (NSCLC). Studying the growth properties of SCLC and NSCLC cell lines *in vitro* we and others were able to show that the polypeptide growth factors IGF-1 and IGF-2 (insulin-like growth factors 1 and 2) can modulate the growth of SCLC and NSCLC cell lines in an autocrine/paracrine way [1–3]. Although most of the circulating IGF are secreted by the liver, many other tissues produce these peptide growth factors. We have shown previously that lung cancer cell lines express IGF-2-specific mRNA but transcripts coding for IGF-1 could not be detected [4]. While IGF-1 postnatally mediates many of the growth promoting effects of growth hormone (GH) through binding to its cell surface receptor (IGF-1 receptor type 1) the physiological role of IGF-2 mainly expressed during embryonic development still remains obscure. Both IGF-1 and IGF-2 probably mediate their growth stimulating activities through IGF-receptor type 1 [5]. It is believed that insulin-like growth factors play an important role in the growth control of human tumours [4–7].

Usually, IGF, unlike many other peptide hormones, circulate in serum bound to specific IGF binding proteins (IGFBP) [8–10]. Six different IGF binding proteins termed IGFBP-1 to IGFBP-6 have been cloned so far [8, 9, 11–17]. The physiological role of these IGFBP is not yet clear but IGF potentiating as well as inhibiting activities have been reported [10, 13, 18–21]. We have shown previously that SCLC and NSCLC cell lines secrete IGFBP of different molecular weights into their growth media [22–24]. Northern analysis revealed a differential expression pattern between SCLC and NSCLC cell lines. All tested SCLC cell lines express mRNA for IGFBP-2 but none of them express mRNA for IGFBP-1 or IGFBP-3 [24]. In contrast to this, we frequently detected IGFBP-1 and IGFBP-3 in NSCLC cell lines whereas IGFBP-2 could be detected in only two out of 11 NSCLC cell lines [23].

With different oligonucleotides from IGFBP-2 we found several transcripts differing in length from normal IGFBP-2 mRNA. The protein patterns showing IGF binding activity in conditioned media revealed a panel of bands that let us suggest that they could not be attributed to the expression of only IGFBP-1 to IGFBP-3. We, therefore, discussed the probability that lung cancer cell lines express IGF binding proteins other than IGFBP-1 to IGFBP-3 [22–24]. To elucidate these additional mRNA transcripts we screened two cDNA libraries from SCLC cell lines, one constructed in our laboratory from SCLC cell line NCI-N417 and one from NCI-H69 (Clontech). Furthermore, we supplemented our investigations concerning the IGFBP distribution in SCLC and NSCLC cell lines, in lung tumour tissue and normal lung by northern analysis with probes specific for IGFBP-4, IGFBP-5 and IGFBP-6.

MATERIALS AND METHODS

Cell lines

The cell lines used were established in our laboratory (SCLC-16H, EPLC-32M1, EPLC-65H, EPLC-272H, ADLC-5M2,

Correspondence to B.R. Wegmann.
B.R. Wegmann, H.J. Schöneberger, P.E. Kiefer, G. Jaques and K. Havemann are at the Division Hematology/Oncology, Department of Internal Medicine, Philipps University Marburg, Baldingerstraße, D-3550 Marburg; and D. Brandscheid is at the Rohrbach Clinics, D-6900 Heidelberg, F.R.G.
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LCLC-97TM1, LCLC-103H), donated by Drs Carney, Gazdar and Minna, NCI, Bethesda, Maryland (NCI-H69, NCI-H82, NCI-H526, NCI-N417, NCI-H146, NCI-H510, NCI-H209, NCI-H322, NCI-H596, NCI-H23, NCI-H125, A549), Drs Pettengill and Sorensen (DMS79), Dr Bergh, Department of Pathology and Oncology, University of Uppsala, Sweden (U1752, U1810) and Dr Reeve, MRC centre, Cambridge, U.K. (FRE). Cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum (R10). For reference of cell lines see [1, 22–25].

