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REVIEW

Biology of small cell lung cancer: an overview

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Biology of small cell lung cancer: an overview. P. Weynants, Y. Humblet, J.L. Canon, M. Symann.

ABSTRACT: Despite disappointing results in the treatment of small cell lung cancer (SCLC), major progress in our understanding of SCLC biology has occurred in the past decade. Advances in the technique for culturing SCLC tumours *in vitro* have greatly facilitated the study of the biological properties of this tumour. The major progress in our understanding of SCLC includes: 1) the availability of nonspecific biological tumour markers such as neuron-specific enolase (NSE), the BB isoenzyme of creatine phosphokinase (CPKBB), bombesin/gastrin releasing peptide (GRP) and chromogranin A; 2) the generation of monoclonal antibodies raised against the neural and epithelial features of SCLC tumours; 3) the identification of several autocrine growth factors such as bombesin/GRP, insulin-like growth factor (IGF), transferrin and physalaemin; 4) the close study of cytogenetic abnormalities leading to the discovery of a unique chromosomal deletion of the short arm of chromosome 3 (del 3p 14–21), and to changes in oncogenic expression, *e.g.* c-myc, L-myc and N-myc, accounting for known biological and treatment results. These data suggest that all lung cancers arise from a common stem cell of endodermal origin. The information derived from these biological studies represents the most promising avenue towards new treatment strategies in SCLC.

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Lung cancer remains the most common and lethal human cancer in Western countries, showing a continually rising incidence, especially among women [1, 2].

Small cell lung cancer (SCLC) accounts for 20–25% of all new cases of bronchogenic carcinoma, but differs clinically and biologically from the other three major histological types (squamous, adeno- and large cell carcinomas), collectively referred to as non-SCLC. For non-SCLC, surgery remains the treatment of choice [1]. Until 1970, surgery and radiotherapy were the primary therapeutic modalities for SCLC, although the cure rate was less than 1%. Since then, "combination chemotherapy" has become the cornerstone of therapy for SCLC, with an objective response achieved in almost 90% of SCLC patients [1, 2–6]. However, despite this progress in tumour response and the resulting fivefold increase in survival duration, only 3–5% of these patients are cured by the current chemotherapeutic regimens [1, 2–5].

Although few important advances in the management of SCLC patients have occurred in the past decade, an explosion of new information on the basic cellular and molecular biology of this tumour has been discovered [7, 8]. Much of this progress arises from advanced cell culture methods leading to the establishment of a large number of human SCLC cell lines [9–12].

This review will focus on recent progress in the biology of SCLC (table 1), and outline the potential applications of these properties in the management of SCLC patients. Indeed, at this time, information derived from biological findings represents the most promising avenue for a breakthrough in the treatment of SCLC.

Small cell lung cancer lines

Establishment of SCLC cell lines

The studies on the biology of SCLC initially necessitated the establishment of continuous cell lines from tumour specimens [10]. The major difficulty in culturing tumour cells is that normally they require the presence of serum which frequently encourages the overgrowth of non-malignant cells such as fibroblasts.

Initial attempts to culture SCLC tumour cells using conventional culture media, were rarely fruitful with a success rate of approximately 10% [9]. Subsequently, a success rate of 50% was reported, by using conditioned media from already established

Table 1. – Biological properties of SCLC and non-SCLC cell lines

Characteristics	Cell type		
	SCLC		NON-SCLC
	Classic	Variant	
Growth morphology	Tight aggregates	Loose aggregates	Adherent
Cytology	SCLC	SCLC/LC	Non-SCLC
Doubling time	±72 h	±33 h	±50 h
Cloning efficiency	1–5%	10–30%	0.5–40%
Radiation sensitivity	Sensitive	Resistant	Resistant
Biomarkers			
Peptide hormones	++	+/-	-
LDDC	++	-	-
BN/GRP	++	-	-
NSE	++	+	-
CPKBB	++	++	-
Chromogranin A	+	+	-
Growth factor receptors			
BN_R	+	-	-
IGF_R	+	+	?
$Transferrin_R$	+	+	+
EGF_R	-	-	+
Surface Ag.			
HLA class I	-	-	++
Leu 7	+	+	-
Intermediate filaments			
Cytokeratin	+	+	+
Neurofilaments	+/-	+	-
Oncogene amplification			
c-myc	No	Yes	Sometimes
N-myc	Sometimes	Sometimes	No
L-myc	Sometimes	?	No
Chromosome abnormalities			
3p deletion	+	+	+/-

This table summarizes the currently known biological properties of small cell carcinoma (SCLC) in comparison with non-small cell carcinoma cell lines (non-SCLC). LDDC: L-Dopa decarboxylase; NSE: neuron-specific enolase; BN/GRP: bombesin, gastrin releasing peptide; CPKBB: creatine-phosphokinase; EGF_R: epidermal growth factor receptor; IGF: insulin growth factor receptor; HLA: histocompatibility locus antigen; SCLC/LC: small cell/large cell.

SCLC cell lines. This suggested that growing SCLC cells produce necessary "mitogenic factors" absent from conventional serum supplemented media [9]. The NCI group following the lead of Sato [13, 14] studied the replication of an established SCLC cell line: "NCI - H69" in serum free medium sequentially supplemented with a large panel of "growth factors and hormones". They were able to define the optimal combination of factors permitting the most effective continuous replication of this cell line over a 12 month period [11]. This supplemented RPMI medium is called HITES based on the added ingredients: hydrocortisone (10^{-8} M), insulin (15 $\mu g \cdot ml^{-1}$), transferrin (100 $\mu g \cdot ml^{-1}$), 17 beta-oestradiol (10^{-8} M), selenium ($3 \cdot 10^{-8}$ M).

