Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin

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Abstract

Lung cancer is the most common cause of cancer deaths worldwide. The two broad histological subtypes of lung cancer are small-cell lung cancer (SCLC), which is the cause of 15% of cases, and non-small-cell lung cancer (NSCLC), which accounts for 85% of cases and includes adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. Although NSCLC and SCLC are commonly thought to be different diseases owing to their distinct biology and genomic abnormalities, the idea that these malignant disorders might share common cells of origin has been gaining support. This idea has been supported by the unexpected findings that a subset of NSCLCs with mutated EGFR return as SCLC when resistance to EGFR tyrosine kinase inhibitors develops. Additionally, other case reports have described the coexistence of NSCLC and SCLC, further challenging the commonly accepted view of their distinct lineages. Here, we summarise the published clinical observations and biology underlying tumours with combined SCLC and NSCLC histology and cancers that transform from adenocarcinoma to SCLC. We also discuss pre-

clinical studies pointing to common potential cells of origin, and speculate how the distinct paths of differentiation are determined by the genomics of each disease.

Introduction

Lung cancer is the leading cause of cancer death in both men and women worldwide. WHO classifies lung cancer into two broad histological subtypes: non-small-cell lung cancer (NSCLC) which is the cause of about 85% of cases, and small-cell lung cancer (SCLC), which accounts for the remaining 15%. NSCLC is further subdivided into adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. The approval by the US Food and Drug Administration to introduce targeted therapies against EGFR, such as erlotinib and afatinib, has revolutionised the treatment of a subset of adenocarcinomas that have *EGFR*-activating mutations;[1–6] however, acquired resistance develops after about 12 months.[7,8] Repeat biopsy samples from patients with *EGFR*-mutant cancers have shown that several mechanisms bring about the acquired resistance, including the rare but consistent observation of histological transformation from adenocarcinoma to SCLC.[9,10] Transformation to SCLC suggests that both adenocarcinoma and SCLC arise from a common cell type. Here, we explore the cellular and molecular relationship of adenocarcinoma to SCLC by discussing the clinical experience of combined-histology tumours and transformation from adenocarcinoma to SCLC.

Combined SCLC and NSCLC histology

Treatment strategies for lung cancer are based on the assumption that an individual patient's cancer is purely of one subtype.[11,12] For example, limited-stage SCLC and localised NSCLC are both potentially curable diseases, but their treatment strategies differ substantially.[11,13,14] Surgery has been largely abandoned as a treatment modality in patients with limited-stage SCLC, which is now treated with concurrent chemotherapy and radiotherapy.[11] By contrast, patients with localised stage I or II NSCLC are treated mainly with surgery possibly followed by adjuvant chemotherapy, although radiotherapy is typically not part of the strategy.[15] In advanced stage lung cancer, chemo therapy is the mainstay of treatment for both NSCLC and SCLC but the drugs used are distinct for each subtype. The initial response to chemotherapy is much greater for patients with extensive-stage SCLC than patients with metastatic adeno carcinoma,[11,12] which suggests inherent differences in tumour biology. For example, pemetrexed is one of the most active agents in meta static adeno-carcinoma,[12] but it is fairly inactive in extensive-stage SCLC.[16] This difference could result from the high levels of thymidylate synthase in patients with SCLC,[17] which can cause resistance to pemetrexed.[18]

In practice, combined-histology lung cancers can make treatment decisions difficult. SCLC with a large-cell component accounts for about 10% of SCLC cases; at present, these patients are given standard SCLC chemo-therapy regimens.[11,14] Two large case series have investigated the frequency of tumours with combined SCLC and NSCLC histology.[19,20] In the first study,[19] 176 SCLC tumours were analysed and 17 (10%) tumours also had an NSCLC component. Seven of these patients who had originally been diagnosed with NSCLC and given chemotherapy or radiotherapy were found to have combined histology at