Tumour tissue and normal lung tissue

Tumour samples were obtained from 7 patients with squamous carcinoma (TU-24, TU-26, TU-25, TU-27, TU-3, TU-4, TU-13), one specimen was derived from adenocarcinoma (TU-28), one specimen showed adeno/squamous differentiation (TU-12), one specimen was obtained from a squamous carcinoma with areas of SCLC (TU-14) and one tissue sample is derived from small cell lung cancer (TU-33). N-24 is histopathologically normal, adult lung tissue obtained from the same patient as TU-24 (tissue adjacent to TU-24). All samples were snap frozen in liquid nitrogen and stored at −70°C until RNA was prepared.

Preparation of RNA

Cells growing in R10 were collected in logarithmic growth phase, washed twice in phosphate buffered saline (PBS) and dissolved in guanidinium thiocyanate solution (6 mol/l guanidinium thiocyanate/25 mmol/l sodium citrate/0.5% sodium lauryl sarcosine). Frozen lung tissue was pestled under liquid air, dissolved in guanidinium thiocyanate solution and mechanically homogenised. The homogenates were layered on CsTFA (caesium trifluoroacetate) cushions and RNA was pelleted in a Beckman centrifuge (24 h, 15°C, 125000 *g*).

Construction of a cDNA library of NCI-N417

Poly A RNA was obtained by extraction of total RNA through oligo (dT) columns. One microgram of poly A RNA was reverse transcribed using M-MuLV-RT (Gibco-BRL). Complementary DNA was methylated with EcoR1-Methylase, ligated to EcoR1-linkers, digested with EcoR1 and ligated to EcoR1-digested λ gt 10 arms (Amersham). DNA was packed with λ phage packaging extracts (Promega) and phages were propagated in *E. coli* L87. The cDNA library consisting of 2×10^6 phages was amplified.

Screening of cDNA libraries and characterisation of positive phages

The cDNA libraries of NCI-H69 (Clontech) and NCI-N417 were plated at a density of 2×10^4 plaque-forming units/15-cm plate. Plaques were lifted to nitrocellulose membranes and hybridised to radiolabelled IGFBP-2 cDNA [8] under low stringent conditions. Phages from positive plaques were rescreened to homogeneity. cDNA were subcloned into pBluescript II ks(+). For characterisation the inserts were mapped with the restriction enzymes Rsa I, Sau3A and Sst I. Selected clones were sequenced according to the method of Sanger using Sequenase (USB).

Northern blot analysis

After heat denaturation in the presence of glyoxal and DMSO, 20 μg of RNA was size fractionated on 1.4% agarose gel and electroblotted onto Hybond N membranes (Amersham). cDNA probes were radiolabelled using the Multiprime labelling Kit (Amersham) and [α-^{32}P]-dCTP to specific activities $\geqslant 10^8$ cpm/μg DNA. For hybridisation with radiolabelled oligonucleotides 10 pmoles were kinased with [γ-^{32}P]-ATP ($\geqslant 10^7$ cpm/pmol). Hybridisation was performed in 6 × SSC, 5 × Denhardt, 0.5% SDS and 100 μg/ml salmon sperm DNA. Filters were incubated with radiolabelled cDNA at 60°C or with oligonucleotide at 55°C for 12 h and stringent washing was performed in 0.1 × SSC, 0.1% SDS at 65°C or 1 × SSC at 65°C, respectively. The following probes were used for hybridisation: a 505 bp EcoR1/Hind111 fragment from the coding region of IGFBP-4 cDNA (pHBP4-503) [14]; the nearly full length cDNA coding for IGFBP-5 (H5) cloned in our laboratory; a synthetic 30 mer antisense oligonucleotide, O-IGFBP-61, complementary to the 5′ region of IGFBP-6 with the sequence 5′TGG GGT TTA CTC TCC TTA GGA TTC TCC TCT 3′.

RESULTS

Screening of two amplified cDNA libraries of the human small cell lung cancer cell lines NCI-H69 (Clontech) and NCI-N417 established in our laboratory with a full length cDNA of IGFBP-2 [8] under non-stringent conditions revealed 28 positive plaques. After subcloning into pBluescript ks(+), restriction enzyme mapping with Rsa I, Sau3A and Sst I and partial sequencing, the inserts of 17 phages could be characterised as IGFBP-2-specific cDNA. Three clones of weaker hybridisation signals and divergent restriction enzyme maps were sequenced according to the method of Sanger. We obtained cDNA clones enclosing nearly the whole coding region of IGFBP-5. Figure 1 shows a sequence comparison between our three cDNA of IGFBP-5 (H5, H6, H8/2), the cDNA derived from a human osteosarcoma cDNA library [16], the cDNA published by Shimasaki and coauthors [15] isolated from a human placenta library and the rat IGFBP-5 cDNA derived from ovary [15]. Our longest IGFBP-5-specific cDNA exceeds in its 5′region the already published sequences [15, 16] in 23 nucleotides and ends at bp 814—3 bp in front of the proposed stop signal (the first base from the start codon is numbered 1). Apart from this, our sequence data are identical to the published ones.