Subsequently, CARNEY *et al.* [14] demonstrated that HITES medium can support proliferation of cells in culture from both a fresh SCLC tumour specimen obtained directly from patients or of heterotransplanted SCLC tumour cells removed from nude mice. Currently, the establishment of SCLC cell lines has a success rate of about 70% for all tumour containing specimens prepared by experienced workers [15]. Nevertheless, after selection of SCLC cell lines in HITES medium, the established cell lines grow better when they are maintained in conventional media containing 10% foetal calf serum.

In most cases, the original tumour specimens come from metastatic sites, including mainly pleural effusions, bone marrow aspirates or lymph nodes. Only a few cell lines have been derived from the primary tumour itself (10%). This could reflect an intrinsic difference in the properties of these different tumoral locations [16], as well as the small number of viable cells obtained from endobronchial biopsies (Crushing specimen).

Usually, SCLC cell lines retain a morphological, cytological and histological appearance similar to the original biopsy specimens from which they were established [9, 17].

Characteristics of established SCLC cell lines

SCLC cell lines can be classified into two major groups, called "classic" and "variant" cell lines.

Seventy percent of established SCLC cell lines retain properties of differentiated neuroendocrine cells from the amine precursor uptake and decarboxylation (APUD) system. This includes the presence of cytoplasmic dense core "neurosecretory granules" and the expression of elevated levels of 4 biomarkers including the key APUD enzyme L-Dopa decarboxylase (LDDC), the APUD enzyme neuron-specific enolase (NSE), the peptide hormone bombesin (gastrin-releasing peptide) (BN), and the BB iso-enzyme of creatine phosphokinase (CPKBB) [9]. With few exceptions, these markers are not expressed in non-SCLC cell lines. In addition, light microscopy demonstrates that SCLC lines retain typical intermediate subtype morphology, they grow as tightly packed floating cellular aggregates, with lengthy doubling times and low colony forming efficiency in soft agar. These cell lines also remain tumorogenic in nude mice.

The above characteristics defined the so-called "classic SCLC cell lines" [17]. In contrast, 30% of established SCLC cell lines have discordant expression of the biochemical markers and growth characteristics, and are thus called "variant SCLC cell lines" [18, 19]. They have significantly lower concentrations of NSE, lack LDDC and bombesin immunoreactivity but retain high concentrations of CPKBB. Morphologically, they resemble large cell undifferentiated carcinoma and grow as loosely attached floating aggregates with short doubling times and high cloning efficiencies. Finally, they are radioresistant *in vitro* and express a high level of the c-myc oncogene [20, 21].

Taken altogether, these data suggest a more malignant behaviour for the variant SCLC cell line, maybe, in part, related to c-myc amplification in these cells (see below).

Clinically, the variant phenotype probably represents the large cell-type in SCLC tumours whose presence is associated with a worse prognosis [22, 23]. A gradual change in tumour morphology from small cells to large cells has been observed both in tissue culture and *in vivo* [24, 25]. This transformation to large cells is accompanied by a loss of APUD properties. This *in vitro* transformation mimics the histological changes observed in as many as 35% of patients relapsing with SCLC who become resistant to both radio- and chemotherapy [20, 24].

Tumour markers

Clinically, the first application to come from the biology of SCLC was in the identification of useful tumour markers. Although abnormal levels of the peptide hormones produced by SCLC tumours, such as adrenocorticotrophin (ACT), arginine vasopressin (AVP), and calcitonin, may be detected in the serum of approximately 50% of untreated SCLC patients, the level of these substances in the blood has never been correlated with either the stage of disease or the histological subtype [26, 27].

In recent years, considerable interest has been generated in screening patient body fluids for those biological markers frequently detected in SCLC cell lines or their culture supernatants. These markers would be useful for identifying, staging, monitoring treatment or predicting relapse of SCLC patients [28]. However, none have been shown to be either sensitive or specific enough to use for screening asymptomatic patients [28]. Among "potentially useful markers", the four that have been most extensively tested are NSE (neuronspecific enolase), CPKBB (creatine phosphokinase), GRP (gastrin releasing peptide) and chromogranin A [29].

NSE

Enolase is a glycolytic enzyme that is widely distributed throughout mammalian tissues and is composed of three distinct subunits alpha, beta and gamma. Three combinations of dimers have been found in the central nervous system (CNS) (alpha-alpha, alpha-gamma, gamma-gamma) [30], and NSE was identified as iso-enzymes gamma-gamma. Large amounts of NSE have been detected in neuroendocrine tumours (Apudome) including SCLC by immunostaining and radioimmunoassay [31]. Serum levels of NSE are potentially a biological marker of SCLC [32–37].

In agreement with others, we found elevated levels of serum NSE (>15 $\mu g \cdot l^{-1}$) in 61 out of 95 patients (64%) with SCLC, including 45% with limited disease (LD) and 90% with extensive disease (ED). However, only 8 out of 60 patients (13%) with non-SCLC and 0 out of 71 (0%) with benign pulmonary disease had elevated levels of serum NSE [38].

