Expression of the proto-oncogenes c-met and c-kit and their ligands, hepatocyte growth factor/scatter factor and stem cell factor, in SCLC cell lines and xenografts

K. Rygaard¹, T. Nakamura² & M. Spang-Thomsen³

¹University Institute of Pathological Anatomy, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark; ²Department of Biology, Faculty of Science, Kyushu University, Fukuoka, 812, Japan; ³University Institute of Pathological Anatomy, Frederik V's Vej 11, DK-2100 Copenhagen, Denmark.

Summary We examined a panel of 25 small cell lung cancer (SCLC) cell lines and nude mouse xenografts for expression of the proto-oncogenes c-met and c-kit, and for expression of the corresponding ligands, hepatocyte growth factor (HGF) (also known as scatter factor (SF)), and stem cell factor (SCF), respectively. Expression of mRNA was detected by Northern blotting, and c-met and c-kit protein expression was detected by Western blotting and immunocytochemistry. c-met and c-kit mRNA was expressed in 22 of the examined cell lines or xenografts, and coexpression of the two proto-oncogenes was observed in 20 tumours. Expression of c-met and c-kit protein paralleled in the mRNA expression. HGF/SF mRNA was expressed in two of the examined tumours, and only one of these also expressed the c-met proto-oncogene. SCF mRNA was expressed in 19 of the examined tumours, and in 18 of these coexpression of c-kit and SCF was present. The high percentage of SCLC tumours expressing c-met and c-kit indicates that these proto-oncogenes may have an important function in this disease. The rare coexpression of c-met and HGF/SF is evidence that an autocrine regulatory pathway is not present for this receptor/ligand system in SCLC, while the frequent coexpression of c-kit and SCF indicates that this receptor/ligand system may have an autocrine function in SCLC.

Overexpression of proto-oncogenes is a common feature of human cancer. Proto-oncogenes may be divided into a number of groups according to their cellular functions. One prominent group is the tyrosine kinase family of proto-oncogenes (Cantley et al., 1991), a subgroup of which encodes proteins which are cell surface receptors, and which phosphorylate tyrosine residues on intracellular proteins when an appropriate ligand is bound to the receptor. This subgroup includes the c-met (Dean et al., 1985) and c-kit (Yarden et al., 1987) genes, as well as other known proto-oncogenes.

Recently, c-met was shown to be identical to the hepatocyte growth factor receptor (HGFR) (Bottaro et al., 1991; Naldini et al., 1991a; Naldini et al., 1991b), which has hepatocyte growth factor (HGF) (Nakamura et al., 1989; Miyazawa et al., 1989; Nakamura, 1991) as one known ligand; HGF is identical to the molecule known as scatter factor (SF) (Naldini et al., 1991b; Weidner et al., 1991), which is known to increase the motility of many cell types including carcinoma cells (Stoker, 1989; Gherardi et al., 1989; Weidner et al., 1990). HGF/SF has the ability to promote growth of hepatocytes (Nakamura et al., 1989), inhibit growth of certain tumour cells (Shiota et al., 1992; Tajima et al., 1991), and increase motility of many cell types (Stoker, 1989; Gherardi et al., 1989; Weidner et al., 1990). These diverse effects of HGF/SF could be of importance for the behaviour of c-met/HGFR expressing SCLC cells.

The c-kit proto-oncogene has recently been identified as the receptor for stem cell factor (SCF) (Zsebo et al., 1990; Williams et al., 1990), alternatively termed mast cell growth factor (Anderson et al., 1990) or hematopoietic growth factor KL (Huang et al., 1990).

Examination of the level of expression of tyrosine kinase growth factor receptors in tumour cells may be of particular importance in instances where an appropriate ligand is available to the tumours. In such cases the binding of the ligand to the surface receptors on tumour cells may contribute to the growth regulation of the cells. This could be in an endocrine, paracrine or autocrine manner, depending on the site of production of the ligand.

We examined the expression of c-met and c-kit mRNA and protein in a panel of 25 SCLC cell lines and nude mouse xenografts in order to determine if these genes are expressed in SCLC. In addition, we examined the panel for expression of HGF/SF and SCF which are known ligands for the c-met and c-kit tyrosine kinase growth factor receptors, respectively.