Keeping in mind the growth stimulating activities of IGF on lung cancer cell lines our main interest is to identify which IGFBP are expressed in lung cancer cell lines and tumour tissues with a view to understanding what effects these components exert in mediating the action of IGF and thus being part of the autocrine/paracrine regulation mechanism in lung cancer.

As a supplement to our recent investigations concerning the expression of IGFBP-1, -2 and -3 [23, 24], we now present the expression patterns of IGFBP-4, -5 and -6 in small cell lung cancer cell lines (Fig. 2), in non-small cell lung cancer cell lines (Fig. 3) and in different specimens of lung cancer as well as in normal lung (Fig. 4).

The mRNA transcripts detected in lung cancer cell lines after hybridization of northern blots with a [^{32}P]-labelled cDNA for IGFBP-4 (IGFBP-4-503) were 2.6 kb in length. IGFBP-4 is expressed in nine of 11 NSCLC cell lines (Fig. 3), in all tested SCLC cell lines (Fig. 2), in all NSCLC tumour tissue samples and in normal lung tissue (Fig. 4). The steady state level of IGFBP-4 is very high in the SCLC cell lines NCI-H69, NCI-H510 and in NCI-N417 but rarely detectable in SCLC-16Hc and NCI-H146. In NSCLC cell lines we found the highest expression of IGFBP-4 in A549, EPLC-32M1 and in U1752; no hybridisation signal could be detected in LCLC-103 and in NCI-H322. We found higher amounts of IGFBP-4 mRNA in normal lung than in tumour tissues derived from non-small cell lung cancer patients and the only SCLC specimen (Tu33) as well