The level of NSE has also been strongly correlated with tumour burden and the number of metastatic sites [34]. Osterlind *et al.* [39] demonstrated that NSE is one of the most sensitive prognostic indicators for SCLC. A good correlation has been drawn between the initial decline in plasma NSE concentra tion and response to treatment [40]. A persistent rise in serum NSE as much as 12 weeks before clinical identification of a relapse has also been demonstrated [34].

Recently, Viallard *et al.* [41] have suggested that the ratio of NSE/NNE (NNE represents the non-neuronal enolase) is a more specific index for SCLC than NSE alone.

CPKBB

In 1981, Gazdar *et al.* [42] showed that both SCLC tumour specimens and their cells in continuous

culture are characterized by high levels of the BB isoenzyme of creatine phosphokinase. Creatine phosphokinase is an enzyme which reversibly catalyses the transfer of a high energy phosphate from creatine phosphate to ADP. CPK levels in clinical specimens and established SCLC cell lines are 10–100 times higher than in either normal lung tissue and non-SCLC tumours or cell cultures. Furthermore, "variant" SCLC cell lines that lose LDDC activity retain high levels of CPKBB [18].

In two recent clinical studies, CPKBB was found to be elevated in about 41% of untreated SCLC patients with extensive disease, but in only 2% of limited disease patients [42, 43]. There was also a correlation between the number of metastatic sites and the level of serum CPKBB. Furthermore, elevated levels of serum CPKBB had a significantly adverse impact on survival, *i.e.* elevated levels at diagnosis were associated with significantly shorter survival times (5 months) compared to patients with normal levels (13 months) [42, 43].

More recently, BORK *et al.* [44] found frequent elevation of CPKBB levels among untreated SCLC patients, with abnormal levels in 82% of extensive disease and 50% of limited disease patients. These differences can be explained by the use of different assays and reference limits.

Recently, CPKBB levels in cerebrospinal fluid (CSF) have been shown to be useful for distinguishing between meningeal carcinomatosis (MC) and parenchymal metastases [45].

Bombesin/GRP

Bombesin (BN) is a 14-amino-acid peptide factor first isolated from the skin of the frog *Bombina bombini* [46]. Gastrin releasing peptide (GRP) is the mammalian counterpart of bombesin, characterized by its ability to release gastrointestinal hormones. A bombesin-like immunoreactivity has been found in normal human tissues including the brain, peripheral nerves and pulmonary endocrine cells of the bronchial tree [47–49].

Bombesin and GRP share homologous carboxy-terminal regions and the same cellular receptor. BN/GRP was found to be mitogenic for 3T3 mouse fibroblasts, normal human bronchial epithelial cells and SCLC cell lines [50–52]. Clinical interest in BN/GRP resides mainly in its potential use as a "growth factor for SCLC" (discussed below), although it appears to be of little use as a serum tumour marker because it is quickly degraded in the peripheral blood [53]. In fact, patients with high amounts of BN/GRP in their tumour tissue have undetectable concentrations in their plasma [49]. Elevated levels of BN/GRP have been found in the CSF of 75% of SCLC patients suffering from meningeal carcinomatosis, regardless of cytologically positive analysis of the cerebrospinal fluid [54]. Recently, PEDERSEN *et al.* [54] showed that by using both calcitonin and BN/GRP, the overall detection rate of patients with central nervous disease approaches 93%.

Therefore, these markers are a useful aid for the diagnosis of CNS metastases in SCLC, while conventional methods are capable of diagnosing approximately 20–50% of CNS dissemination.

Chromogranin A

Chromogranin A is a 68,000 dalton protein found in the neurosecretory granules of normal and malignant APUD cells, including small cell lung carcinomas [55–59]. Elevated concentrations of serum chromogranin A have been detected in 53% of patients with LD and 72% with ED [55].

In the study of SOBOL *et al.* [55], 4 patients originally classified as having non-small cell lung carcinoma with elevated chromogranin A levels were subsequently found to have mixed SCLC and non-SCLC tumours. Further serum radioimmunoassays for this protein in the serum have demonstrated the same results [56].

Several other less specific tumour markers have been suggested for the evaluation of SCLC such as LDH and CEA [60, 61]. The latter is mainly a potential prognosis factor, while SCULIER *et al.* [61] demonstrated a negative correlation between survival time and the serum level of CEA. Finally, BORK *et al.* [44] suggested using a panel of tumour markers.

However, at the present time, the level of NSE in the serum remains the most sensitive and accurate marker for the management of SCLC, with only a small improvement on adding CEA [62].

Monoclonal antibodies

The development of monoclonal antibodies raised considerable enthusiasm about their potential for providing tools for the diagnosis, staging and treatment of lung cancer as well as helping elucidate the origin of this tumour. Currently, more than 100 monoclonal antibodies reacting with SCLC have been reported, but intercomparison of this data is difficult due to the variety of methods and the range of target tissues tested [63, 64]. Therefore, in 1986, SOUHAMI *et al.* [65, 66] organized the first SCLC antigen workshop, modelled after the leucocyte differentiation antigen workshops which have successfully introduced order into the organization of human leucocyte antigens.