surgery or autopsy. Additionally, in autopsies of 40 patients originally diagnosed with SCLC, two were reported to have only adenocarcinoma. In the second case series,[20] the histology of 429 SCLC tumours was studied; nine (2%) tumours contained NSCLC (six adenocarcinoma, three squamous-cell carcinoma). Repeat biopsy samples from six of these nine patients showed one dominant histology (three SCLC, two adenocarcinoma, one squamous-cell carcinoma). Although one possible ex planation for these results is the transformation from one phenotype to another, another possibility is that core biopsy samples or fine-needle aspirates used to make the initial diagnosis did not provide sufficient pathological material to determine the presence of combined histology to be identified at diagnosis. Whether the results from these autopsy studies represent transformation or com bined-histology tumours, these striking observations pose several practical questions that could affect clinical practice. First, in localised, early-stage, combined-histology tumours, how does the presence of adenocarcinoma affect the decision on whether to offer surgery? Alternatively, should patients with tumours that have an SCLC component always be given concurrent chemotherapy and radio therapy? Furthermore, in an advanced-stage setting, if tumours do not respond as initially expected or if the responses among two or more different lesions are discordant, is repeat biopsy indicated to rule out a dominant histology that was not identified at initial diagnosis?

EGFR-mutant adenocarcinoma and transformation to SCLC

Three EGFR inhibitors—erlotinib, gefitinib, and afatinib—are now used worldwide for first-line treatment of lung cancers that have *EGFR*-activating mutations, and their use has greatly changed clinical practice. However, within an average time of 12 months,[7] resistance develops, and samples from repeat biopsies have shown several distinct mechanisms of acquired resistance to EGFR inhibitors. The most common resistance mechanism is a Thr790Met mutation in *EGFR*, which increases the affinity of the receptor for ATP and allows for continued EGFR signalling in the presence of the inhibitor.[21] Thr790Met mutations are reported in 50–60% of samples from resistant tumours.[9,10] Mechanisms that bypass the requirement for EGFR signalling, such as *MET* and *HER2* amplification, could account for another 15–20% of resistance to EGFR inhibitors.[9,10,22]

Another mechanism of resistance to EGFR inhibitors is histological transformation of *EGFR*-mutant adeno-carcinoma to SCLC. This process was first described in 2006 in a 45-year-old woman with *EGFR*-mutant adenocarcinoma who had never smoked; she was originally diagnosed with adenocarcinoma and given erlotinib.[23] The tumour responded for 18 months, but at the time of progression, the repeat biopsy sample showed SCLC with the original *EGFR* exon 19 deletion mutation. Since this first case, several other case series have been reported of *EGFR*-mutant adenocarcinoma transforming to SCLC as a mechanism of resistance to EGFR-inhibitor treatment (table 1).[9,10,23–28] In all reported cases, small-cell tumours were identified by morphology and positive immunohistochemical staining for synaptophysin, chromogranin, or NCAM. In a case series in which repeat biopsies were done when resistance developed in patients given EGFR inhibitors,[9] transformation to SCLC was reported in 14% of cases. In another case series, trans formation to SCLC was apparent in less than 5% of repeat biopsy samples.[10] Genomic sequencing of *EGFR* from both the original and repeat biopsy samples at the time of resistance shows that every

transformed SCLC tumour sample retained its original *EGFR*-activating mutation (table 1),[9] which suggests that these were not independent de-novo cancers, but a transformed phenotype as a mechanism of resistance to treatment instead. Another hypothesis for this observation is that patients had tumours with combined histology at the time of initial diagnosis, which was not apparent on the diagnostic biopsy sample, and then the SCLC component became dominant as the adenocarcinoma component was successfully treated with the EGFR inhibitor. However, the clinical course of the reported cases has not typically been consistent with this hypothesis because most patients responded well to EGFR inhibitors for several months to years (table 1)[9] and clinically had greater tumour growth at the time when SCLC was diagnosed. If tumour heterogeneity accounted for the development of SCLC in these patients, a less dramatic response to EGFR inhibitors and acquired resistance at an earlier time would be expected.