The panel of SCLC tumours included cell lines and nude mouse xenografts established in five different laboratories in Europe and USA, and is thus likely to be widely representative for this disease. Most tumours were propagated both as cell lines and as xenografts in order to explore the possible role of the growth conditions on the expression of the examined proto-oncogenes and their ligands.

Both c-met and c-kit mRNA was expressed in the majority of the examined SCLC cell lines and xenografts (together referred to as 'tumours'), and expression of the corresponding proteins was found to parallel the expression of their mRNAs. Only two tumours expressed detectable amounts of HGF/SF mRNA transcripts, and only one of these two also expressed c-met mRNA. In contrast to this, several tumours expressed both c-kit and SCF.

Our results are the first to demonstrate expression of c-met and HGF/SF in SCLC, and the data presented also provide the first demonstration of c-kit protein in this disease.

Materials and methods

Cell lines and xenografts

Twenty-five tumours established from 20 patients were investigated. Five tumours were grown only as xenografts, six tumours only as cell lines, while the remaining 14 were investigated both as cell lines and as xenografts.

Cell lines were grown at 37°C in a humidified atmosphere containing 5% CO₂. Media contained 10% foetal calf serum. Tumours designated CPH were established in Copenhagen, Denmark (Engelholm et al., 1986), DMS tumours at Dartmouth Medical School, NH, USA (Pettengill et al., 1980), the NCI tumours at the National Cancer Institute, MD, USA (Carney et al., 1985), and GLC tumours at University...
Hospital of Groningen, The Netherlands (de Leij et al., 1985; Berendsen et al., 1988). CPH-54A and CPH-54BN are in vitro established subclones of the same original tumour (Engelholm et al., 1985), and CPH-136A and CPH-136B were established from the same patient before and after chemotherapy, respectively. GLC-14, GLC-16, and GLC-19 were established from the same patient during longitudinal follow-up (Berendsen et al., 1988), the same was the case for GLC-26 and GLC-28. The cell line MAR-24H was established at Marburg, Germany (Bepler et al., 1987). Cell lines were regularly tested and found free of Mycoplasma infection.

Cells for investigation were harvested in mid- to late exponential growth phase. Harvesting was done by scraping with a rubber policeman for cells growing attached to the bottom of culture flasks, and by scraping as floating aggregates. The cells were washed in sterile buffer (150 mM NaCl–10 mM EDTA–10 mM Tris–pH 8.0), spun down, immediately frozen in liquid nitrogen, and stored at –80°C until further processing.

Xenografts were established in the flanks of nude mice by s.c. inoculation of 106–107 cells from cell lines, or directly from patients by inoculation of 2-mm-diameter tumour biopsies (Engelholm et al., 1985). Northern blots were established by scraping the immortalised xenograft into liquid nitrogen, and stored frozen at –80°C for several months before testing and found free of Mycoplasma infection.

**RNA extraction and Northern blotting**

RNA was extracted by the acid guanidinium phenol chloroform method (Chomczynski & Sacchi, 1987), dissolved in diethyl pyrocarbonate treated water, and the concentration determined by spectrophotometry. Twelve μg total RNA samples were electrophoresed through 1% agarose gels containing 2.2 M formaldehyde (Sambrook et al., 1989), and transferred to nylon membranes (GeneScreen Plus, NEN DuPont) in 10 × SSC (saline sodium citrate; 1 × SSC is 150 mM sodium chloride – 15 mM sodium citrate).

Membranes were prehybridised at 42°C in 50% (v/v) formamide – 1% (w/v) sodium dodecyl sulfate – 1 M sodium chloride – 5% (w/v) dextran sulfate – 100 μg ml−1 salmon testes DNA, and hybridised to denatured probes (see below) for 18–19 h in the same hybridising buffer. Maximal washing stringency was 63–65°C in 2 × SSC – 1% (w/v) sodium dodecyl sulfate for 1 h. Membranes were exposed to X-ray film for 1 × 106–107 c.p.m. per film.