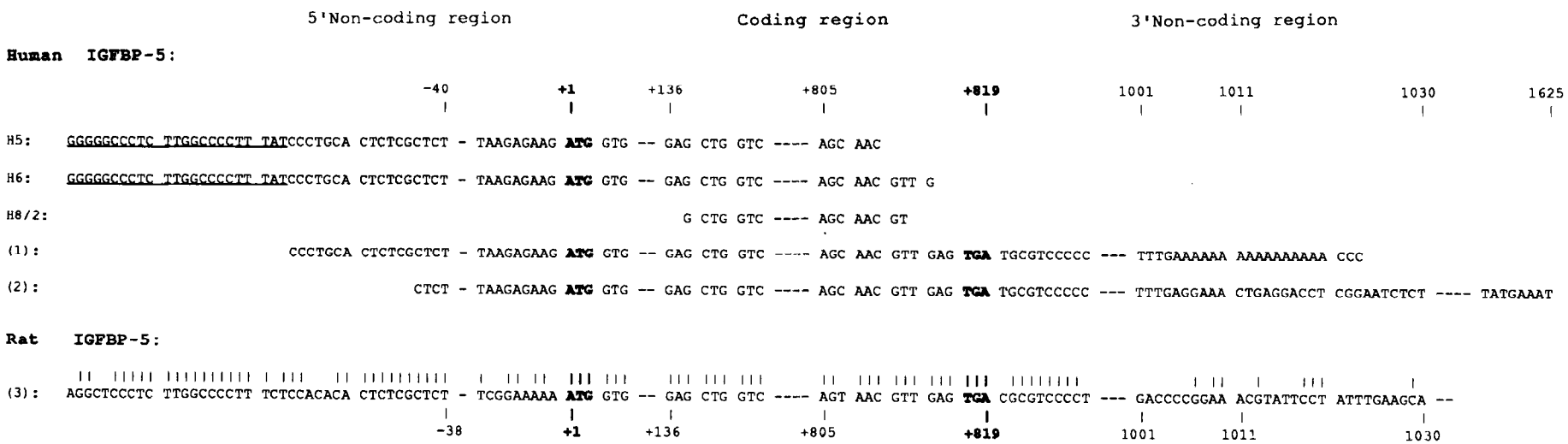

Fig. 1. Sequence comparison between different IGFBP-5 specific cDNA: H5, H6, H8/2 were cloned from cDNA libraries of SCLC cell lines in our laboratory, (1) was cloned from human placenta library [15], (2) from human osteosarcoma library [16] and (3) from rat ovary [15]. Start and stop codons are printed in bold. The first nucleotide of the coding region is numbered + 1. The proximal 23 bp of H5 and H6 exceed the published sequences and show high homology to the corresponding region in rat IGFBP-5.

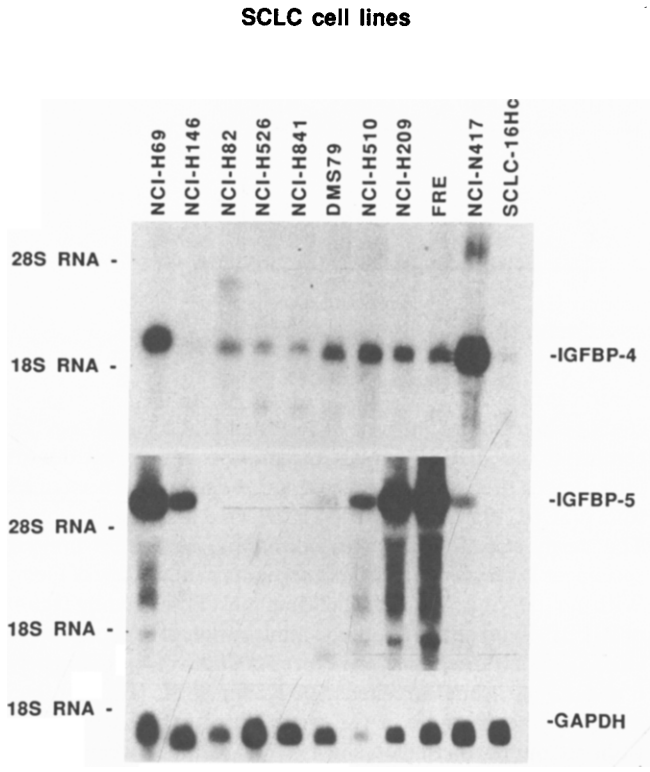

Fig. 2. Northern analysis showing the expression of IGFBP-4 (2.6 kb), IGFBP-5 (6.0 kb, 1.7 kb and several transcripts between 2.1 and 4.8 kb) and GAPDH (as a control) in 11 SCLC cell lines.

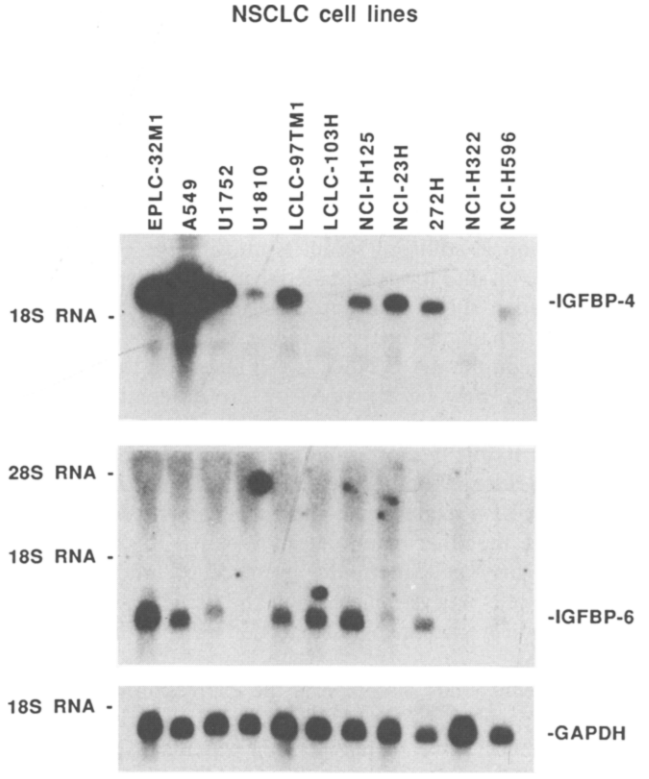

Fig. 3. Northern analysis showing the expression of IGFBP-4 (2.6 kb), IGFBP-6 (1.3 kb) and GAPDH (as a control) in 11 NSCLC cell lines.