Fifty one monoclonal antibodies (MoAbs) were submitted from 17 laboratories studying SCLC. From the results presented, 60% of the submitted MoAbs were classified into six clusters based on their pattern of reactivity:

Cluster 1 included eleven MoAbs and another two associated (1-A) with the cluster. They recognize an antigen of 124–165 kDa which has strong "neural

reactivity" and is expressed on the majority of SCLC cancers as well as neuroblastoma, carcinoid, renal carcinoma, and only weakly on non-SCLC. These antibodies also react with large granular lymphocytes. For example, anti-Leu-7 staining is positive, both on SCLC and on natural killer cells [67].

Cluster 2 contains four monoclonal antibodies recognizing a 35–40 kDa antigen present on many "epithelial" malignancies including SCLC, non-SCLC, and carcinoid tumours but not neuroblastoma.

Cluster 3 antibodies appear to recognize an intracellular antigen, that is associated with proliferation.

Cluster 4 antibodies show wide reactivity with both renal and epithelial tissues, including both SCLC and non-SCLC.

Cluster 5 antibodies stain an antigen of 95–110 kDa, abundant on SCLC, breast adenocarcinomas, melanoma and neuroblastoma.

Cluster 6 antibodies react with selected neural tissue SCLC, non-SCLC and weakly with neuroblastoma and melanoma.

However, no evidence was presented to suggest that these antigens are specific for SCLC, or universally present on all the SCLC specimens examined. This indicates that there is considerable heterogeneity in the expression within and between SCLC tumours [66, 68, 69].

Obviously, the clinical use of monoclonal antibodies against lung tumour antigens is only in its infancy. We will discuss three major applications of MoAb technology in SCLC, including the immunodetection of micrometastases in bone marrow, the expression of histocompatibility locus antigen (HLA) on the SCLC surface and the study of intermediate filament proteins by those tumour cells.

Detection of micrometastases

Detection of SCLC micrometastases in bone marrow specimens and purging contaminated bone marrow of tumour cells was potentially one of the first clinical uses for monoclonal antibodies raised against SCLC.

Our group and others have shown that nearly 30–50% of patients who otherwise have limited disease exhibited bone marrow micrometastases detectable only through immunodetection with one or, better, a panel of anti-SCLC monoclonal antibodies that do not react with haematopoietic cells [70–73]. Although immunodetection with monoclonal antibodies markedly increases the detection of bone marrow micrometastases, particularly in limited disease patients, its prognostic importance is still unknown. However this data could have considerable impact in the management of this group.

Only truly limited disease patients are considered to possibly benefit from either additional local treatment such as radiotherapy or surgery, or from late intensification chemotherapy with autologous bone marrow transplantation (ABMT) rescue [74]. Recently, in a randomized study, our group demonstrated that late intensification chemotherapy with ABMT rescue increased the complete response (CR) rate and resulted in a statistically significant increase in relapse free survival, but had little impact on the overall survival [75]. The reinoculation of clonogenic tumour cells with autologous marrow is one potential cause of relapse. We and others have recently reported successful *in vitro* purging of bone marrow SCLC micrometastases using a panel of complement mediated cytotoxic monoclonal antibodies used with or without cytotoxic drugs [76–80].

Nevertheless, the long-term impact of this purging remains to be defined in prospective clinical studies.

HLA expression

Another class of antigen evaluated in lung cancer cells includes the class I major histocompatibility complex antigens (HLA - A, B, C).

Recently, DOYLE *et al.* [81] demonstrated that HLA class I antigens and beta$_2$-microglobulin are expressed at very low levels in SCLC as opposed to non-SCLC. They demonstrated that the gene coding for class I molecules remains intact but that the corresponding messenger ribonucleic acid (mRNA) is expressed at low levels. However, our group and others have reported that incubation of SCLC cell lines with low doses of gamma interferon lead to the expression of HLA class I antigen at the same level as that expressed on a B cell line [81, 82].

Based on the role that these molecules play in immune recognition, we have shown that SCLC cells can be lysed by allogenic cytolytic T-cells (CTL), provided that they were previously treated with gamma interferon (IFN) [82]. Based on these results, it seems worthwhile to stimulate an autologous CTL response against SCLC cell lines treated with gamma IFN, and to test further the *in vivo* relevance of the immunomodulating effects of gamma IFN in CR SCLC patients. Results obtained with several mouse tumours have shown that tumour specific antigens often fail to elicit a rejection response *in vivo* due to insufficient class I molecule expression [83–85]. By raising the level of class I expression, it becomes possible to elicit an immune response against tumour associated antigen (TAA). Currently, several co-operative groups in the USA are considering randomized trials of recombinant interferon treatment in responding SCLC patients.

Intermediate cell filaments

Another possible application of monoclonal antibodies comes through the large panel of MoAb raised against intermediate filament proteins (IFP).

All nucleated cells have a complex cytoplasmic network of filamentous structures including microfilaments, microtubules and intermediate filaments, which are collectively called the cyto-skeleton.

