Clin Transl Oncol (2008) 10:189-197
DOI 10.1007/s12094-008-0181-1

EDUCATIONAL SERIES Blue Series*

# Genetic changes in small cell lung carcinoma

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Received: 12 January 2008 / Accepted: 6 February 2008

**Abstract** Small cell lung carcinoma (SCLC) accounts for approximately 15% of all lung cancer cases. Despite a frequently good response to first-line treatment with chemotherapy and/or radiotherapy, early relapse occurs in the majority of patients and 5-year survival is only about 5%. Therefore, there is a need to develop novel treatments to improve the outcome of patients with SCLC. To fulfil this need, it is critical to gain further understanding on the molecular basis of SCLC and specifically to identify novel therapeutic targets. Clinical trials with molecularly targeted agents have been performed with little success in the past, but recently many promising oncogenic pathways have been discovered and novel targeted therapies are under evaluation. In this review, we summarise the most relevant genetic and signalling pathway alterations reported to date in SCLC and discuss the potential therapeutic implications of such events.

**Keywords** Small cell lung carcinoma · Genetic changes · Expression profiles · Oncogenic pathways

*Supported by an unrestricted educational grant from GlaxoSmithKline.

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## Introduction

Small cell lung carcinoma (SCLC) accounts for approximately 15% of all lung carcinomas [1]. This histological subtype of lung cancer is strongly associated with tobacco smoking. The incidence of SCLC has decreased slightly in recent years, probably related to the decrease of the number of smokers and to changes in the characteristics of cigarettes [2].

The behaviour of SCLC is unique within solid tumours. Initially, it exquisitely responds to chemotherapy or radiotherapy. However, at relapse, which occurs early in the majority of cases, the tumour is resistant to available therapy and eventually will cause the death of the patient [3]. This results in an overall 5-year survival of approximately 5% for the whole population of patients diagnosed with SCLC [4]. This dismal prognosis has not significantly changed in past years.

In an attempt to better understand the biology of this disease and to find potentially relevant alterations to be targeted by new agents, many studies focusing on genetic alterations of these tumours have been performed. As a result, multiple genetic changes have been implicated in the development of SCLC, but unfortunately, to date, these findings have not been translated into a clinical benefit for patients.

**Table 1** Recurrent genomic imbalances in SCLC

| | Chr. arm | Region | Genes | Prevalence | Ref. |
|---|---|---|---|---|---|
| Losses | 3p | 3p21.3 | *RASSF1* | 80–90% | [96] |
| | | 3p14.2 | *FHIT* | | [97] |
| | 4q | 4q32–35 | *PDGFC* | 60–70% | [10, 12] |
| | 5q | 5q13-21 | P85α PI3K, *APC* | 90% | [5] |
| | 10q | 10q24-26 | *PTEN* | 60–70% | [10, 12] |
| | | | *TNFRSF6* | | [15] |
| | 13q | 13q14.2 | RB1 | 78%–90% | [5, 7] |
| | 16q | _ | _ | 60–70% | [10, 12] |
| | 17p | 17p13 | TP53 | 70% | [8] |
| Gains | 1p | 1p34.2 | MYCLTNFRSF4 | _ | [12, 98] |
| | | | 1p36 | | |
| | 2p | 2p24.3 | MYCN | _ | [12, 98] |
| | 3q | 3q26-29 | NLGN1, CLDN1 | 60–70% | [98] |
| | 5p | 5p12-13 | NNT, FGF10 | 60–70% | [98] |
| | 8q | 8q24.1 | MYC | 40% | [12, 99] |
| | 19q | 19q13.1 | PDCD5 | 40% | [12] |
| | 20q11.21 | | BCL2L1 | | [15] |
| | dmin | _ | MYC | 10% | [99, 100] |
| | hsr | _ | MYC | _ | [99, 100] |

*Chr*, chromosome; *Ref*, reference; *dmin*, double minutes; *hsr*, homogeneously staining regions

In this article, we sought to review the current knowledge on genetic changes that might be of relevance in the biology of SCLC and that offer novel potential anticancer targets.