**Studies of c-met, c-kit and HGF/SF expression in normal adult human tissues**

Membranes were transferred to a Hybond-N+ membrane (Amersham) and hybridised with 32P-labeled probes. The following probes were used:

- **c-met** probe: a 1.3 kb EcoRI fragment of the plasmid pRc-RS3 (kindly provided by G.F. Vande Woude) or a 0.5 kb EcoRI fragment of the plasmid pmetS obtained from the American Type Culture Collection (ATCC) (Park et al., 1987); the poh1 probe recognizes both translated and untranslated c-met as well as c-met transcripts of different sizes (G.F. Vande Woude, personal communication), while the pmetS probe recognizes only a single untranslated human c-met transcript (Park et al., 1987). The HGF/SF probe was a 0.4 kb Smal-SalI fragment of the plasmid pRcRRE/SF (Gunning et al., 1993), and the c-kit probe was a 1.2 kb SrfI fragment of the plasmid phkit-171 obtained from the ATCC (Yarden et al., 1987). The SCF probe was a 0.4 kb Smal-HindIII fragment of the plasmid pGEM3:SCF-#9 (kindly provided by K. Zsebo (Zsebo et al., 1990)) and the human β-actin probe was a 2.1 kb BamHI fragment of the plasmid pHFβA-1 (Gunning et al., 1983).

**Protein extraction, electrophoresis and c-met immunoblotting**

Cell and tissue samples for protein extraction were homogenised in lysis buffer (25 mM Tris (pH 7.5) – 50 mM NaNCl – 0.5% (w/v) sodium deoxycholate – 1% (v/v) Nonidet P-40 – 0.1% (w/v) sodium dodecyl sulfate – 1 mM phenylmethylsulfonyl fluoride – 500 KIE ml−1 aprotinin (Trasylol, Bayer)), further homogenised by ultrasonication, and centrifuged for 15 min at 12,000 g. The supernatant was transferred to a new tube and the protein concentration determined with a commercial kit utilising bienechonic acid (Pierce, France). Sample buffer containing β-mercaptoethanol was added to the supernatant to give a final protein concentration of 2 μg ml−1. Samples containing 50 μg total protein were boiled for 5 min and size-fractionated by electrophoresis through SDS containing 7.5% polyacrylamide gels. Molecular weight markers in the 42–200 kD range (Bio-Rad, CA) were coelectrophoresed. The electrophoretically separated proteins were transferred to nitrocellulose membranes (0.45 μm, Schleicher and Schuell, Dassel, Germany) by semidy electroblotting (Kyse-Andersen, 1984) according to the manufacturer’s instructions (JKA, Copenhagen, Denmark).

Membranes were blocked for 1 h in 50 mM Tris (pH 7.4) – 150 mM NaCl – 0.1% Tween-20 – 4% Non-fat dry milk (Carnation, USA) – 10 mM sodium azide. After three washes in Tris buffered saline (TBS) the primary c-met specific antibody, 19S, was added. This antibody is monoclonal and raised in mouse against a 50 kD protein (p50met) from the carboxy-terminal part of the human c-met protein. The antibody was used as ascites fluid in a dilution of 1:1000 in 50 mM Tris (pH 7.4) – 150 mM NaCl – 0.05% Tween-20 – 2% bovine serum albumin (BSA) – 10 mM sodium azide; incubation time was 3 h. Following three washes in TBS, a secondary alkaline phosphatase coupled rabbit anti-mouse antibody (Dakopatts, Glostrup, Denmark) was added in dilution 1:2000 for 1 h in buffer identical to that used for the primary antibody. After washing, bound antibody was visualised by a chromogenic reaction catalysed by the conjugated alkaline phosphatase using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as chromogenic substrate.

Controls included incubation without primary antibody and incubation with primary antibody which had been preincubated with the p50met protein which was used for immunization (1 μg peptide per 1 ml ascites fluid). Both these control experiments completely eliminated binding to proteins with molecular weight of 50 kD or equivalent to the c-met specific bands (data not shown). The c-met antibody and the corresponding blocking protein, p50met, was a generous gift of Dr Marianne Oskarsson, NCI-Frederick, USA. The available c-met specific antibody was not suitable for use in immunocytochemistry.

**Western blotting and immunocytochemistry of c-kit protein expression**

Western blotting was performed as described for c-met except that the buffer used for blocking and incubation was 100 mM Tris (pH 7.4) – 150 mM NaCl – 10% foetal calf serum – 2% BSA – 0.1% Triton X-100. The antibody was a mouse monoclonal (Boehringer Mannheim, Germany), and was used at a concentration of 10 μg ml−1. A blocking peptide was not available for this antibody.