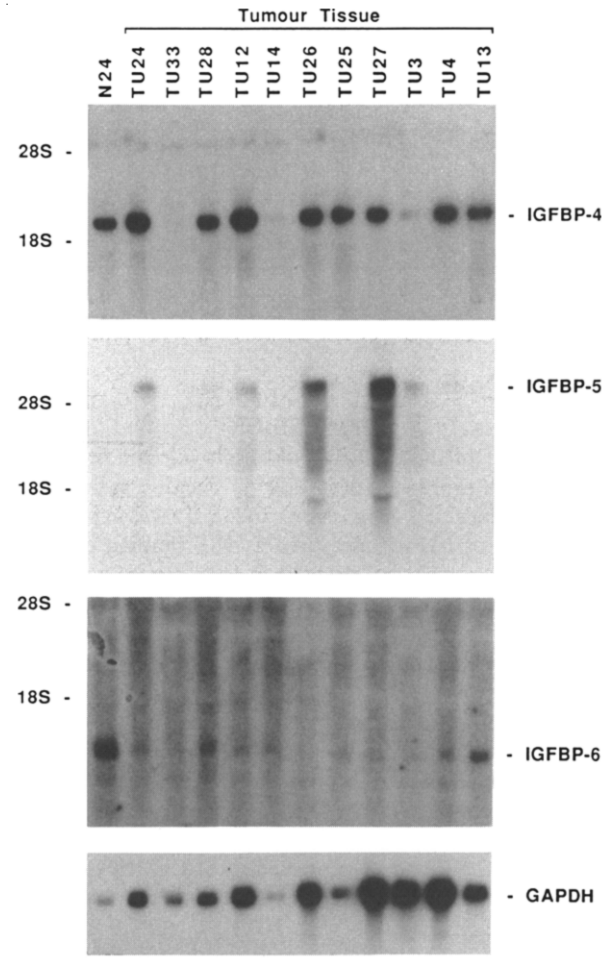

Fig. 4. Northern analysis showing the expression of IGFBP-4 (2.6 kb), IGFBP-5 (6.0 kb, 1.7 kb and several transcripts between 2.1 and 4.8 kb), IGFBP-6 (1.3 kb) and GAPDH (as a control) in primary lung tumour tissues [squamous CA (TU-24, TU-26, TU-25, TU-27, TU-3, TU-4, TU-13), adeno CA (TU-28), adeno/squamous CA (TU-12), squamous CA with SCLC areas (TU-14), SCLC (TU-33)] and in normal lung (N24).

as the heterogenously differentiated tumour enclosing SCLC islands (Tu14) showed the lowest expression of IGFBP-4.

IGFBP-5 is expressed in seven out of 11 SCLC cell lines (Fig. 2) but we could detect it under the same conditions in only one of 11 tested NSCLC cell lines, in NCI-H322 (data not shown). IGFBP-5 is expressed weakly in normal lung tissue and is detectable in different amounts in all of the tumour tissues (Fig. 4). In our northern analysis we found IGFBP-5 transcripts of approximately 6 kb and 1.7 kb as described by Kiefer *et al.* [16] but in addition we found several transcripts ranging in length from 2.1 to 4.8 kb (Figs 2–4).

IGFBP-6-specific transcripts were not detected in any of the tested SCLC cell lines but are frequently expressed in NSCLC cell lines (nine out of 11) (Fig. 3). We could demonstrate mRNA expression of IGFBP-6 in normal lung tissue and to a lesser extent in all of the tested tumour samples (Fig. 4). The length of IGFBP-6 mRNA transcripts detected in human lung and NSCLC cell lines is 1.3 kb. A brief summary of the expression pattern of all six IGFBP in SCLC and NSCLC cell lines, human lung and in primary lung cancer tissue is given in Table 1.