## Cytogenetic changes and genomic copy number variations

The analysis of karyotypic changes in SCLC has revealed a number of recurrent copy number alterations that are implicated in the development of this tumour [5, 6]. These changes are usually numerous and involve regions encompassing genes described as oncogenes and tumour suppressor genes (TSG) [7, 8]. The use of comparative genomic hybridisation (CGH), arrayCGH technology and allelotyping studies has further refined the regions of loss and gain in these tumours and interesting genes have emerged in these studies as potential therapeutic targets [9–15].

Table 1 summarises the most frequent copy number changes found in SCLC studies. The cited studies have evaluated fresh tumour samples in some cases, cell lines in others or both sample types. Consistent genetic gains and losses are found in these studies irrespective of the sample type. As shown in Table 1, SCLC is characterised by multiple genetic gains and losses, which are found quite commonly in this disease. It is interesting to note that regions containing *TP53* and *RB* genes known to act as TSGs in many tumours are often lost in SCLCs. The loss of function of these TSGs has been described in other tumour types but it is particularly prevalent in SCLC. Moreover, these alterations are exclusive to cancer cells, when compared to other tumours. Thus, they can be used as tumour-specific "markers". Generating compounds that target these frequent and specific alterations can be effective in the treatment of cancer cells, avoiding toxicity in normal tissue. An example of this therapeutic approach will be discussed below for p53.

Furthermore, amplification of several members of the *MYC* family (*MYCN, MYCL1, MYC*) is quite common in SCLC [15]. Interestingly, overexpression of these oncoproteins is more common in chemorefractory disease [16]. A comprehensive analysis of *MYC* amplification in SCLC cell lines and their expression profiles identified a set of genes whose expression is correlated with *MYC* amplification, but with little overlap within the different members of the *MYC* family (*MYCN, MYCL1, MYC*). The dysregulated genes in *MYC* amplified samples were involved in regulation of apoptosis, and the authors conclude that this might be a relevant mechanism in the pathogenesis of SCLC mediated by *MYC* amplification. Other genes of interest encompassed in the consistently lost regions are, for instance, *PTEN* (10q24-26), PI3K (5q13-21) [17]. This is translated into dysregulation of the pathways regulated by these gene products [18, 19]. Therapeutic implications of these changes will be discussed below.

In addition to the genetic alterations present at diagnosis of SCLC, treatment with chemotherapy and radiotherapy may also induce genetic changes, which provide a survival advantage for subclones of the tumour. This has been demonstrated in SCLC cell line studies [20–23]. These changes occur and are clearly represented by the dramatic differences on the karyotypes observed in stably chemoresistant SCLC cell lines vs. the parental cells, as shown in Figs. 1 and 2. The specific genetic changes that participate in the resistant phenotype of SCLC human tumour samples