Immunocytochemistry was employed to determine the localisation of c-kit protein in the SCLC cells. Cell lines growing attached to the bottom of culture flask were grown on 8-well slide glasses until attached, while cells growing in suspension culture were washed in phosphate buffered saline (PBS), placed on 8-well slide glasses in a small amount of PBS and allowed to dry completely. Cells on slide glasses were fixed in 1% formaldehyde in PBS for 10 min, washed in PBS and blocked with 10% foetal calf serum in PBS for
30 min. After washing in PBS, a polyclonal c-kit specific antibody was added at a concentration of 10 μg ml⁻¹ in PBS containing 4% BSA, and incubated for 4 h at room temperature. The primary antibody was raised in rabbits against a peptide with the sequence GSTASSQPLLHVDDV, representing amino acids 961–976 in the C-terminal part of c-kit protein (Oncogene Science, Uniondale, NY). The specimens were washed in PBS containing 0.1% Tween-20 and incubated for 1 h with a FITC-conjugated swine anti-rabbit antibody (Dakopatts, Glostrup, Denmark) diluted 1:20 in PBS containing 4% BSA. An epifluorescence microscope (Aristoplan, Leica) equipped with filters appropriate for FITC fluorescence was used for viewing and photographing. Controls included incubation without the primary antibody and incubation with primary antibody which had been pre-incubated with the peptide used for immunisation (Oncogene Science). Preincubation was done with a 10-fold excess (by weight) of peptide.

Results

The expression of mRNA transcripts of c-met, HGF/SF, c-kit and SCF in SCLC cell lines and xenografts is summarised in Table I. There was no systematic difference in expression pattern in the two model systems. In most cases there were similar expression levels in the two systems, but in some cases expression was slightly higher in a cell line than in the corresponding xenograft and vice versa.

Expression of c-met mRNA was detected in 22 of the 25 examined SCLC tumours (Figure 1). Most of the tumours expressed a single transcript with an electrophoretic mobility corresponding to a size of 7.5 kb. However, a few tumours (e.g. CPH-167 and DMS-456) also expressed a mRNA of about 6 kb (Figure 1), which was detectable only with the phospho probe (see Materials and methods).

The protein encoded by c-met was expressed in tumours expressing c-met mRNA (Figure 4), and in general the expression levels of c-met protein corresponded well with the levels of c-met mRNA. On the Western blots two bands with Mₛ of 145,000 (p145MET, (Giordano et al., 1989b)) and 170,000 (p170MET) were observed in all positive tumours, the p145MET band being most prominent. In a few tumours (e.g. DMA-273 xeno and NCI-H69) the level of protein expression was relatively high despite the fact that c-met mRNA was quite low.

The level of c-met expression detected in different tumours varied widely (Figure 1). In general, tumours expressing c-met did so both when grown as cell lines and as nude mouse xenografts. However, a few tumours (e.g. CPH-54A, CPH-54B) expressed very low levels of c-met when grown in vitro as cell lines, whereas no expression was detectable in the corresponding xenografts.

Expression of c-met mRNA was also examined in normal adult human tissues, and high levels were detected in placenta and lung, while moderate expression was observed in heart, brain, liver, skeletal muscle and kidney (Figure 3). In pancreas, only trace levels of c-met expression was found. In the examined SCLC tumours, expression of mRNA for the c-met ligand, HGF/SF, was detectable only in DMS-114 and NCI-N417 (Figure 1). Expression of HGF/SF was determined in various normal adult human tissues (MTN Blot, see Materials and methods), and expression was detected in heart, placenta, lung, liver, muscle (weak) and kidney (Figure 3).

Expression of c-kit mRNA was demonstrated in 22/25 SCLC cell lines (Figure 2). As was the case for c-met, the

