THERAPEUTIC ADVANCES in
Medical Oncology

Review

Histologic transformation in lung cancer: when one door shuts, another opens

Ther Adv Med Oncol

2022, Vol. 14: 1–18

DOI: 10.1177/17588359221130503

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***Abstract*:** Histologic transformation (HT) is a major cause of drug resistance to therapy in patients with lung cancer. HTs to small-cell lung cancer (SCLC) have been reported frequently in patients with epidermal growth factor receptor (*EGFR*)-mutated lung cancer. Although HTs have an impact on the clinical outcomes in patients owing to a high refractoriness to treatments, there is limited data on the prevalence, causes, mechanisms, treatment efficacy, and future treatment strategies. In this review, we assess the literature regarding HTs comprehensively, including those describing EGFR-tyrosine kinase inhibitors, other molecular targeted drugs, and immune checkpoint inhibitors. Furthermore, we discuss the mechanisms of HTs and the lineage plasticity to SCLC and squamous cell carcinoma in lung cancer. In addition, we summarize the treatment efficacy and future perspectives of HTs in patients with lung cancer, and propose better management strategies for this group of patients.

Keywords: EGFR, histologic transformations, lineage plasticity, non-small-cell lung cancer, small-cell lung cancer

Received: 20 June 2022; revised manuscript accepted: 12 September 2022.

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide.[1] According to the WHO classification of lung tumors, there are two distinctive histologic subtypes of lung cancer: non-small-cell lung cancer (NSCLC) which comprises 85% of cases and high-grade neuroendocrine carcinoma (HGNEC) which comprises 15% of cases.[2] NSCLC is further categorized into various subtypes. Among these, adenocarcinoma and squamous cell carcinoma (SqCC) are common, while sarcomatoid carcinoma is rare. HGNEC is also divided into two subtypes: small-cell lung cancer (SCLC) and large-cell neuroendocrine carcinoma. Throughout the course of treatment, some patients may experience histologic transformations (HTs), resulting in anticancer drug resistance. In particular, HTs to HGNEC in patients with an epidermal growth factor receptor (*EGFR*) mutation-positive NSCLC on EGFR-tyrosine kinase inhibitor (TKI) treatment are most frequent. However, a comprehensive review of HTs in patients with lung cancer has been limited. In this review, we describe the characteristics, prevalence, genetic background, and future perspectives of HTs in patients with lung cancer.

HTs in patients with *EGFR* mutation-positive NSCLC

Transformation as a resistance mechanism

Somatic mutations in the tyrosine kinase domain of the *EGFR* gene are present in 15–50% of European and Asian patients with advanced NSCLC. As a result, treatment with EGFR-TKIs has extended progression-free survival (PFS) relative to chemotherapy as a first-line therapy and has been established as standard care.[3,4] Although these patients demonstrate a high objective response rate to EGFR-TKIs, most develop an acquired resistance after approximately 12 months. The most common mechanism of the acquired resistance is the *EGFR* exon20 T790M mutation from the first or second generation (G) EGFR-TKIs.[5] Currently, the third G EGFR-TKI, osimertinib, which is a potent inhibitor of active *EGFR* and T790M mutations, has been

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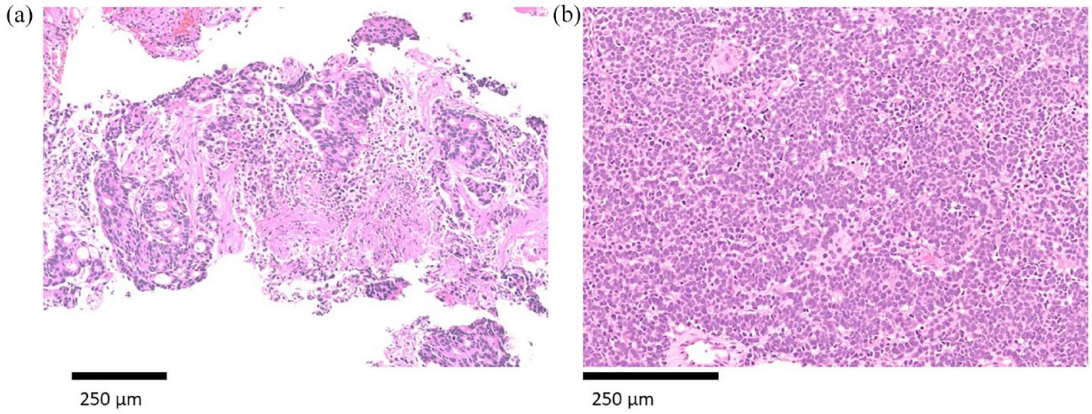