Table 1. Expression of IGFBP-1, -2, -3, -4, -5 and -6 in adult human lung, in SCLC and NSCLC cell lines and in tumour tissue from patients with lung cancer

	IGFBP-1*	IGFBP-2*	IGFBP-3*	IGFBP-4	IGFBP-5	IGFBP-6
Adult human lung	−	+	+	+	+	+
SCLC cell lines	0/10	12/12	0/10	11/11	7/11	0/11
NSCLC cell lines	6/11	2/11	9/11	9/11	1/11	9/11
Lung tumour specimens	0/9	9/9	7/9	11/11	11/11	9/11

* The data of IGFBP-1 to -3 were in part published recently [23, 24]. −, no expression detected; +, specific mRNAs detected; number of positive samples/total number of samples investigated.

DISCUSSION

The results of these investigations provide the first demonstration of the expression of IGFBP-4, -5 and -6 in human lung cancer cell lines and lung cancer tissue. It was reported that serum levels of IGFBP measured as IGF binding capacity in crosslinking analysis are elevated in lung cancer patients and that the smaller IGFBP ranging from 32 to 37 kDa are especially involved [26].

In our previous publications we showed ligand blots from conditioned media of SCLC and NSCLC cell lines with radiolabelled IGF-1 and we demonstrated heterogeneity in the expression of IGFBP on the protein level [23, 24]. The additional bands we had detected in northern analysis after hybridisation with different oligonucleotides derived from the sequence of IGFBP-2 caused us to screen cDNA libraries looking for other IGFBP than IGFBP-1, -2 and -3 expressed in SCLC cell lines. From cDNA libraries of NCI-H69 and NCI-N417 we could isolate several clones hybridising with IGFBP-2 cDNA under non-stringent conditions and sequence comparison showed that SCLC cell lines probably express the same IGFBP-5 mRNA present in human placenta and human osteosarcoma [15, 16]. The coding region of our IGFBP-5 cDNA clone shows 100% identity with the published sequences. Because all our clones lack the stop signal and at least two nucleotides in front of it, we cannot exclude that IGFBP-5 mRNA transcripts of SCLC cell lines have different 3'termini (Fig. 1). Interestingly, the sequence isolated from placenta differs in its 3' non-coding region from the one cloned from osteosarcoma (Fig. 1). The shorter placenta-derived cDNA from which 148 bp of the 3' non-coding region were sequenced ends with 16 adenine and 3 cytosine residues [15], while the 3' non-coding region of the cDNA from osteosarcoma encloses 753 bp.

They diverge at bp 131 downstream from the transcriptional stop signal. It is interesting that the larger cDNA isolated from osteosarcoma and published by Kiefer and coworkers [16] (at first named IGFBP-6 but re-designated IGFBP-5 at the International IGF Symposium in San Francisco) is homologous to the 3' region of rat-specific IGFBP-5 reported by Shimasaki *et al.* [15], while the human sequence of the latter authors does not show homology to the rat sequence in this part of the molecule (Fig. 1). It was reported that IGFBP-5 appears as transcripts of different lengths, one of 6 kb and one of 1.7 kb [16]. These observations lead to the hypothesis of the usage of different 3' exons. The fact that we did not isolate a clone enclosing a stop signal although we had isolated clones of different length leaves the possibility that different 3' termini even in the coding region of IGFBP-5 may exist.

Our northern blot analysis revealed expression of IGFBP-5 in seven of 11 SCLC cell lines but positive hybridisation signals could be detected in only one of 11 tested NSCLC cell lines. In contrast to this, IGFBP-5 mRNA transcripts are expressed in all lung tumour tissues although most of these samples are obtained from NSCLC tumours. IGFBP-5 is detected in normal lung too. The steady state RNA level in normal lung and most tumour specimens is very low with higher amounts in only a few of them. With our cDNA probe (H5) enclosing most of the coding region of IGFBP-5 and a small 5' non-coding region of 79 bp we were able to detect transcripts of the expected length of approximately 6 and 1.7 kb, thus agreeing with Kiefer *et al.* [17] who had described the existence of two transcripts of these lengths. In addition, our northern blots show several bands between 2.1 and 4.8 kb. Like the 1.7 kb transcript, these additional bands are less intense than the 6 kb transcript and could never be detected in the absence of the 6 kb transcript. DMS79 shows a weak 6 kb band and, in addition, a band of 1.5 kb of similar intensity. The additional bands in SCLC cell lines and tumour tissues may be other transcripts of IGFBP-5 or may be attributed to the homology of IGFBP-5 with thyroglobulin, with a gastrointestinal tumour-associated antigen or with the invariant chain of class II histocompatibility antigen (MHC) described by Kiefer and coworkers [16]. It cannot be totally excluded that all of these additional bands are fragments of IGFBP-5 mRNA.