IFP are unique in that biochemically and immunologically they include a highly heterogeneous family of proteins. Furthermore, their expression is remarkably cell and tissue-type specific, and this specificity is retained after neoplastic transformation [86]. However, because of differences in fixation techniques and antibody selection or specificity, conflicting results were reported for lung cancer. For instance, BERGH *et al.* [86] claimed that SCLC contains neurofilaments while BROERS *et al.* [87] found cytokeratin but not neurofilaments. Others identified both neurofilaments and cytokeratins in the same SCLC cells [88]. BROERS *et al.* [87] went further to show that classical SCLC cell lines contain cytokeratin proteins but not neurofilaments, while variant SCLC cell lines partially express neurofilaments but not cytokeratin [88–90]. Recently, they used a large panel of monospecific anti-cytokeratin antibodies, and claim that it is possible to distinguish between the main subtypes of lung carcinoma as well as to detect the degree and type of heterogeneity within each tumour [91]. For instance, in both SCLC and adenocarcinoma, cytokeratin 18 is preferentially expressed while cytokeratin 10 is detectable in squamous carcinoma only [90].

Taken altogether, these data suggest a common epithelial cell origin for SCLC and are consistent with a common stem cell for all lung cancers (see below). The potential clinical relevance of this immunohistological classification system remains to be established.

At this time, the role of monoclonal antibodies specific for lung cancer remains to be identified and several problems must still be overcome. Problems that could diminish or hamper the effectiveness of monoclonal antibodies include: the presence of free circulating antigen, antigenic modulation, immunogenicity caused by the foreign mouse monoclonal antibodies, the ability of the monoclonal antibody to reach the tumour and the finite capacity of humoral effector cells inside the tumour tissue.

Certainly the use of antibodies coupled to cytotoxic agents, radioactive substances or toxins is now being explored in addition to imaging of SCLC tumours with radiolabelled monoclonal antibodies, and the effectiveness of antibodies against growth factors such as bombesin. Clear therapeutic uses have not emerged for monoclonal antibodies, but the preliminary data merit carefully controlled clinical trials and have strong potential for a role in the future SCLC strategy [92–94].

Autocrine growth factors

The ability of cancer cells to produce, secrete and respond to growth factors has become a central theme in studying the mechanisms of growth regulation in human tumour cells [95]. Furthermore, it is also likely that many oncogenes function in malignant cells by altering the production or receptor binding of autocrine growth factors (see below).

The production of peptides is a hallmark of SCLC [27] and the fact that conditioned medium from established SCLC lines was able to support the growth of SCLC cells in otherwise serum free medium, suggests that SCLC cells secrete self-mitogenic growth factors [9]. Some of them have already been identified and molecularly cloned.

Bombesin/GRP

The predominant member of this group is gastrin releasing peptide (GRP, mammalian bombesin) [96, 97]. Bombesin/GRP fulfils the requirements of an autocrine growth factor for SCLC lines: 1) SCLC secrete GRP into their culture medium [98]; 2) they express high affinity receptors for GRP on their cell surface [98, 99]; and 3) their growth in culture can be stimulated by adding GRP to the culture medium [100, 101].

CUTTITTA *et al.* [53] confirmed these observations by showing that a monoclonal antibody (2A11) with specificity for the carboxy-terminal portion of bombesin blocks the binding of bombesin-GRP to its receptor and inhibits clonal growth of SCLC cells *in vitro*. Also, the growth of some SCLC xenografts in athymic nude mice can be significantly inhibited by this monoclonal antibody [53]. Molecular genetic studies demonstrated that three different mRNAs are produced by the prepro GRP gene, giving rise to three different GRP gene-associated peptides, expressed in SCLC cells and human foetal lung [50, 102].

In addition to its autocrine action, GRP can also stimulate growth of normal bronchial epithelial cells, suggesting a secondary role as a paracrine growth factor [51]. Recently, AGUAYO *et al.* [103] reported high levels of bombesin-like immunoreactivity in the bronchoalveolar lavage of smokers. This observation implies a role for this growth factor in the early pathogenesis of lung cancer.

Insulin-like growth factor I and II (IGF I-II)

The effect of insulin-like growth factor I (IGF I) on the growth of SCLC cell lines has recently been studied. The IGF-I precursor molecule has been detected by Western blot analysis of SCLC medium, by specific IGF-I receptor binding on SCLC cell lines, by IGF-I mediated growth stimulation and by inhibiting basal cell growth with a monoclonal antibody to the IGF-I receptor. Taken altogether, this suggests that an IGF-I like molecule functions as an autocrine growth factor for human SCLC cell lines *in vitro* [104]. Furthermore, IGF-I can be mitogenic for many types of cells, both *in vitro* and *in vivo*, including human tumour cell lines such as cultured human fibroblasts and breast carcinomas.

On the other hand, a related member of the IGF family, IGF-II, binds with lower affinity to the IGF-I

receptor and is therefore less potent in stimulating the biological events mediated through that receptor [104].

Unlike bombesin/GRP, which is not synthesized by fast growing variant SCLC lines, IGF-I is mitogenic for both classic and variant SCLC lines [105]. MACAULY *et al.* [106] measured serum IGF-I concentrations from 42 SCLC patients, and concluded that IGF-I levels do not correlate with tumour bulk, or the therapeutic responsiveness of SCLC patients. This is probably due to low concentrations of this potent peptide being secreted by tumour cells to insure that they have only a local effect (autocrine-paracrine) [106].

Transferrin

Recently, VOSTREIJS *et al.* [107] described an SCLC cell line that produces a transferrin molecule with immunological and biochemical characteristics similar to serum transferrin. This molecule apparently acts as part of an important autocrine mechanism, allowing continued proliferation of the cells in transferrin-free culture conditions. Transferrin is the major iron transport protein and is essential for cellular proliferation [108–110]. The use of specific agents that affect iron metabolism, including MoAb against transferrin or transferrin receptors as well as gallium nitrate, could provide new strategies for the treatment of SCLC.