Figure 1. Representative images of HT to SCLC from lung adenocarcinoma with an *EGFR* mutation. (a) hematoxylin–eosin staining of adenocarcinoma samples obtained *via* transbronchial lung biopsy before administering EGFR-TKI. (b) Hematoxylin–eosin staining of surgical biopsy samples of SCLC in lymph node obtained from patients exhibiting resistance to EGFR-TKI.
EGFR, epidermal growth factor receptor; HT, histologic transformation; SCLC, small-cell lung cancer; TKI, tyrosine kinase inhibitor.

recognized as standard care for *EGFR* mutation-positive lung cancer.[6] The detection of T790M mutations is important in the treatment decision after the development of resistance to the first or second G EGFR-TKIs. Therefore, as a routine clinical practice, a re-biopsy is conducted.[7] The use of a repeat biopsy after clinical resistance to TKI therapy has aided the understanding of the molecular mechanisms underlying the acquired resistance to EGFR-TKIs.

In 2006, the first case of HT in a patient with lung cancer was reported by Zakowski *et al.*[8] A 45-year-old woman with no history of smoking and *EGFR* del19-positive lung adenocarcinoma who had undergone erlotinib treatment for 18 months was found to have developed SCLC after a second tumor biopsy. The second biopsy of the SCLC sample retained its original *EGFR* mutation, which suggested that SCLC may arise from adenocarcinoma cells. Since that report, HTs have been recognized as a mechanism of resistance to EGFR-TKIs in patients with *EGFR* mutation-positive NSCLC. As a result, a number of case reports, systematic reviews, retrospective cohort studies, and pathological analyses of HTs in patients with lung cancer have been reported.[9–13] The representative hematoxylin–eosin staining of biopsy samples from patients with HT is shown in Figure 1.

Debate on HTs or de novo *combined SCLC*

SCLC can be categorized as pure or combined SCLC (c-SCLC). The latter features a mixed tumor histology of SCLC and NSCLC. In a previous study, c-SCLC was observed in 10% of 176 autopsied patients diagnosed with SCLC, which suggested that a small biopsy is not adequate for an accurate diagnosis.[14] In practice, the presence of a c-SCLC can hinder the identification of the characteristics of HTs. The clonal selection hypothesis states that HTs may occur if the SCLC component becomes dominant when the adenocarcinoma component is killed by the EGFR inhibitors in patients with c-SCLC at the time of the initial diagnosis.[15] Although this hypothesis has existed for more than a decade, strong evidence has been lacking. Generally, patients with HTs respond well to EGFR-TKIs for several months and experience greater tumor growth at the time of acquired resistance due to HT to SCLC. If the clonal selection hypothesis is true for the development of SCLC, a less dramatic response to EGFR inhibitors and earlier acquired resistance would be expected. Therefore, HTs in patients with lung cancer may be the reason for the detection of SCLC upon re-biopsy, at the time of the acquired resistance.

Prevalence of HTs in patients with EGFR *mutation-positive NSCLC*

The first comprehensive study using a genetic assessment was conducted by Sequist *et al.* in 2011, who reported a prevalence of HTs of 14% in patients with lung cancer.[11] The results of large cohort studies on HTs in patients with lung cancer are summarized in Table 1. Most of the current publications have focused on HTs to

Table 1. Summary of HTs in patients with *EGFR* mutation-positive NSCLC.

Author	Journal	Year	*N*	The number and prevalence (*N*, %) of HTs	HT histology	TKI (G)	3rd G TKI (N)	Time to HT (Mo)
Fujimoto *et al.*[10]	*Eur J Cancer*	2022	2624	59 (2.2) 15 (0.6)	HGNEC another NSCLC	1st–3rd	14	21.6
Sequist *et al.*[11]	*Sci Transl Med*	2011	37	5 (14)	SCLC	1st	0	14.1
Yu *et al.*[16]	*Clin Cancer Res*	2013	155	4 (2.6)	SCLC	1st	0	13
Nosaki *et al.*[7]	*Lung Cancer*	2016	314	12 (3.8)	SCLC	1st–2nd	0	N.R.
Zeng *et al.*[17]	*Lung Cancer*	2020	103	3 (2.9)	SCLC	1st	0	N.R.
Piotrowska *et al.*[18]	*Cancer Discov*	2018	32	2 (6.3) 1 (3.1)	SCLC SqCC	3rd	32	N.R.
Lin *et al.*[19]	*Lancet Res Med*	2018	53	2 (3.8) 1 (1.9)	SCLC SqCC	3rd	53	N.R.
Oxnard *et al.*[20]	*JAMA Oncol*	2019	41	6 (15)	SCLC	3rd	41	N.R.
Schoenfeld *et al.*[21]	*Clin Cancer Res*	2020	62	3 (4.8) 6 (9.7)	SCLC SqCC	3rd	62	13.6