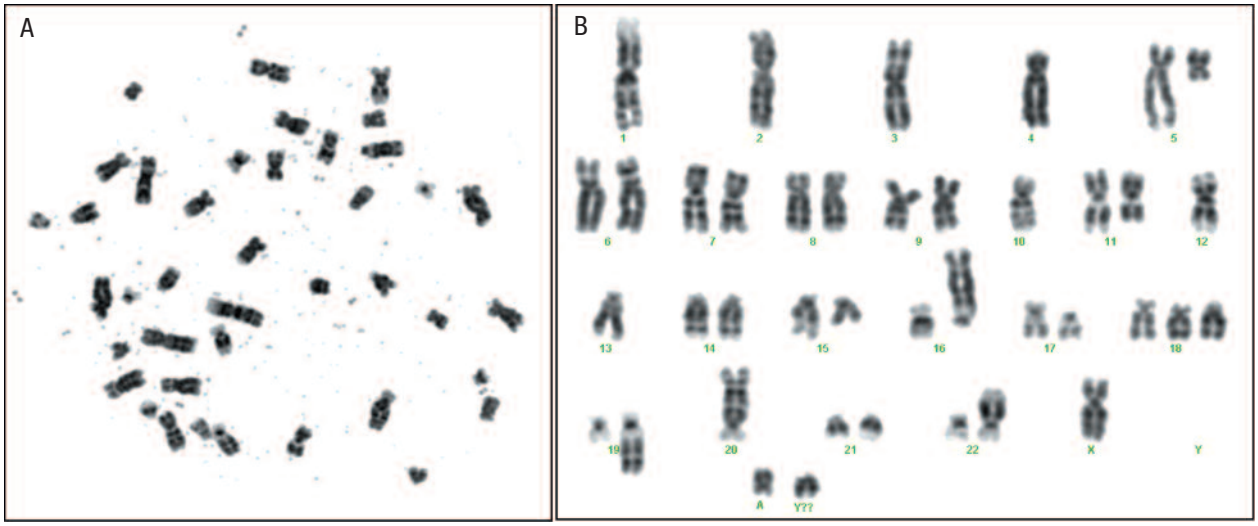


**Fig. 1** Karyotype of the H69 SCLC cell line (sensitive to adryamicin)
**A**: G- banding stained metaphase from H69 cell line presenting double minutes (dmins); the arrow indicates one dmin. **B**: Karyotype of this cell line; ISCN 2005 formulation:40,XY,-1,-2,-3,-4,del(5)(q13),-10,del(11)(q23),-12,add(12)(p13),-13, del(15)(q22),der(16)t(5;16)(q11;p13)del(16)(q12),del(17)(p13),+der(18), add(19)(q13),-20,der(20)t(1;20)(q21;p13),der(22) t(12;22)(p11;q11),+2mar,+3-100dmins [cp20]

remain to be identified. Specific approaches against these genetic alterations might represent an additional tool in the treatment of chemorefractory disease.

## Gene expression profiles

To better define the genetic changes that occur in SCLC, several works evaluating the expression profiles of SCLC have been reported [24–29]. These studies have used different expression microarray platforms (cDNA and oligonucleotide arrays). They analyse a combination of non-small-cell lung cancer (NSCLC) samples, usually low numbers of SCLC samples along with cell lines or cell lines only. Despite these limitations, interesting conclusions can be drawn from the data.

One common finding is that SCLC samples (tumours and cell lines) cluster together in hierarchical clustering analysis and can clearly be distinguished from NSCLC