Physalaemin

BEPLER *et al.* [111] used a soft agarose clonogenic assay to evaluate the influence of several neuro-endocrine peptides (closely related to SCLC cells) on the *in vitro* proliferation of SCLC cell lines. All of them failed to influence SCLC growth except "physalaemin" which inhibits the clonal and mass culture growth of SCLC lines. First isolated in 1962, physalaemin is a peptide of the tachykinin family. It was extracted from the skin of the frog *Physalaemus fuscumaculatus* [112, 113]. This molecule can apparently act as a negative regulatory factor for SCLC cells.

At present, therapies targeted to interfere with growth factor-mediated tumour proliferation are beginning their clinical evaluation. The first anti-growth factor therapies utilizing monoclonal antibodies have recently begun at the National Cancer Institute, and involve a monoclonal antibody to GRP. To date, none of the treated patients have demonstrated a significant tumour response [114, 115]. One explanation could be that a particular tumour produces and responds to more than a single growth factor. This point was recently emphasized by studies on the classical SCLC cell line, NCI H-345. This line has been shown to produce and respond to three different autocrine growth factors, including GRP, insulin-like growth factor, and transferrin [114–116].

The possibility that combinations of molecules mediate the observed autocrine effects has increased the complexity of successful therapeutic intervention [114, 117].

Genetic analysis

Recent advances in molecular biology have allowed the study of molecular events involved in the development of transformed cells. For SCLC, the successful establishment of permanent cell lines has greatly facilitated cytogenetic analysis and uncovered series of genetic aberrations including numerical abnormalities, chromosomal rearrangements, deoxyribonucleic acid (DNA) deletions and gene amplifications.

Analysis of the DNA by flow cytometry has revealed considerable heterogeneity in DNA content, ranging from hypodiploid to tetraploid in most cases. However, unlike other tumours, a correlation has not been shown between DNA content and response to therapy and survival of SCLC patients, with the exception of one study [118–120].

Chromosomal and DNA deletions

Loss of DNA sequences on the short arm of Chr 3 is the most common chromosomal abnormality reported for SCLC [121]. This deletion has been observed by karyotype analysis [122–124] and DNA restriction fragment length polymorphism analysis (RFLP). Although this deletion has variable breakpoints, it usually includes chromosomal bands 3p14–3p23. However, despite the loss of alleles at 3p observed in both classic and variant SCLC cell lines [123] and in tumour samples obtained either before or after chemotherapy [125], it is not always found in all SCLC cell line specimens [126–129]. Further more, 3p deletion has also been observed in other malignancies including non-small cell lung cancer, and sporadic renal carcinomas [125, 130].

The fact that changes in 3p21 (the shortest region of overlap analysis) are found in all types of lung cancer, suggests that this area is central in the development and pathogenesis of lung cancer [121–125]. In addition, increased fragility of this region is seen in the normal cells of cigarette smokers [131].

A deletion can lead to tumorogenesis by one of two mechanisms: 1) a change in the location of a gene so that it becomes adjacent to a strong promotor [132]; or 2) the unmasking of a mutated gene on the allelic chromosome [133, 134]. This finding raises the possibility that the fragments of DNA that are deleted reflect the loss of a putative tumour suppressor gene. It is thought that this suppressor gene, called also "antioncogene" controls other genes which if uninhibited (uncovered by the deletion) may contribute to malignant transformation [135].

Recently, DNA losses have been reported for other chromosomes including Chr 13 - 17 - 11

[136, 137]. For Chr 13, the DNA loss is restricted to the rb locus, suggesting that SCLC have absent or dramatically reduced expression of the rb gene product [138].

Future advances in molecular genetic biology could permit us to correct the defect by introducing into the lung cancer cells a normal copy of the gene [139, 140]. This has already been demonstrated for Wilms tumour and retinoblastoma [141, 142].

Chromosomal and DNA amplification

In addition to chromosomal deletions, double-minute chromosomes (DM) and homogeneous staining regions (HSR) have been observed in both fresh specimens and SCLC cell lines. These cytogenetic abnormalities are thought to represent amplified DNA associated with drug resistance [143] or with the increased expression of specific proto-oncogenes.

It is generally accepted that viral oncogenes arise from a group of normal cellular genes (proto-oncogenes) during the passage of retroviruses through the host cell. Proto-oncogenes play a major role in normal cellular proliferation and differentiation. However, several lines of evidence link proto-oncogenes to the induction or maintenance of cancer [144]. When they are altered by any insult (*e.g.* radiation, chemical carcinogens, chromosomal alterations, point mutation or translocation), the genes become active in the pathogenesis of abnormal cell growth. Currently, more than 40 proto-oncogenes have been identified [145].

Oncogenes from the myc family have been found to change genetically in SCLC tumours, through gene amplification and/or overexpression. The myc family includes a minimum of three closely related genes, called c, N and Lmyc genes, respectively, all encoding nuclear proteins with DNA binding properties involved in the regulation of the cell cycle [146]. These oncogenes have been mapped to chromosome 8 for c-myc, Chr 2 for N-myc and very near or within band p32 on Chr 1 for L-myc [147–150].