| Tumour | c-met | HGF/SF | c-kit | SCF |
|--------|-------|--------|-------|-----|
| CPH-54A | (+) | NA | (+) | NA |
| CPH-54B | (+) | NA | (+) | NA |
| CPH-136A | NA | ++ | NA | ++ |
| CPH-136B | NA | ++ | NA | ++ |
| CPH-167 | (+) | NA | NA | ++ |
| CPH-186 | NA | + | NA | ++ |
| CPH-187 | NA | + | NA | ++ |
| DMS-53 | ++ | + | + | + |
| DMS-79 | ++ | ++ | ++ | ++ |
| DMS-92 | + | ++ | + | ++ |
| DMS-114 | - | ++ | + | + |
| DMS-153 | + | ++ | + | ++ |
| DMS-273 | + | + | + | + |
| DMS-406 | - | NA | NA | ++ |
| DMS-456 | ++ | -- | -- | -- |
| GLC-2 | - | NA | NA | NA |
| GLC-3 | + | + | + | + |
| GLC-14 | + | + | + | + |
| GLC-16 | + | + | + | + |
| GLC-19 | + | + | + | + |
| GLC-26 | + | NA | NA | NA |
| GLC-28 | + | NA | NA | NA |
| NCI-H69 | (+) | NA | NA | NA |
| NCI-N417 | + | NA | NA | NA |
| MAR-24H | + | NA | NA | NA |

The level of expression was rated visually as none: ‘-‘, trace: ‘(+),’ low: ‘+’, intermediate ‘++’, and high: ‘+++’. Xenografts marked ‘NA’ were not established as cell lines and vice versa.

Table 1 Expression of c-met, HGF/SF, c-kit and SCF in SCLC cell lines and nude mouse xenografts and in normal adult human tissues
Figure 1 Northern blots demonstrating expression of the c-met proto-oncogene and of HGF/SF in SCLC cell lines a, and xenografts b. The two upper blots were probed with the phosl and the pmet5 probes, respectively. A 7.5 kb band was detected by both c-met probes, and an additional c-met specific band of approximately 6 kb (arrow) was seen in some tumours on blots probed with the phosl probe (top). The lower two blots demonstrate expression of a 6 kb HGF/SF transcript and of β-actin, respectively. An asterisk in b, marks non-specific hybridisation to the 4.8 kb ribosomal RNA band. Each lane contains 12 μg total RNA. The positions of the coelectrophoresed size markers or of the 18S (1.9 kb) and 28S (4.8 kb) ribosomal RNA bands are indicated.
Figure 2  Northern blot demonstrating expression of the c-kit proto-oncogene and of SCF in SCLC cell lines a, and xenografts b. The upper panel was probed with a c-kit specific probe, the middle panel with a SCF specific probe, and the bottom panel with a β-actin specific probe. Each lane contains 12 μg total RNA. The positions of the 18S (1.9 kb) and 28S (4.8 kb) ribosomal RNA bands are indicated.
expression of c-kit seemed independent of the model system in which the tumours were grown. Coexpression of c-met and c-kit mRNA was found in 20 tumours.

Western blotting of c-kit protein demonstrated that there was very good correlation between the level of c-kit protein expression and c-kit mRNA expression (Figures 2 and 5). Two bands with M.s of 145,000 and 120,000 were detected. The M, 145,000 band is the c-kit receptor while the M, 120,000 band is most likely a precursor (Blume-Jensen et al., 1991).

Immunocytochemical analysis of cell lines with and without detectable expression of c-kit mRNA was also performed. The examined tumours were the c-kit mRNA positive DMS-92, DMS-133, DMS-273, DMS-406, GLC-14, GLC-16, GLC-19 and NCI-H69, and the c-kit mRNA negative CPH-54A, CPH-54B and DMS-114. In all tumours expressing c-kit mRNA, clear cell-membrane staining was observed together with some cytoplasmic staining with nuclear shadowing (Figure 6). In cell lines in which c-kit mRNA and protein were not detectable on Northern and Western blots (e.g. CPH-54A and CPH-54B), very weak membrane staining was detected. In all cases the staining could be completely eliminated by preincubation of the primary antibody with the peptide used for immunisation (Figure 6) or by omission of the primary antibody.

The ligand for c-kit, SCF, was expressed in 19/25 tumours, and coexpression of the c-kit receptor and its ligand was found in 18 of the examined tumours.

Normal adult human tissues frequently expressed both c-kit and SCF mRNA (Figure 3).

Discussion

Twenty-two of 25 tumours (88%) expressed detectable amounts of c-met mRNA transcripts and c-met protein. Two bands were detected by the 19S c-met monoclonal antibody on Western blots of proteins electrophoresed under reducing conditions. The smaller band represents the c-met β-chain (p145MET) while the 170,000 band most likely represents uncleaved c-met precursor, p170MET (Giordano et al., 1989a; Giordano et al., 1989b). The α-chain with a M, of 50,000 (p50MET), which is presumed to be derived from the amino-terminal part of the p170MET precursor (Tempest et al., 1988), is likely not to be detected by the 19S antibody which was raised against a carboxyterminal c-met-protein (p50met).