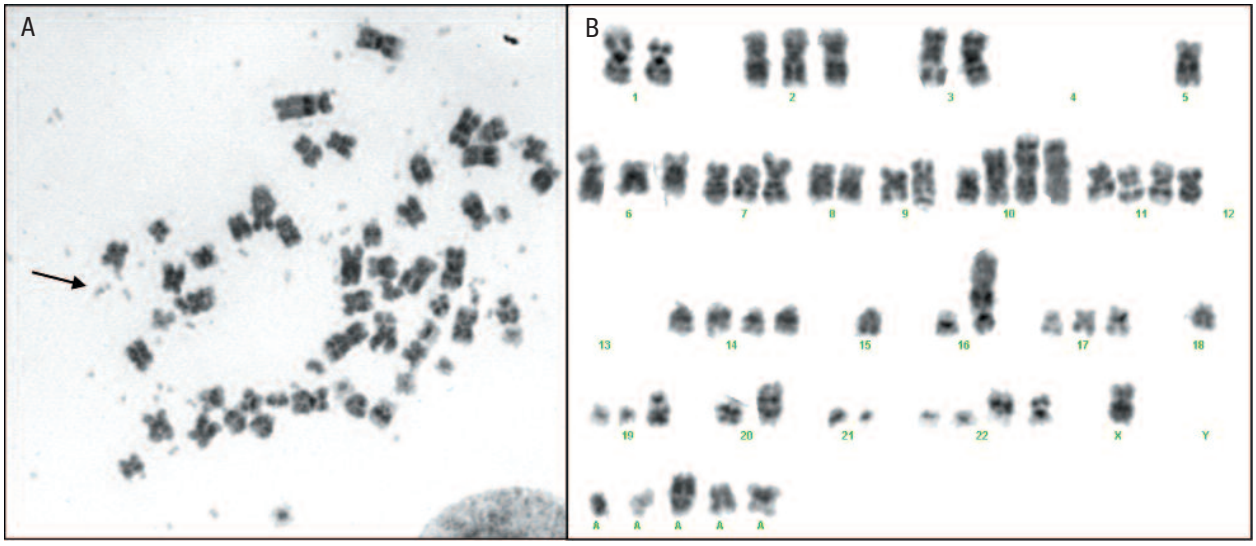


**Fig. 2** Karyotype of the H69AR (adryamicin resistant) SCLC cell line
**A**: G- banding stained metaphase from H69AR cell line presenting double minutes (dmins); the arrow indicates one dmin. **B**: Example of a karyotype of this cell line;ISCN 2005 formulation:50-60,XY,der(X),der(1)t(1;12)(p12;q11),+2,der(3),der(4), der(5),+6,+7, add(7)(p22),add(7)(q22),der(9)t(9;21)(q34;?),der(10)t(4;10)(q21;p11)x2,del(11)(q23),-12,add(15)(p11),der(16)add(16)(p13), +17,del(17)(p13),+der(18), der(19)add(19)(q13),20,der(20)t(1;20)(q21;p13),der(22)t(12;22)(p11;q11),+5-6mar,+3-100dmins [cp20]

samples. Therefore, SCLC can be considered a distinct entity reproducing the currently used histopathological classification. Moreover, as demonstrated by Virtanen et al. [29] and Pedersen et al. [26], although cell lines are not a perfect model for the study of human tumours, gene expression profiles of the SCLC cell lines reproduce the expression profiles obtained from tumour samples quite reliably. However, the authors observed a large number of genes consistently overexpressed by cell lines but not by the tumours. Therefore, adequate data filtering is necessary to focus further studies on clinically cancer-relevant gene products.

In these studies [24, 27–29], although very few samples were analysed, overlapping overexpressed genes were reported. These are summarised in Table 2. Highlighted in bold are the genes that are overexpressed in at least 2 studies. Interestingly, the achaete scute homologous protein (*ASCL1*) and the insulinoma associated 1 (*INSM1* or *IA-1*) genes are upregulated in 4/5 studies. These genes could be used for diagnostic purposes as they are neuroendocrine markers [30, 31], but there are data showing that they may also play a role in the progression of tumours, and therefore could also be used as therapeutic targets [32, 33]. Other consistently overexpressed novel genes whose implication in tumour development is unknown are KIAA0282 and Forkhead box G1B. The role of these genes in SCLC remains to be defined.

Another consistent finding in these studies is that SCLC is characterised by overexpression of neuroendocrine genes. Of note, SCLC samples cluster together with typical and atypical carcinoids, and large cell neuroendocrine carcinomas (LCNEC), and separately from NSCLC in hierarchical clustering analysis [28]. These reports define sets of genes differentially expressed between neuroendocrine tumours and NSCLCs.

A more detailed analysis of the data reveals subgroups within the neuroendocrine tumours. Typical carcinoid tumours, for instance, although clustered more closely to high-grade neuroendocrine tumours (HGNT) than to normal tissue or NSCLCs [29], belong to an independent branch and overexpress a set of genes that may account for their distinct (more benign) behaviour in the clinical setting. These genes are largely neuronal differentiation markers (i.e., neurofascin) [28].

Furthermore, these expression profile analyses define gene sets that are able to separate HGNT according to prognosis. The study by Jones et al. [28] defines two prognostically different subtypes of HGNT with SCLC and LCNEC samples distributed within both groups. Interestingly, cell lines had overexpression of the genes that were overexpressed in the poor prognosis subgroup. However, paradoxically, these "poor prognosis" genes were also upregulated in typical carcinoid tumours. The authors therefore suggest that these genes are not the direct cause of the poor prognosis. Moreover, the genes upregulated in the different expression profiling studies do not correspond to the copy number changes described above. These results underscore the relevance of pursuing validation of the multiple genes that are highlighted in the microarray studies, of which many will end up being non-crucial for the oncogenic process. No prospective studies have been reported evaluating the prognostic significance of these sets of genes for SCLC.