G, generation; HGNEC, high-grade neuroendocrine carcinoma; HT, histologic transformation; N.R., not reported; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; TKI, tyrosine kinase inhibitor.

HGNEC. These studies have reported that 2–15% of patients with lung cancer experience HTs to HGNEC upon the acquisition of resistance. Our largest cohort study reported among 2624 re-biopsy cases that the prevalence of HTs to HGNEC was 2.2% while that to other types of NSCLC was 0.6%.[10] Overall, the time from initial diagnosis of HT to HGNEC ranged from 13 to 22 months. Four cohort studies reported that most cases included the first or second G EGFR-TKIs, and HT after the third G TKI therapy. The results indicated collectively, that a HT in patients with lung cancer is recognized as a mechanism of resistance, irrespective of the TKI generation.

HTs to another subtype of NSCLC in patients with EGFR *mutation-positive NSCLC*

Although uncommon, HTs from one subtype of NSCLC to another have been reported in 0.6% of in patients with lung cancer.[10] Most of the reports were case reports or small case series. In our previous cohort study, most TKIs that were used during HTs were first or second generation. In some reports that analyzed pre- and post-HT samples of patients treated with osimertinib, the prevalence of SqCC transformations was 2–10%; thus, larger studies on third G TKIs are needed.[18,19,21] Moreover, in a systematic review of 17 patients with HTs to SqCC, most of the patients were female (82%), 41% were former smokers, and the median time to SqCC onset was 11.5 months.[22]

HTs to subtypes other than HGNEC or SqCC are extremely rare. For example, there have been several reports on transformations to sarcomatoid carcinoma.[23,24] Specifically, six sarcomatoid HTs [five cases with *EGFR* mutations and one with c-ros oncogene 1 (*ROS1*) mutations] were reported, and the interval from initial diagnosis to HT was 9–88 (median: 31.5) months.[25] Giant cells were the most common form of sarcomatoid transformations, and overall survival (OS) after HTs in patients with lung cancer was reported to be 2.5 months, which was shorter than for HTs to HGNEC.

Current problems of HTs in patients with EGFR *mutation-positive NSCLC*

After the FLAURA study, the mainstay first-line EGFR-TKI changed to osimertinib.[26] No

standard treatment has yet been developed for those with osimertinib-resistant NSCLC, and a re-biopsy after osimertinib resistance is not conducted as routine clinical practice. Therefore, no large cohort studies have been conducted and the precise incidence of *EGFR* mutation-positive NSCLC with HTs after osimertinib has not been determined. Currently, the major focus is on overcoming osimertinib resistance by combining new drugs, such as a mesenchymal epithelial transition (*MET*) inhibitor (selumetinib), a vascular endothelial growth factor receptor (*VEGFR*)-2 blocker (ramucirumab), and a bispecific antibody that blocks *EGFR* and *MET* receptors simultaneously (amivantamab).[27–29] HTs in patients with lung cancer that occur after treatment with these agents should also be investigated in future studies.

HT induced by agents other than EGFR-TKIs

Programmed cell death 1/programmed cell death ligand 1 inhibitors

HTs have been reported mainly in patients with *EGFR* mutation-positive lung cancer. However, they have also been reported in patients with NSCLC undergoing treatment without EGFR-TKIs and in those with other cancers.[30–33] For example, HTs to SCLC after programmed cell death 1 (PD-1) inhibitor treatment has also been reported. In addition, HTs of adenocarcinoma to SqCC and vice versa have been reported after treatment with PD-1 inhibitors such as nivolumab, pembrolizumab, and sintilimab.[34–38] In contrast, HTs after programmed cell death ligand 1 (PD-L1) inhibitor administration has been reported rarely, likely due to differences in the timing of its introduction into clinical practice and its infrequent use as a single agent for advanced NSCLC.[39] HTs to SCLC were detected in only one case after treatment with nivolumab followed by atezolizumab.