Does transformation to SCLC result solely from EGFR inhibition, or does *EGFR*-mutant adenocarcinoma have the potential to transform into SCLC? Furthermore, are adenocarcinomas that do not have *EGFR* mutations similarly able to transform to SCLC? Synchronous development of adenocarcinoma and SCLC was noted in *EGFR*-mutant tumours before treatment with an EGFR inhibitor.[26] This observation suggests that the development of SCLC in *EGFR*-mutant cancers is not exclusively the result of EGFR inhibition. Furthermore, this series[26] included two cases of adenocarcinoma that transformed to SCLC in tumours that did not have *EGFR* driver mutations in the original biopsy sample; this finding suggests that transformation can occur independently of *EGFR* mutational status. This was unlikely to be the result of false-negative EGFR testing because in both cases the specimens tested were resected tumours and one patient was reported to have a *KRAS* mutation. In a case series reported in 1986, before the discovery of *EGFR*-activating mutations, when resistance to conventional chemotherapy or radiotherapy developed, about 5% of patients originally diagnosed with NSCLC had SCLC at relapse.[19] Whether the tumours of these patients did have *EGFR*-activating mutations is unknown, but the patients did show transformation to SCLC independently of EGFR inhibition. In their 2011 study, Sequist and colleagues[9] found no cases of transformation to SCLC in 79 surgical specimens from patients with stage III NSCLC who received chemotherapy and radiotherapy,[9] which suggests that *EGFR*-wild-type NSCLC is less likely to transform to SCLC than *EGFR*-mutant NSCLC. Larger studies will be needed to identify the precise frequencies of SCLC transformation in *EGFR*-mutant and *EGFR*-wild-type NSCLC, but the information available at present suggests that transformation to SCLC is more common in *EGFR*-mutant cancers treated with EGFR inhibitors than in wild-type cancers.

EGFR mutations in de-novo SCLC

Two large case series[29,30] have investigated whether *EGFR* mutations occur de novo in classic SCLC (table 2).[26,29–35] In the series reported by Tatematsu and colleagues,[30] five of 122 patients had *EGFR*-mutated SCLC. These patients had a history of heavy smoking (average of 30 pack-years), but a significantly lower pack-year smoking history than that of patients who did not have *EGFR* mutations. Three of the five patients with *EGFR*-mutant SCLC had combined small-cell and adeno carcinoma histology. Two of the patients, both of whom had *EGFR* amplification, were given gefitinib and responded to treatment. One

responder had combined SCLC and adenocarcinoma with *EGFR* amplification reported only in the adenocarcinoma component. The other responder had small-cell histology with *EGFR* amplification and had a rare Gly719Ala *EGFR* mutation. Of note is another case report of a patient with de-novo SCLC with an *EGFR*-activating mutation that did respond to gefitinib.[31] Immunohistochemistry showed that this patient had high expression of EGFR. In another large case series from Taiwan,[29] 76 patients with SCLC were analysed for the presence of *EGFR* mutations. Two patients had *EGFR* mutations, who did not have previously known *EGFR*-mutant adenocarcinoma. One patient was treated with gefitinib and showed no response to treatment.[29]

In a phase 2 trial,[36] responses to gefitinib were tested in 19 patients with SCLC previously given chemotherapy. The participants were not tested specifically for *EGFR* mutations.[29,30] Only two of the patients had stable disease and there were no partial or complete responses.[36] Analysis of *EGFR*-mutant tumours that transformed to SCLC from adenocarcinoma as a mechanism of acquired resistance shows that, at transformation, the cancers lose protein expression of EGFR and have lower levels of *EGFR* amplification.[37] In the past, SCLC was reported to have lower protein expression of EGFR than NSCLC.[38,39] The mechanisms underlying low expression of EGFR in SCLC remain unknown, but this could explain the absence of response to EGFR inhibitors in several case reports.[23,28,29] Overall, response to EGFR inhibitors in SCLC patients with *EGFR* mutations do not seem to match those of *EGFR*-mutant adenocarcinoma, perhaps because of mechanisms that suppress EGFR protein expression in these tumours. However, a case report by Araki and colleagues[31] suggests that patients can have *EGFR*-mutant SCLC that does express EGFR and responds to EGFR inhibition. In view of individual case reports of patients with rare SCLC with *EGFR* amplification, larger studies will be needed to correlate protein expression with EGFR inhibition to find out whether other patients with SCLC could benefit from EGFR inhibition.