## MicroRNAs

MicroRNAs (miRNA) are small non-coding RNAs that regulate diverse biological processes. Mutations or misexpressions of miRNA have recently been associated with cancer and its prognosis (reviewed in [34]). These studies indicate that miRNAs can act as oncogenes or TSGs. Several publications have evaluated the role of miRNAs in NSCLC, showing a potential prognostic value for miRNAs in these tumours [35, 36].

As an emerging field in cancer research, few publications have addressed the role of miRNAs in SCLCs yet. A work by Hayashita et al. [37] demonstrated that overexpression of miR-17-92 cluster, located in intron 3 of the C13orf25 gene at 13q31.3, may play a role in the development of lung cancers, in particular, SCLC [37]. Other reports [38, 39] have suggested a potential oncogenic role of other miRNAs in SCLC, but this remains to be validated in the clinic. This approach seems to be complementary to the previous studies with expression arrays and may provide additional information to improve the treatment of patients with SCLC.

## Oncogenic pathways

Finally, the study of the key features in malignant transformation and the associated signalling pathways has resulted in the characterisation of genes with a relevant oncogenic role in SCLC. Whether targeting these pathways will result in clinical benefit is now being evaluated. Here, we briefly discuss the most relevant pathways that are dysregulated in SCLC and whose clinical development seems more promising.

### Insulin-like growth factor-I receptor (IGF-IR)

Activation of this receptor tyrosine kinase (RTK) induces tumorigenesis and proliferation. This pathway has been implicated in the development and growth of SCLC [40, 41]. SCLC cell lines show overexpression of IGF-I and its receptor, suggesting a role for inhibition of these molecules as a therapeutic approach [42, 43]. Treatment of these cell lines with targeted inhibitors against IGF-IR (NVP-ADW742) leads to apoptosis and has shown highly specific and potent antitumour activity [44, 45]. This approach may translate into clinical activity and trials are ongoing.

**Table 2** Genes overexpressed in SCLC tumours and cell lines in expression profile studies

| Pedersen et al. (26) | Bhattacharjee et al. (27) | Sugita et al. (24) | Garber et al. (25) | Virtanen et al. (29) |
|---|---|---|---|---|
| Neutral amino acid transporter B | Tubulin beta polypeptide | **ASCL1** | **IA-1** | Fibroblast growth factor 12 |
| KIAA0120, thiopurine S-methyltransferase (TPMT) | **Insulinoma associated 1 (IA-1)** | Chromogranin B | **ASCL1** | Sex determining region Y-box 4 (SRY) |
| Hypothetical protein FLJ12443 | Extra spindle poles, yeast homologue | Chromogranin C | Tyrosine phosphatase receptor N2 | Catenin delta 2 |
| Thyroid transcription factor 1 (TTF1) | Core-binding factor, alpha subunit 2 | Dopa decarboxylase | Amyloid beta A4 precursor-like 1 | PDZ domain protein |
| DEAK/H (DDX11) | Guanine nucleotide binding protein 4 | **IA-1** | Glutaminyl cyclase | Reticulon 1 |
| Sodium channel, nonvoltage-gated 1 alpha (SCNN1A) | **ASCL1 1** | **KIAA0280** | 7B2 protein | DnaJ(Hsp 40) homologue, subfamily C, member 6 |
| Hepatoma-derived growth factor (HDGF) | CDKN2C (p18) | MAGE-A2 | **ISL1 transcription factor** | Internexin neuronal intermediate filament protein alpha |
| Nuclear receptor co-repressor N-Cor (NCOR1) | **Forkhead box G1B** | MAGE-A3 | **Forkhead box G1B** | **IA-1** |
| **Achaete scute homologous protein (ASCL1)** | **Thymosin beta, neuroblastoma** | MAGE-A6 | KIAA0805 gene product | Kinesin family member SC |
| Extra spindle poles like 1 (ESPL1) | **ISL1 transcription factor** | MAGE-A10 | Neuronal protein | Hypothetical protein FLJ23468 |
| Gastrin-releasing peptide (GRP) | Distal-less homeo box 6 | MAGE-A12 | **Thymosin beta** | Deoxycytidine kinase |
| Uncoupling protein homologue (UCP2) | Transcription factor 12 (HTF4) | Sodium-potassium ATPase | KIAA1051 protein | Kinesin heavy chain member 2 |
| Cell division cycle 25 B (CDC25B) | PC4 and SFRS1 interacting protein 2 | NEFL | Imprinted in Prader Willi syndrome | Minichromosome maintenance deficient 3 and 4 |
| Inhibitor of DNA binding 2, dominant negative helix-loop-helix (ID2) | | NY-ESO-1 | Hypothetical protein FLJ21935 | CDC7-like 1 |
| Neurite growth-promoting factor 2 (MDK) | | | v-myc viral oncogene homologue 1 | Uracil-DNA glycosylase |
| Programmed cell death 6 (PDCD6) | | | Neuronal cell adhesion molecule | Leukaemia-associated phosphoprotein p18 |
| Immunoglobulin superfamily 4 (IGSF4) | | | **KIAA0282** | High-motility group protein 17 |
| Cofilin 1 (CFL1) | | | Hypothalamus protein HBEX2 | |
| Calmodulin 1 (CALM1) | | | Lim homeobox protein 2 | |
| Tyrosine 3-/triptophan 5-monooxygenase activation protein zeta (YWHAZ) | | | Internexin neuronal filament | |
| Nuclear receptor subfamily 2, group F, member 1 (NR2F1) | | | | |
| Sex determining region Y-box 2 (SOX2) | | | | |
| Exostoses (multiple)-like 3 (EXTL3) | | | | |
| Inositol polyphosphate-5 phosphatase-like 1 (INPPL1) | | | | |
| Tripartite motif-containing 28 (TRIM28) | | | | |
| Tyrosine phosphatase type IVA, member 3 (PTP4A3) | | | | |
| VEFG related factor isoform VRF186 (VEGFB) | | | | |
| Transport-secretion protein 2.2 (TTS-2.2) | | | | |
| Lysophosphatidic acid acyltranferase alpha (AGPAT1) | | | | |
| Protective protein of beta-galactosidase (PPGB) | | | | |