It has been suggested that the size (9 kb) often reported for the c-met mRNA species expressed in various tissues (Park et al., 1986; Park et al., 1987; Prat et al., 1991) is likely to be an overestimate (Park et al., 1987). Examination of overlapping human c-met cDNA clones and heteroduplex analysis with full length mouse met cDNA, which is close to 7 kb long (Iyer et al., 1990), indicated that the human c-met transcript is likely to be of approximately the same size (Park et al., 1987). In accordance, the two c-met specific probes (pmet5 and pphs5) used in the present study both detected a band with an electrophoretic mobility of 7.5 kb. One of the c-met probes used, pmet5, is known to recognise only one band on Northern blots (Park et al., 1987). Examination of several Northern blots where an RNA size marker which included a band of 7.46 kb was coelectrophoresed, and of MTN Blots where the position of size marker bands are marked, repeatedly showed a c-met specific band with an electrophoretic mobility of 7.5 kb (Figures 1 and 3). To ascertain the authenticity of our pmet5 probe, we performed Southern blotting of TaqI digested human DNA and obtained the expected 3 bands (Dean et al., 1987) of approximately 1.8, 3.2 and 11.0 kb (data not shown). Our results is further evidence that the actual size of the human c-met mRNA is close to 7.5 kb.

A few tumours expressed an additional c-met specific mRNA species with a size of approximately 6 kb detectible with the phosil probe but not with the pmet5 probe. In some tumours the phosil probe detects up to three different c-met specific transcripts reported to be 6, 7, and 9 kb (Park et al., 1987), and a 5 kb transcript of a fusion gene resulting from translocation of tpr (translocated promoter region) sequence from chromosome 1 to c-met sequence on chromosome 7 (Park et al., 1986; Park et al., 1987). As judged from its size, the 6 kb band is not likely to represent the 5 kb tpr-met fusion transcript, but rather one of the two other c-met transcripts of 6 and 7 kb. Thus, the examined SCLC tumours expressed only one additional c-met transcript when probed with the phosil probe, while other tumour cells have been reported to express at least two transcripts smaller than the 7.5 kb transcript detected by both c-met probes (Park et al., 1986; Park et al., 1987; Prat et al., 1991).

If the c-met protein detected in the examined SCLC tumours encodes a functional receptor, this may be of importance for the behaviour of SCLC tumours in patients, since the c-met ligand, HGF/SF, is expressed in many normal tissues (Figure 3), and is present in the plasma (Nakamura et al., 1989; Zarnegar et al., 1990). We detected HGF/SF expression in several normal tissues (Figure 3), thus confirming that this growth factor is widely expressed (Rubin et al., 1991; Higashio et al., 1990; Yanagita et al., 1992). However,

![Figure 3](http://example.com/figure3.png)
among the examined SCLC tumours, only two expressed HGF/SF (Figure 1), and coexpression of c-met and HGF/SF was found in only one tumour (NCI-N417). Therefore the results do not indicate that an autocrine regulatory loop involving this receptor/ligand system is frequently active in SCLC.

Several normal tissues coexpressed c-met and its ligand HGF/SF, and c-kit and its ligand SCF (Figure 3). This suggests that these receptor-ligand systems may play a role in normal growth regulation in an autocrine or paracrine manner. It may be speculated that the expression of HGF/SF and SCF in many normal tissues could be of importance for the ability of SCLC tumour cells to metastasise to these tissues, or that HGF/SF or SCF produced in various organs might reach the lungs or sites with metastatic tumour spread and modulate the growth of c-met and c-kit positive SCLC tumours in situ. It is not known whether these ligands stimulate or inhibit SCLC growth; recent studies of various tumour cell lines, not including SCLC, have shown that HGF/SF may inhibit tumour cell growth (Tajima et al., 1991; Shiota et al., 1992), despite the fact that HGF/SF is a potent mitogen for hepatocytes (Nakamura et al., 1989).

The fact that normal lung tissue expresses c-met mRNA does not necessarily imply that c-met is expressed in SCLC. The SCLC progenitor cell has not been identified with certainty, and it may represent only a minority of the cells present in normal lung tissue.