Genomic analysis of SCLC and adenocarcinoma

Genomic analyses have improved understanding of the pathways that are dysregulated in each specific tumour type. These efforts aim to identify oncogenic driver mutations, copy number changes, and translocations that can be targeted therapeutically. In adenocarcinoma, these sequencing efforts have resulted in the identification of several mutated oncogenic drivers and tumour suppressor genes and have validated the high prevalence of *KRAS* and *EGFR* mutations.[40] In clinical practice, the standard of care is to genotype every patient with a new diagnosis of metastatic adenocarcinoma for *EGFR* mutations and *ALK* translocations, since targeted therapies are now approved in cancers that have these abnormalities.[7,41,42] *EGFR* mutations and *ALK* translocations are estimated to account for about 30–50% of adenocarcinomas in patients who have never smoked, and the proportion might vary according to race.[43,44] Additionally, many centres now undertake multiplexed genetic analyses to identify the presence of other prevalent genetic abnormalities, since therapeutics are under development for those cancers also.[45]

Two large-scale genome-sequencing projects have been completed in SCLC.[46,47] They included whole-genome, transcriptome, exome, and copy-number analyses. Both studies

identified a high prevalence of mutations in *TP53* and *RB1,* validating previous human sequencing studies. Indeed, mouse models of SCLC have been developed by conditional inactivation of *Tp53* and *Rb1*.[48] Although some findings overlapped between these two studies, several findings were unique to each study, perhaps because of the heterogeneous patient samples in terms of stage and exposure to previous treatment. Known oncogenic drivers reported in adenocarcinoma, such as *EGFR* or *KRAS*, were not statistically significantly mutated in either SCLC genome-sequencing study. Of note, *MYC* amplification was seen in 16% of cases.[46] *MYCL1* knock-down resulted in decreased cell proliferation in SCLC cell lines,[47] which suggests that *MYC* functions as an oncogenic driver in a subset of small-cell tumours. Activators of ERK signalling, such as *EGFR* and *KRAS*, are mutated more commonly in adenocarcinoma. By contrast, *RB1* loss seems to be much more common in SCLC.[46–48] Together these genomic efforts suggest that fundamental genetic differences drive the development of SCLC and lung adenocarcinoma.

RB1 inactivation as a defining feature of SCLC

Inactivation of *RB1* has long been known to have an important role in the tumorigenesis of SCLC. In 2003, a mouse model of SCLC was developed by means of conditional knockout of *Tp53* and *Rb1*.[48] Further studies with this model have shown that *Rb1* inactivation is essential for the development of SCLC. Mutation or loss of *RB1* has been found in 100% of the human SCLC tumours sequenced.[46] Furthermore, a proteomic study that compared SCLC and NSCLC cell lines showed that RB1 was quantitatively the most downregulated protein in SCLC compared with NSCLC.[17] However, mice with loss of *Rb1* alone do not develop small-cell tumours, which suggests that loss of *Rb1* is necessary but not sufficient for SCLC tumorigenesis. Analyses of repeat biopsy samples from patients with *EGFR*-mutant adenocarcinoma that transform to SCLC revealed that 100% of these patients have loss of *RB1*;[37] loss of *RB1* seems to be a universal event leading to transformation from adenocarcinoma to SCLC.