In bold, genes overexpressed in more than one study

### C-KIT

The *c-Kit* gene encodes a RTK of the platelet-derived growth factor (PDGF). Although up to 70% of SCLCs express *c-Kit* and its ligand, the stem cell factor (SCF) establishing autocrine regulation [46–48], imatinib (small molecule inhibitor against *c-kit*), has failed to show clinical activity in these tumours in the studies reported to date. Probably, activating mutations of this pathway are required to cause oncogenic "addiction" for survival [49]. Only in these cases, as described in gastrointestinal stromal tumours, inhibition of this pathway results in dramatic clinical benefit. However, there seems to be a subpopulation of SCLC cell lines, with an activated SCF/*c-kit* autocrine loop, where imatinib, in combination with the above-mentioned IGF-IR inhibitors, may play a therapeutic role [45]. This deserves further studies, to better characterise the role of *c-kit* in SCLC treatment.

### Angiogenesis: VEGFR, FGFR

SCLC is a highly angiogenic tumour. Pretreatment high vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) serum levels have been associated with poor prognosis and lower response rates to conventional chemotherapy [50–53]. The successful activity of antiangiogenic therapy in other tumours [54] and preclinical activity in SCLC cell lines and xenografts [55] has prompted evaluation of these drugs in SCLC patients. Unfortunately, recent results of a phase III trial evaluating the effect of the antiangiogenic drug thalidomide in combination with standard chemotherapy have failed to show a benefit from this drug [56]. Moreover, vandetanib, an inhibitor of vascular endothelial and epidermal growth factor receptors, tested in patients with SCLC that responded to initial therapy, has shown no benefit as maintenance therapy [57]. Other antiangiogenic compounds are under evaluation.