The tissue distribution of IGFBP-5 in the rat as reported by Shimasaki *et al.* [15] differs from the other IGFBP which are mostly expressed in liver. IGFBP-5 is expressed in all tissues examined but the highest amount was found in kidney. Its concentration in adult rat serum is much lower compared to IGFBP-3 or -4, thus it was supposed that IGFBP-5 may regulate the local action of IGF in an autocrine and/or paracrine manner [16]. The fact that IGFBP-5 is expressed in tumour tissue of NSCLC origin but not in NSCLC cell lines with the exception of NCI-H322 may be explained by the different regulatory factors present *in vivo* possibly secreted by stroma tissue. That means that IGFBP-5 expression is possibly switched off under culture conditions. Another explanation is that NSCLC cell lines lacking IGFBP-5 expression could have a growth advantage in culture. On the other hand, detection of IGFBP-5 in tumour specimens may be due to the presence of other IGFBP-5 producing cells, e.g. stroma cells in the tissue sample. *In situ* hybridisation or immunological detection will give the answer to this question.

Our present study also reports the expression of IGFBP-4 and -6 in a panel of SCLC and NSCLC cell lines, in healthy lung and in tumour tissue of lung cancer patients. IGFBP-4 was cloned by Shimasaki and coworkers [14] and LaTour and coworkers [13] from cDNA libraries of human placenta, liver, ovary and from a human osteosarcoma cell line, respectively. The amino acid sequence deduced from the cDNA of IGFBP-4

encloses 237 amino acids and contains two additional cysteines compared to all other IGFBP identified so far. Purified rat IGFBP-4 inhibits binding of IGF-1 to its receptor and thus diminishes IGF-1 stimulation of [^{3}H]thymidine incorporation in B104 rat neuronal cell line. Cheung *et al.* [27] suggested that at least in the rat there are multiple forms of post-transcriptionally modified IGFBP-4 and Ceda *et al.* [28] reported on the expression of two IGFBP-4 mRNA in rat tissue, an abundant transcript of 1.8 kb and a less abundant one of 2.4 kb. Shimasaki *et al.* [14] examined the expression of IGFBP-4 in several rat tissues and reported the expression of a 2.6 kb transcript in gland, testis, spleen, heart, lung, kidney, liver, stomach, hypothalamus and brain cortex. The highest level of expression was detected in liver. In contrast to this, neither we (data not shown) nor LaTour *et al.* could detect IGFBP-4 in the hepatoma cell line HepG2. LaTour *et al.* described the expression of IGFBP-4 mRNA transcripts of 2.1 kb in human osteosarcoma cell line TE89, in normal bone cells and in skin fibroblasts but not in two other osteosarcoma cell lines (MG63, U2-OS) and in term placenta. Our data presented here show the expression of a 2.6 kb IGFBP-4-specific transcript in 11/11 human SCLC cell lines, in nine of 11 NSCLC cell lines, in 11/11 lung tumour specimen and in healthy human lung tissue. The different amounts of IGFBP-4 mRNA cannot be correlated with the tumour types. The highest levels were detected in two squamous cell lines (EPLC-32M1, U1752) and one derived from adenocarcinoma (A549). Cell lines that lack IGFBP-4 expression are one large cell lung cancer cell line (LCLC-103H) and one cell line derived from a tumour showing bronchoalveolar differentiation (NCI-H322). In tumour tissues derived from lung cancer patients IGFBP-4 is expressed in lower amounts than in healthy lung tissue. The lowest amounts were found in the only tested SCLC tumour (TU33), in one specimen from squamous carcinoma (TU3) and in one tumour classified as squamous lung cancer with areas of SCLC. Speculating that IGFBP-4 shows inhibitory activity on the growth stimulating action of IGF in lung, a deficiency of this IGF antagonist in lung cancer may stimulate tumour growth.