Prostate cancer is another example of a cancer in which small-cell differentiation can occur after treatment. In one case series, 90% of small-cell prostate tumours had loss of *RB1*.[49] Furthermore, loss of *RB1* selectively occurred in castration-resistant prostate cancer, which suggests that loss of *RB1* is a late event in tumorigenesis.[50] *RB1* loss is well known to be frequent in many non-neuroendocrine tumour types also.[51] Why neuroendocrine differentiation occurs only in some tumour types with loss of *RB1* is not known. Evidence from animal models supports a role for loss of *Rb1* selectively affecting tumours of neuroendocrine origin. For example, *Rb1*+/− mice form pituitary and thyroid tumours after loss of the wild-type allele.[52] Additionally, conditional inactivation of *Rb1* in lung tissue results in increased numbers of pulmonary neuroendocrine cells during development.[53] The three cancers with the highest frequency of *RB1* mutations or deletions are SCLC, prostate cancer, and bladder cancer.[51] Interestingly, both prostate and bladder cancers are known to undergo small-cell differentiation.[49,54] Additional mechanistic insight into the role of *RB1* loss in transformation to SCLC will help elucidate the biology of SCLC, how transformation from adenocarcinoma to SCLC occurs, and possibly help create new therapeutic targets to treat SCLC.

Adenocarcinoma and SCLC cells of origin

The appreciation that some adenocarcinomas and SCLC have the plasticity to switch histologies raises the possibility of the existence of shared cells of origin and greater plasticity than originally appreciated. Historically, SCLC was thought to arise from neuroendocrine cells within the distal part of the conducting airway because SCLC expresses neuroendocrine markers and typically develops in a central location. By contrast, adenocarcinomas are more commonly localised peripherally and were believed to originate from alveolar type II cells. However, studies in mouse models of lung cancer have yielded insights that could explain the shared ancestry of some SCLCs and NSCLCs.

Four studies have used cell-type specific Cre-recombinase conditional knockout mice to identify the cell of origin of SCLC[55,56] and *KRAS* mutant adeno carcinoma[57,58] in murine models of lung cancer. Two independent groups crossed Cre-recombinase under the control of cell-type specific promoters with mice that had floxed alleles of *Tp53* and *Rb1*,[48] a previously established SCLC model.[48] Small-cell tumours were identified by morphological appearance and positive immuno histochemical staining for synaptophysin or NCAM.[55,56] These groups noted that the CGRP promoter, which is specifically active in neuroendocrine cells, gave rise to SCLCs.[55,56] The proportion of mice in which SCLC formed was similar for those expressing Cre-recombinase under the CGRP promoter or under the CMV promoter, which targets all cells in the lung; this finding suggests that small-cell tumours are mainly derived from neuroendocrine cells.[55] Similarly, two additional studies showed that targeted disruption of *Tp53* and *Rb1* in neuroendocrine cells yields SCLC.[56,59] These results were as expected. However, Sutherland and colleagues[55] also used Ad5-SPC-Cre selectively to target deletion in alveolar type II cells. Surprisingly, they reported that targeted disruption of *Tp53* and *Rb1* in alveolar type II cells led to the development of SCLC. By contrast, Park and colleagues[56] did not note the formation of SCLC in mice when they used an ER-inducible Ad-SPC-Cre to drive acute deletion of *Tp53* and *Rb1* on administration of tamoxifen in alveolar type II cells. One explanation for this discrepancy is that the mice developed by Sutherland and colleagues had loss of *Tp53* and *Rb1* in all alveolar type II cells throughout lung development. Thus, alveolar type II cells might also have the potential to give rise to SCLC, albeit at a much lower frequency than for neuroendocrine cells[55] (figure 1). Future studies are needed to establish the capacity for alveolar type II cells to give rise to SCLC and to reconcile the differences between the two mouse studies.[55,56]

Two studies that used the same principles of cell-type specific promoters in mouse models of *KRAS* mutant adenocarcinoma found that both clara cells and alveolar type II cells could give rise to adenocarcinoma (figure 1).[57,58] Furthermore, in transgenic mice, expression of the *EGFR* L858R mutation under the control of the SPC promoter led to tumorigenesis,[60] which suggests that alveolar type II cells can also serve as the cell of origin of *EGFR*-mutant adenocarcinoma. Together, these studies[55,57,58,60] suggest that alveolar type II cells can give rise to both adenocarcinoma and SCLC (figure 1). Thus, alveolar type II cells seem to have the capacity to develop both SCLC[55] and *EGFR*-mutant adenocarcinoma,[57,58] and the range of genetic mutations is likely to affect the type of cancer that develops. Thus, *EGFR*-mutant lung cancers that develop in alveolar type II cells might have the potential to transform into SCLC.