### Epidermal growth factor receptor (EGFR)

EGFR-activating mutations and amplifications have been described as an oncogenic pathway in lung cancer. Selective inhibition of this molecule has led to the successful treatment of patients with EGFR-mutated NSCLCs. Studies on patients with SCLC have demonstrated mutations of EGFR and clinical response to gefitinib (an orally available EGFR inhibitor) [58, 59]. However, a phase II trial of patients with SCLC treated with gefitinib failed to demonstrate benefit from this drug [60]. This might be because EGFR mutations are infrequent in the general population of SCLC but when present, EGFR inhibitors may play a relevant role.

### C-MET

*c-Met* is an oncogene that, when activated by its natural ligand, the hepatocyte growth factor (HGF), initiates downstream signalling that leads to increased proliferation, motility and invasion [61]. *c-Met* is overexpressed in SCLC and a number of gain-of-function mutations have been described in cell lines and tumour samples [62, 63]. The inhibition of the pathway with small molecule inhibitors has demonstrated decreased proliferation and invasiveness and increased apoptosis in cell lines and xenografts [64–66]. These encouraging preclinical results have led to the development of ongoing clinical trials.

### PI3K/Akt/mTOR and PTEN

The activation of this pathway results in increased proliferation and survival of malignant cells [67, 68]. SCLC samples show expression of phosphorylated Akt in a high percentage [18, 19]. Preclinical studies have shown that inhibition of this pathway can induce apoptosis and also leads to reversion of resistance to chemotherapy in SCLC cell lines [69–71]. *PTEN*, a negative regulator of this pathway, is frequently altered in SCLC. Loss of the genomic region encompassing *PTEN* is prevalent in these tumours and loss of the *PTEN* gene can be found in approximately 10% in SCLC [17]. Moreover, mutations of *PTEN* have also been identified in a small subset of SCLC tumours (2–4%) [17]. This would lead to activation of this pathway and therefore targeting it with specific inhibitors could be a promising approach to the treatment of SCLC. Nevertheless, hitherto, clinical trials have failed to demonstrate survival benefit with inhibitors of this pathway, but additional studies are ongoing.

### BCL-2

*Bcl-2* is a member of the antiapoptotic family of proteins involved in tumour development. SCLC with upregulation of *Bcl-2* presents a phenotype resistant to chemotherapy [72]. Overexpression of this protein has been demonstrated in most SCLC cell lines and tumours [73, 74]. In addition, treatment with *Bcl-2* antisense oligonucleotides has emerged as a promising strategy for SCLC patients [75–77]. A recent report testing the potential benefit of oblimersen has failed to demonstrate a survival gain with the addition of this agent to conventional chemotherapy [78].

### Gastrin-releasing peptide (GRP)

As previously described in the microarray data, *GRP* is overexpressed in SCLC cell lines [26] and *GRP* receptor is expressed in a high percentage of SCLCs [79]. This autocrine activation loop results in the activation of proliferation pathways and angiogenesis [80, 81]. Inhibitors of this pathway have been evaluated and have shown activity in SCLC [82, 83]. These early results need to be further explored.

DNA topoisomerase II α

Topoisomerase II alpha (*TOP2A*) inhibitors, doxorubicin and etoposide, are part of standard chemotherapy in the treatment of SCLC. *TOP2A* amplification seems to be predictive of benefit of treatment with anthracyclines in other tumours [84]. The expression of the protein was an unfavourable prognostic factor in a study of SCLC patients treated with *TOP2A* inhibitors [85]. The role of *TOP2A* in SCLC remains unclear, and it is not used as a prognostic/predictive marker in the clinic. A new synthetic anthracycline, amrubicin, has shown interesting activity in the treatment of sensitive and resistant SCLC [86, 87]. However, the status of *TOP2A* has not been correlated with the activity of amrubicin in SCLC trials. Studies in cell lines indicate high levels of expression of *TOP2A* in chemosensitive SCLC cell lines (H69) and lower levels in resistant isogenic cell lines (H69AR) [21]. These findings are in contrast to the high percentage of responses of chemorefractory SCLC to amrubicin (up to 50%). This partial non-cross-resistance with other *TOP2A* inhibitors might be explained by additional mechanisms of action attributed to amrubicin. Amrubicin demonstrated the ability for DNA–protein complex formation, inhibition of cell growth and the induction of double-stranded DNA breaks in cell cultures [88]. The activity of this drug in sensitive and refractory patients and its lack of cardiotoxicity makes it an interesting option for the treatment of SCLC patients.