The c-myc amplification has been described for a variety of cancer cell lines and tumour specimens including leukaemia, colon cancer, breast cancer, gastric cancer, glioblastoma, small and non-small cell lung cancer [151]. The c-myc amplification has been found in SCLC cell lines with the variant phenotype only [18, 19, 21, 152]. The c-myc amplification is also more common in tumour cell lines established from treated relapsing patient tumours rather than from untreated patient tumours, 44% to 11%, respectively [153]. Furthermore, c-myc amplification found in treated patient tumour cell lines was associated with shortened median survival, 33 weeks *versus* 53 weeks, respectively [153].

To investigate whether or not the amplified c-myc gene directly causes the "variant" phenotype, with its more aggressive behaviour, JOHNSON *et al.* [154] transfected a classical SCLC cell line with an amplified c-myc gene. The c-myc transfectant acquired some characteristics of the variant phenotype including resistance to radiation, growth and morphological changes, but did not lose neuroendocrine markers like L-Dopa decarboxylase or bombesin/GRP immunoreactivity.

On the other hand, the N-myc oncogene has been shown to be amplified in tumours and cell lines with neuroendocrine phenotypes, such as neuroblastoma, retinoblastoma, SCLC and more recently in a primary adenocarcinoma with some neuroendocrine properties [155, 156]. In contrast to c-myc, N-myc amplification was demonstrated in both classic and variant SCLC cell lines [156]. FUNA *et al.* [157], used *in situ* hybridization to demonstrate that increased expression of N-myc in primary biopsies from 15 untreated SCLC patients was strongly associated with poor response to chemotherapy, rapid tumour growth and short survival. They suggested that SCLC patients with increased N-myc expression in their primary biopsies should be considered for more aggressive therapy due to the more aggressive phenotype of these tumours. WONG *et al.* [155], studying both the N-myc and c-myc gene amplification in fresh human tumour specimens, demonstrated that patients did not display both N and c-myc overexpression in the same tumour, suggesting that expression of these oncogenes is independent.

Finally, a more recent study identified a third member of the myc family, called "L"-myc because of its initial discovery in a lung cancer [150]. Its overall structure appears similar to the c and N-myc genes. NAU *et al.* [150], screening SCLC cell lines with an L-myc specific probe, demonstrated amplification and overexpression of the oncogene; however, some non-SCLC were also shown to express L-myc although there was no apparent amplification [138]. Recently, certain L-myc haplotypes have been implicated in the metastatic behaviour of lung cancer of several histological types [158].

Given the structural and sequence homology between the three members of the myc gene, it is likely that they have similar functions in lung cancer cells. GEMMA *et al.* [159] demonstrated that lung cancers with myc family gene amplification were more tumorogenic in nude mice than lung cancer cells without this abnormality. Furthermore, myc-family oncogene abnormalities are more frequently observed in cell lines compared with fresh biopsy specimens, and in both groups are usually observed in specimens obtained at relapse from prior therapy. These data suggest that myc family oncogenes may contribute to the more aggressive growth behaviour observed at relapse but are not important in early events in the pathogenesis of SCLC [160–161].

Finally, GRIFFIN and BAYLIN [162] recently reported that the oncogene c-myb is differentially expressed in SCLC, emphasizing the increasing evidence that cell transformation may require two or more oncogenes acting in cancer [163]. Based on this evidence, we must consider that new prevention and treatment

strategies should be directed against the deregulated expression of the SCLC nuclear proto-oncogenes.

Although not immediately notable, new insights into the pathogenesis of lung cancer resulting from the discovery of these "cellular proto-oncogenes", should ultimately help in the treatment of SCLC patients. In addition, study of alterations in proto-oncogenes may aid in the (sub)classification of lung cancer and yield new prognostic information [151].

Histogenesis of SCLC

In the past, it has been proposed that SCLC lung carcinoma was of neural crest origin and belonged to the APUD system of endocrine cells [164] which accounted for the many differences observed both clinically and biologically in the behaviour of SCLC and non-SCLC lung cancer [56]. More recently, based on the positive staining of two SCLC cell lines with macrophage-associated antibodies, RUFF and PERT suggested that SCLC arises from bone marrow macrophages [165]. However, these hypotheses have been challenged by substantial data suggesting that lung cancer consists of a continuum of cancer types, and that the different histologically recognizable groups represent preferentially expressed differentiation pathways originating from a common endodermal stem cell [24, 166]. In fact, it is now generally accepted that lung cancer is a heterogeneous cancer in which the incidence of different subtypes is the rule rather than the exception. This is especially true when extensive sampling of the entire tumour is studied instead of only a small biopsy specimen [167, 168].

Using conventional light microscopic analysis, about 15% of SCLC tumours contain non-SCLC subtypes at diagnosis whereas 13 to 28% of autopsy specimens from SCLC patients previously treated by cytotoxic therapy demonstrate a mixed histology [168–172]. GAZDAR *et al.* [24] reported that 6% of the untreated primaries displayed histologically mixed characteristics; however, following therapy, 39% of the cases revealed histological features that differed markedly from those of their own primaries. Furthermore, morphological transition between SCLC to non-SCLC may occur *in vitro* where tripartite differentiation has been reported [7, 173]. TERASAKI *et al.* [174] recently reported that changes of the culture medium may, in some cases, induce changes in SCLC morphology from small cells to squamous cells and *vice versa*. In addition, some SCLC cell lines may lose their neuroendocrine features over time [17, 18].