In fact, activation of EGFR signalling could be impor tant for the fully differentiated alveolar-cell phenotype. Transcriptome analyses show that well differentiated alveolar type II cells have high expression of EGFR family members including EGFR, Erbb2, and Erbb3. Alveolar-cell proliferation was inhibited by EGFR-blocking antibodies.[61] Furthermore, *EGFR* knockout mice have low expression of SPC and have defects in alveolarisation; these features support a crucial role for EGFR in the development of alveolar type II cells.[62,63] Thus, we speculate that the presence of the *EGFR* mutation and active EGFR signalling drive both proliferation and differentiation of type II alveolar cells. In fact, this characteristic might be why *EGFR*-mutant lung cancers tend to be well differentiated adenocarcinomas.[64] However, EGFR tyrosine kinase inhibitors might block this proliferation and differentiation. When additional key genetic events such as *RB1* inactivation occur, these same alveolar type II cells might subsequently transform to SCLC and become independent of EGFR signalling (figure 2). However, if other paths of resistance develop instead, such as an *EGFR* Thr790Met gatekeeper mutation, EGFR signalling is restored and the resistant cells resume adenocarcinoma histology.

Conclusions

Current understanding suggests that both combined-histology tumours and transformation are more common in lung cancers that have *EGFR*-activating mutations than in *EGFR*-wild-type tumours. The basis could be that the cell of origin of some *EGFR*-mutant adenocarcinomas, type II alveolar cells, also have the potential to become SCLC. Many examples used in this review reflect experiences with *EGFR*-mutant cancer, but the possibility remains that this clinical experience is biased by a greater number of repeat biopsy samples in *EGFR*-mutant cancers than in non-*EGFR*-mutant adenocarcinoma because biopsy at progression in NSCLC is not generally indicated. However, other cancers arising from type II alveolar cells could have the capacity to transform into SCLC, and transformation from adenocarcinoma to SCLC has occasionally been observed in lung cancers that do not have *EGFR* mutations.[26] These preliminary observations raise some practical considerations for care of patients. Do specific circumstances exist under which a repeat biopsy should be done on non-*EGFR* mutant adenocarcinomas to look for transformation to SCLC? The frequency of documented transformation from adeno-carcinoma to SCLC in non-*EGFR*-mutant cancers is low at present. Future studies might help define which subsets of NSCLC are most prone to SCLC transformation.

We have also discussed case series showing that, although *EGFR* mutations are identified in SCLC, these patients have had mixed responses to EGFR inhibitors.[29] The mixed responses could be due to loss of EGFR expression at the protein level, as shown in transformation to SCLC after development of resistance to an EGFR inhibitor.[37] Thus, on the basis of available evidence, we believe that patients with SCLC combined with adenocarcinoma and SCLC transformed from adenocarcinoma should be given standard therapies for SCLC.[14] Future studies will be needed to elucidate whether measurement of EGFR protein expression in *EGFR*-mutant SCLC might identify rare cases that would still respond to EGFR inhibitors.[31]

Acknowledgments

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Search strategy and selection criteria

We used the following search terms in PubMed and selected articles on the basis of relevance to transformation from adenocarcinoma to SCLC: "adenocarcinoma to small cell transformation", "*EGFR* mutant lung cancer and mechanisms of resistance", "cell of origin of small cell lung cancer", "cell of origin of adenocarcinoma, genomics and small cell lung cancer", and "combined histology small cell lung cancer". All dates and languages were included in the search.