IGFBP-6 was described in human serum [17] and in cerebrospinal fluid [29] and is secreted by human fibroblast cell lines either transformed with SV40 [30] or isolated from adult lung [31]. Among the family of IGFBP, IGFBP-6 is conspicuous because of its markedly higher affinity for IGF-2 than IGF-1 [17, 30]. Shimasaki *et al.* found expression of the 1.3 kb mRNA in rat in all tissues examined with the highest value in lung [32]. Our data presented here shows the expression of IGFBP-6-specific transcripts in nine of 11 NSCLC cell lines, in human lung tissue and in nine of 10 NSCLC tumour specimens but not in SCLC cell lines or in the SCLC tumour specimen. The length of the transcripts of 1.3 kb equals that described in rat tissue. The amount of IGFBP-6 mRNA differs among the NSCLC cell lines and in two of them, U1810 derived from large cell carcinoma and in NCI-H322 derived from bronchoalveolar carcinoma, no expression could be detected. IGFBP-6 message was only rarely detectable in tumour tissues although most of the tumour samples are derived from patients with non-small cell lung cancer, but it is abundant in normal lung tissue. The reason for the discrepancy in the expression of IGFBP-6 between tumour and cell lines may again be the result of the artificiality of the *in vitro* conditions, the result of selection of IGFBP-6-producing cells by growth advantage or, most probably, a dilution effect because possibly only a small fraction of cells in the *in vivo* tumour express IGFBP-6.

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Disease-related Differences in Antibody Patterns Against EBV-encoded Nuclear Antigens EBNA 1, EBNA 2 and EBNA 6

Evelyne T. Lennette, Lars Rymo, Mohan Yadav, Guiseppe Masucci, Karl Merk, Laszlo Timar and George Klein

Antibodies to Epstein–Barr virus (EBV) nuclear antigen family (EBNA) and three of its individual members, EBNA 1, EBNA 2 (A and B) and EBNA 6, were measured by anticomplement immunofluorescence (ACIF) in sera of 75 healthy controls, 13 patients with chronic EBV infection, 38 with non-Hodgkin lymphoma (NHL), 23 with Hodgkin's disease (HD), 105 with nasopharyngeal carcinoma (NPC) and 7 patients with infectious mononucleosis (IM). Their anti-EBV lytic antigens were also measured. We observed that: (1) anti-EBNA 2A and E6 rose in parallel 4–6 weeks after IM, followed by anti-EBNA 1 at 3–6 months, (2) all seropositive individuals had anti-EBNA 1; 74% also had anti-EBNA 2A and E6, (3) anti-EBNA 1 accounted for most of the anti-EBNA reactivity in non-IM sera. Striking disease-associated differences were noted on the humoral responses to the lytic and transformation-associated antigens. Compared to the controls, anti-EBNA 1, -EBNA 2A and -EBNA 6 were simultaneously four to 10 times higher in chronic reactivations, whereas only anti-EBNA 1 was elevated (10 times) in NPC. Individual EBNA titres were normal in NHL or HD patients.
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INTRODUCTION

EPSTEIN–BARR VIRUS (EBV)-encoded antigens can be categorised into growth transformation-associated antigens and lytic antigens. At least six different proteins of the nuclear antigen complex, EBNA 1–6 (abbreviated E1, E2, etc.), and two of the latent membrane antigens (LMP 1 and 2) (reviewed in [1]) are expressed in growth transformed (immortalised) B cells [2]. E1 binds to the origin of latent viral DNA replication. This binding is essential for the maintenance of the viral genomes in the free episomal form [3]. E2 plays an essential role in B cell activation [4]. Two antigenically distinct allelic forms of E2, designated EBNA 2A and 2B, have been identified [5]. E2A carrying viral isolates are more ubiquitous than E2B carriers. The latter are less prevalent and often occur in dual infections with E2A carriers [5, 6]. The functions of EBNA 3–6 are not known. In some EBV-infected cell lines, a small minority of the cells switch on the lytic cycle, manifested by the appearance of the early antigen complex (EA-D and EA-R) and the late viral capsid antigen (VCA).

The cellular expression of growth transformation-associated antigens differs, depending on the cell phenotype. EBV-transformed B cells of normal origin (LCL) express all eight growth transformation antigens. Phenotypically representative Burkitt's lymphoma (BL) cells express only E1 [7]. Nasopharyngeal