CD56

CD56 is encoded by the neural cell adhesion molecule (*NCAM*) [89]. *NCAM* is expressed in a high percentage of SCLC and CD56 is used as an immunohistochemical marker for the diagnosis of this tumour [90]. The specificity of this marker has led to the development of targeted therapies directed against CD56 (BB10901). Early trials with this compound have demonstrated clinical activity and it is being further evaluated.

TP53

This gene is located on the short arm of chromosome 17 and encodes a 53-Kd protein that regulates cell cycle in response to genomic damage, allowing repair or otherwise inducing apoptosis. Mutations on this *TP53* are highly prevalent in SCLC, being detected in approximately 75–90% of cases [16]. A comprehensive study of loss of heterozygosity and mutations on neuroendocrine tumours demonstrates that there was an increasing incidence of loss of heterozygosity and mutations on *TP53*, with increasing severity of the histopathologic subtype of neuroendocrine (from typical carcinoid to SCLC), reaching 90% prevalence in SCLC [91]. The higher expression of p53 in SCLC cells enables its use as a marker of tumour cells. Vaccines constructed to induce p53-specific immune response [92, 93] are being evaluated in early clinical trials.

Retinoblastoma (RB)

The protein encoded by this gene is involved on the regulation of the G1-S cell cycle checkpoint. SCLC commonly presents alterations on the retinoblastoma gene product (RB) in up to 90% of cases [94]. These alterations consist of decreased expression of the protein due to mutations, gene deletions or rearrangements. Despite the high prevalence of RB alterations in SCLC, specific treatments have not been developed in the clinical setting to date.

## Discussion

Patients with SCLC generally have a very poor prognosis. The lack of effective therapies for resistant disease underscores the need for intensive research in this field. To date, studies with rational targeted therapies have been unsuccessful, but new promising targeted agents are under evaluation and may change the scenario for SCLC patients in the near future. One of the main difficulties in performing translational research with these tumours is the lack of sufficient tissue sample at diagnosis and the difficulty in obtaining enough material for biomarker research. For SCLC, cell lines seem to be an acceptable preclinical model, but still, findings need to be validated in patients. It is therefore important to share efforts in the collection and study of these samples.

One of the most intriguing issues in SCLC remains the different behaviour of initial and relapsed disease. The study of mechanisms of resistance is mandatory, as the selection of this resistant component after initial treatment represents a challenge for clinicians in the treatment of patients. Whether this chemorefractory phenotype is caused by primary resistance, in the form of cancer stem cells (underrepresented in primary tumour) [95] or instead, mechanisms of resistance are acquired and/or induced by therapy [20-23] needs to be elucidated.

Cytogenetic alterations and expression array studies demonstrate the high number of genetic changes that characterise SCLCs and may explain its aggressive clinical behaviour. These high-resolution high-throughput tools are adequate for screening a large area of the genome of these tumours. However, many of these changes are not reproduced by additional studies and may therefore be spurious findings. For this reason, the selection of biologically relevant genes or pathways that are consistently highlighted in these works seems to be the better approach in the use of the immense amount of information provided by these techniques. Moreover, further validation in patients' samples is mandatory. The example of *Bcl-2* illustrates this concept.

One of the consistent findings in the cited papers is the gain and upregulation of *BCL2* in SCLC. The genetic gain is translated into overexpression of the protein and the activation of the pathway [15, 73, 74]. Although a robust background supports the development of a targeted drug against this gene, recent studies targeting this pathway have failed to demonstrate a survival gain for SCLC patients [78].

Rational development of targeted therapy is crucial for the success of the treatments. The identification of these relevant genetic changes with translation into activation of an oncogenic pathway will help researchers and clinicians to develop strategies for the treatment of this disease.

**Acknowledgements** The authors thank Dr. Francisco Solé and Marta Salido from the Cytogenetic and Molecular Biology Laboratory in Hospital del Mar for kindly providing the figures of the H69/AR karyotypes.

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