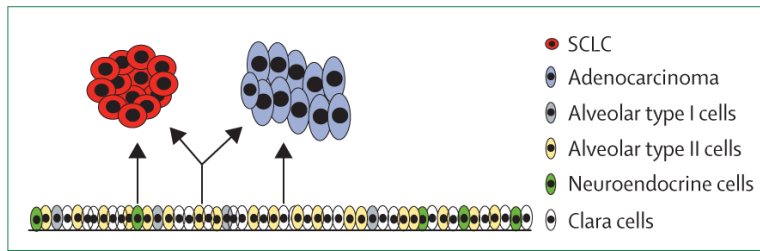**Figure 1. Alveolar type II cells could be a common precursor that can give rise to both adenocarcinoma and small-cell lung cancer (SCLC)**

Diagram depicts the cells of origin of adenocarcinoma and SCLC. Neuroendocrine cells and possibly alveolar type II cells can give rise to SCLC (left and centre), whereas clara cells and alveolar type II cells can give rise to adenocarcinoma (right and centre).

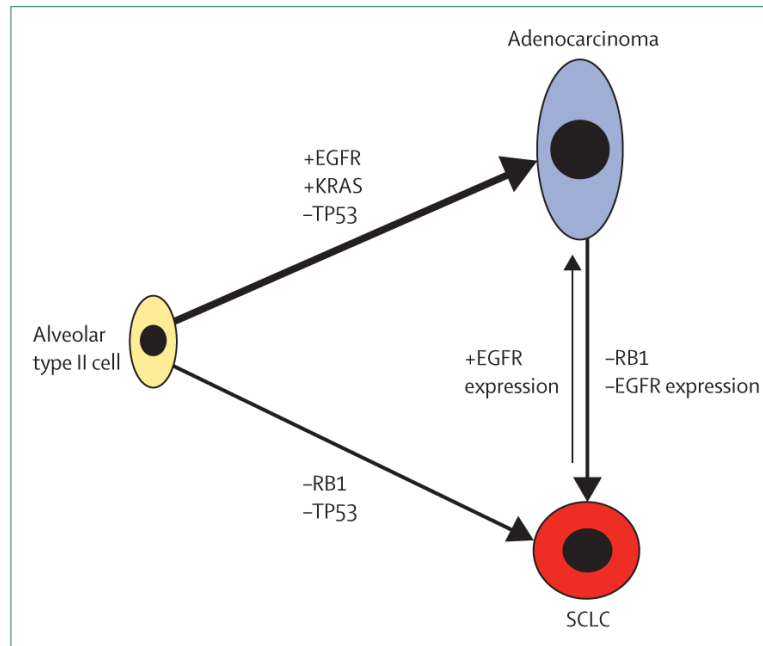**Figure 2. Hypothetical model depicting the molecular events that lead to transformation from adenocarcinoma to small-cell lung cancer (SCLC)**

Alveolar type II cells have the potential to form both adenocarcinoma and SCLC depending on the mutational status of key oncogenes and tumour suppressors. Transformation from adenocarcinoma to SCLC involves the loss of *RB1* and loss of EGFR protein expression.

Table 1

Reported cases of transformation from *EGFR*-mutant adenocarcinoma to SCLC as a mechanism of resistance to EGFR inhibition

	Age (years)	Sex	Smoking history (pack years)	Tumour histology	*EGFR* mutation in initial sample	Treatment	Duration of TKI treatment before transformation (months)	*EGFR* mutation in SCLC	EGFR protein expression in SCLC	Treatment of SCLC with TKI alone
Zakowski and colleagues[23]	45	F	0	Adenocarcinoma	Not tested	Erlotinib	18	Exon 19 deletion	Not tested	Gefitinib (no response)
Sequist and colleagues[9]										
Patient 1	40	F	0	Adenocarcinoma	Exon 19 deletion	Erlotinib	38	Exon 19 deletion	Not tested	No
Patient 2	67	F	0	Adenocarcinoma	Leu858Arg	Erlotinib	22	L858R	Not tested	No
Patient 3	54	F	25	Adenocarcinoma	Exon 19 deletion	Erlotinib	35	Exon 19 deletion	Not tested	No
Patient 4	56	F	0	Adenocarcinoma	Leu858Arg	Erlotinib	14	L858R	Not tested	No
Patient 5	61	F	80	Adenocarcinoma	Leu858Arg	Erlotinib	18	L858R	Not tested	No
van Riel and colleagues[24]	42	F	0	Adenocarcinoma	Not tested	Erlotinib	11	Exon 19 deletion	Not tested	No
Morinaga and colleagues[25]	46	F	0	Adenocarcinoma	Not tested	Gefitinib	10	Exon 19 deletion	Not tested	No
Norkowski and colleagues[26]										
Patient 1	60	F	0	Adenocarcinoma	Exon 19 deletion	Erlotinib	31	E782K	Not tested	No
Patient 2	50	F	0	Adenocarcinoma	Exon 19 deletion	Erlotinib	14	Exon 19 deletion	Not tested	No
Watanabe and colleagues[27]	52	F	0	Adenocarcinoma	Exon 19 deletion	Erlotinib	11	Exon 19 deletion	Not tested	No
Popat and colleagues[28]	46	F	0	Adenocarcinoma	Exon 19 deletion	Erlotinib	12	Exon 19 deletion	Not tested	Afatinib (no response)