In one previous study (Prat et al., 1991), three SCLC patient biopsies were examined for c-met protein expression by immunohistochemistry and none was detected. We detected c-met protein in the vast majority of cultured SCLC tumours. The cause of the apparent difference between our results and the results of Prat et al. (1991) is not clear at present, but it may be necessary to examine larger materials in order to determine whether there is an actual difference between c-met protein expression in SCLC patient biopsies and in cell lines.

The results obtained for the c-kit proto-oncogene confirm recent data demonstrating frequent expression of c-kit mRNA in SCLC (Sekido et al., 1991). In our series 22/25 (88%) SCLC tumours expressed c-kit mRNA, which is in agreement with previous findings (Sekido et al., 1991). Our...
results add important information to these previous findings by demonstrating that the c-kit mRNA is translated into protein. Immunocytochemical detection of very low levels of c-kit protein was possible in some of the cell lines in which c-kit mRNA or protein could not be demonstrated by Northern or Western blotting. This is most likely due to the very high sensitivity of immunocytochemical techniques. SCF mRNA was found to be expressed in a large proportion of SCLC, and coexpression of c-kit and its ligand SCF was demonstrated to be very frequent in SCLC, confirming very recent results (Hibi et al., 1991).

Apparently, SCF is widely expressed in normal human tissues (Figure 3). The production of SCF by SCLC tumour cells and normal tissues may provide SCLC cells expressing the c-kit receptor with a growth advantage and may thus contribute to their malignant phenotype, provided that the receptor and its ligand are functional, and provided that a growth-stimulatory or an otherwise advantageous response is elicited in cells upon binding of SCF.

The proto-oncogenes c-met and c-kit can now be added to the long list of proto-oncogenes which are expressed in SCLC (Birrer & Minna, 1989; Mäkelä et al., 1991). It could be speculated that expression of some of these genes may be a result of a general deregulation of transcription in cancer cells, leading to expression of genes that may not have any function in the tumour cells. However, there is evidence that expression of proto-oncogenes in SCLC is not the result of a non-specific general increase in transcription. For example, some proto-oncogenes, e.g. the c-erbB-2 gene is not expressed in the panel of tumours examined here (data not shown), and neither in another examined panel of SCLC (Schneider et al., 1989). We also examined our SCLC tumour panel for expression of human serum albumin, which can be presumed to be of absolutely no importance for SCLC tumours, and found no detectable transcripts (data not shown). Indirectly, the type of data suggest that only genes which have a function in the tumour cells are expressed.

The fact that expression of c-met and c-kit is found in

**Figure 6** Immunocytochemical demonstration of c-kit protein expression in representative SCLC cell lines. Micrographs on the left show results of incubation with c-kit antibody in a concentration of 10 μg ml⁻¹. Identically prepared samples incubated with primary antibody which had been pre-absorbed with blocking peptide are shown on the right. The cell lines shown are CPH-54A (top), DMS-153 (upper middle), GLC-16 (lower middle), and NCI-H69 (bottom). In all cases, micrographs of cells incubated with primary antibody which had or had not been preincubated with blocking peptide were exposed and reproduced under identical conditions. Magnification: ×1000.
SCLC and in several normal tissues as reported by us and others (Prat et al., 1991; Iyer et al., 1990) suggests that these receptors may be involved in the regulation of cell behaviour in several tissues other than liver and stem cells. Thus, the designation HGF and SCF receptor may not completely describe the function of these genes.

Recently, the ligands for c-met and c-kit, HGF/SCF and SCF, have been cloned and expressed (Nakamura et al., 1989; Miyazawa et al., 1989; Zsebo et al., 1990; Anderson et al., 1990; Huang et al., 1990), and it is possible to produce them in pure form. The availability of the ligands enables further studies of the function of the c-met and c-kit receptors in SCLC. It is of great potential interest that HGF/SCF has been shown to modulate cell growth and motility (Nakamura et al., 1989; Shiotani et al., 1992; Tajima et al., 1991; Stoker, 1989; Gherardi et al., 1989; Weidner et al., 1990). HGF/SCF is produced in normal lung tissue (Rubin et al., 1991; Higashio et al., 1990; Yanagita et al., 1992) and thus may act on SCLC cells in the patient. We are currently investigating the possible effects of HGF/SCF on SCLC cells.

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