At this time, the widespread development of immunohistological analysis using poly- and monoclonal antibodies, as well as ultrastructural analysis, has revealed many hitherto unsuspected relationships between SCLC and non-SCLC lung tumours [175, 176]:

1) A dual ancestry for SCLC was suggested by ultrastructural analysis; on the one hand findings of neuro-microtubules and neuroendocrine granules indicated neural characteristics, and on the other hand, the presence of tonofibrils, desmosomes and junctional complexes indicated epithelial features [8]. In another ultrastructural study, GOULD *et al.* [177] reported convincing evidence of squamous and glandular differentiation in about 30% of previously untreated surgically removed neuroendocrine carcinomas of all types.

2) The reactivity of SCLC with antibodies to epithelial-related antigen was similar to that found in non-SCLC [178, 179].

3) The neuroendocrine (NE) differentiation state of SCLC tends to disappear during the course of the disease, whereas the epithelial differentiation state remains expressed [179].

4) The predominant intermediate filament protein in SCLC is "cytokeratin", which is a common feature of epithelial cells [87, 180].

5) Conversely, neuroendocrine differentiation is demonstrated using immunohistological markers, in up to 20% of both primary tumours and cell lines from non-SCLC subtypes [181]. However, NE markers were most commonly expressed in adeno- and large-cell carcinomas and rarely in squamous cell carcinoma.

6) Finally, embryological studies strongly suggest that the bronchial mucosa is composed entirely of cells of endodermal origin [182].

7) Genetic studies using c-DNA probes specific for cytogenetic deletions demonstrated that the 3p 14–23 deletion previously related with SCLC only is common to most lung cell types [125].

At this time, further studies are underway for clinicopathological evaluation of the relevance of a common stem cell ancestry. For instance, if SCLC has a mixed histology, cytotoxic therapy could eliminate the bulk of sensitive SCLC cells leaving behind the few insensitive non-SCLC cells which could then be removed by surgery. Indeed, recent data indicated a 71% three year survival for this subgroup of patients [183]. In any event, the possibility of morphological transition toward the non-SCLC subtype underscores the need for new biopsies of all patients relapsing in their primary SCLC tumour. One other clinically relevant aspect of a common stem cell ancestry comes from recent studies suggesting that non-SCLC sharing NE features has the same behaviour as SCLC, including a responsiveness to chemotherapy [181, 184, 185].

Finally, it has been shown that lung cancer can be subtyped not only by histological criteria but also by immunohistological and ultrastructural analyses. A new classification may eventually emerge from the application of these new techniques, particularly for adeno- and large-cell undifferentiated subtypes [181, 186]. However, the clinical relevance of any new classification must be carefully established. Recently changing concepts and terminology have led to a new histopathological classification for SCLC as proposed by the IASLC (International Association for Study

of Lung Cancer) [187]. Indeed, the following classification for SCLC is now recommended:
1) Small cell lung cancer includes small cell and intermediate subtypes and represents more than 90% of untreated SCLC.
2) Mixed small cell/large cell carcinoma. This subtype may be associated with a poor prognosis and response to therapy and contains a spectrum of cell types ranging from typical SCLC to larger cells resembling large cell carcinoma.
3) Combined small cell carcinoma, where typical SCLC elements are intimately mixed with areas of differentiated squamous cells or adenocarcinoma.

Conclusions

We have discussed the advances in understanding the biology of SCLC lung cancer and their progress over the past decade. However, all of this information, including biomarkers, genetic abnormalities, antigen expression, as well as the concept of lung tumour heterogeneity has yet to be applied in the clinic in careful and well planned studies for defining new treatment strategies for small cell lung carcinoma. The information derived from biological studies appears to be the most promising avenue toward more effective control of small cell lung carcinoma [5].

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Biologie du cancer pulmonaire à petites cellules. Revue générale. P. Weynants, Y. Humblet, J.L. Canon, M. Symann.

RÉSUMÉ: Malgré les résultats décourageants du traitement du cancer pulmonaire à petites cellules, les progrès importants dans notre connaissance de sa biologie ont été réalisés pendant la dernière décennie. Les progrès dans l'obtention des lignes cellulaires de SCLC, ont facilité largement par l'étude des propriétés biologiques de cette tumeur. Les progrès principaux dans notre compréhension du cancer à petites cellules comportent: 1) l'obtention de marqueurs tumorau biologiques non spécifiques, comme la NSE, CPKBB, bombesin/GRP et chromogranin A; 2) la production d'anticorps monoclonaux contre les aspects neuraux et épithéliaux des tumeurs à petites cellules; 3) l'identification de plusieurs facteurs de croissance autocrine, comme la bombesin/GRP, le facteur de croissance insulin-like (IGF), la transferrine et la physalaemine; 4) l'étude approfondie des anomalies cytogénétiques conduisant à la découverte d'une délétion chromosomique spécifique du bras court du chromosome 3 (del 3p 14–21), et à celle des modifications de l'expression oncogénique, par exemple c-myc, L-myc et N-myc, rendant compte des résultats connus dans le domaine biologique et celui du traitement. Ces données suggèrent que tous les cancers pulmonaires proviennent d'une cellule souche commune d'origine endodermique. Les informations provenant de ces études biologiques représentent la voie la plus prometteuse vers de nouvelles stratégies de traitement du cancer pulmonaire à petites cellules.
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