Rows are individual patients. TKI=tyrosine kinase inhibitor. SCLC=small-cell lung cancer.

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Table 2

Cases of de-novo SCLC with *EGFR* mutations

	Age (years)	Sex	Smoking history (pack years)	Tumour histology	*EGFR* mutation in SCLC initial sample	Treatment with TKI	Response to TKI	EGFR amplification or protein expression in SCLC
Shiao and colleagues[29]								
Patient 1	54	F	0	SCLC	Exon 19 deletion	Gefitinib	Progressed	Unknown
Patient 2	63	M	Heavy smoker	SCLC	Exon 19 deletion	No	NA	Unknown
Tatematsu and colleagues[30]								
Patient 1	36	F	0	Combined SCLC/adenocarcinoma	Leu858Arg	Gefitinib	Partial response	Amplification of EGFR in adenocarcinoma only
Patient 2	81	M	40	SCLC	Gly719Ala	Gefitinib	Partial response	Amplification of EGFR in SCLC
Patient 3	69	M	30	Combined SCLC/adenocarcinoma	Leu858Arg	No	NA	Amplification of EGFR in adenocarcinoma only
Patient 4	89	F	2·5	SCLC	Leu858Arg	No	NA	No EGFR amplification
Patient 5	65	M	67·5	Combined SCLC/adenocarcinoma	Exon 19 deletion	No	NA	Amplification of EGFR in adenocarcinoma only
Araki, Okamoto, and colleagues[31,32]	72	F	0	SCLC	Exon 19 deletion	Gefitinib	Partial response	Immunohistochemistry positive for EGFR protein expression
Norkowski and colleagues[26]								
Patient 1	62	M	0	Combined SCLC/adenocarcinoma	Gly719Ala and exon 21 deletion found in both SCLC and adenocarcinoma	No	NA	Unknown
Patient 2	66	F	80	Combined SCLC/adenocarcinoma	Exon 19 deletion in adenocarcinoma component only	No	NA	NA
Patient 3	65	F	45	Synchronous SCLC and adenocarcinoma	Leu858Arg in adenocarcinoma component only	No	NA	NA
Fukui and colleagues[33]	62	F	0	Combined SCLC/adenocarcinoma	Leu858Arg in both SCLC and adenocarcinoma	No	NA	Unknown
Lu and colleagues[34]								
Patient 1	62	F	0	Combined SCLC/adenocarcinoma	Exon 19 deletion in both SCLC and adenocarcinoma	No	NA	Unknown

	Age (years)	Sex	Smoking history (pack years)	Tumour histology	*EGFR* mutation in SCLC initial sample	Treatment with TKI	Response to TKI	EGFR amplification or protein expression in SCLC
Patient 2	61	M	Smoker	Combined SCLC/squamous-cell carcinoma	Exon 19 deletion in SCLC	No	NA	Unknown
Kurahara and colleagues[35]	65	F	0	SCLC	Leu858Arg	No	NA	Unknown

Rows are individual patients. SCLC=small-cell lung cancer. TKI=tyrosine kinase inhibitor. NA=not applicable.