Among the studies on HTs in patients with lung cancer, after treatment with PD-1 inhibitors, few have examined the causes of HTs. However, several studies have reported that tumor suppressor p53 (*TP53*) mutations were found prior to PD-1 inhibitor administration in cases of HTs to SCLC.[40,41] These findings were similar to those described below for HTs to SCLC after the use of molecular targeted agents, and no report has yet suggested a PD-1 inhibitor-specific mechanism of HT. Therefore, HTs in patients with lung cancer caused by PD-1 inhibitors may also be typical mechanisms of resistance to therapy for NSCLC. However, the rate of HTs among those patients treated with PD-1 inhibitors is unknown. It has been suggested that the frequency of HTs in patients with lung cancer may be underreported because re-biopsy after administration of immune checkpoint inhibitors (ICIs) is not common.[41] Therefore, future large-scale validation is warranted.

Anaplastic lymphoma kinase-TKIs

HTs in patients with lung cancer have been reported with the use of most of the marketed anaplastic lymphoma kinase (ALK)-TKIs, such as crizotinib, alectinib, ceritinib, and lorlatinib, while no report has yet linked ALK-TKIs specifically to HTs.[42–45] Moreover, in most cases, the *ALK*-rearrangement was retained even after the HT.[42–49] As most studies have focused on the mechanisms of resistance to ALK-TKIs, such as secondary mutations, *ALK*-amplification, and bypass signaling pathways, the frequency of HTs as a resistance mechanism to ALK-TKIs has not yet been reported.[50–52]

Several studies have reported the cases of HTs to SCLC in *ALK*-rearranged NSCLC.[42,47,48,53] Similar to HTs to SCLC caused by other drugs, HTs caused by ALK-TKIs have been implicated in the alterations in genes such as *TP53* and retinoblastoma 1 gene (*Rb1*).[45,49] Recently, the first case of a transformed SCLC from *ALK*-rearranged NSCLC using an analysis of the genomic and transcriptomic landscape of paired pre- and post-HT tissues was reported.[54] The findings indicated that no alterations or loss of functions were detected in *TP53* or *Rb1*, suggesting that the mechanism of HT to SCLC in *ALK*-rearranged NSCLC may differ from that in *EGFR*-mutated NSCLC. However, the number of paired-sample analyses was small, requiring further studies to examine the mechanistic differences of HTs between *ALK*-rearrangements and *EGFR* mutations. HTs to SqCC have also been reported after the use of ALK-TKIs in several cases.[55–60] Two reports have demonstrated the paired pre- and post-treatment genomic landscape of the transformed *ALK*-rearranged SqCC; however, much remains unknown about HTs in SqCC.[57,58]

Other molecular targeted agents for NSCLC

HTs in patients with lung cancer are also caused by molecular targeted agents other than

EGFR- and ALK-TKIs. For example, several reports have shown HTs after treatment with ROS1-TKIs or Kirsten rat sarcoma viral oncogene homolog (KRAS) G12C-inhibitors.[61–63]

In the case of *ROS1* rearrangement-positive NSCLC, the whole exome analysis of biopsy and autopsy-derived specimens before and after crizotinib administration have provided detailed confirmation that the SCLC transformation occurred as a mechanism of resistance.[61] This case showed a loss of function of *TP53* and *Rb1*, which was similar to typical HT cases.

In terms of *KRAS*, mouse models and cell lines of *KRAS* G12D have been used frequently to study the mechanisms of oncogenesis of lung adenocarcinoma. In addition, studies using a *KRAS* G12D mutation-positive cell line and a mouse model have indicated that the deletion of liver kinase B1 (*LKB1*) may be closely related to HTs to SqCC.[64,65] However, no molecular targeting agents specific for *KRAS* G12D are currently in clinical application, and the relationship between *KRAS* G12D and HT to SqCC has not yet been demonstrated in clinical practice. With respect to *KRAS* G12C, for which there are molecular targeted drugs are in clinical use, HTs to SqCC have been reported. In a study of *KRAS* G12C-positive NSCLC, the authors reported a resistance mechanism in 11 cases in which disease control was achieved for more than 12 weeks after adagrasib treatment.[63] Of those 11 patients, some amplifications, oncogenic fusions, and loss of function mutations were identified. Among them, two patients exhibited HTs, both to SqCC. In one of the two cases, a circulating tumor DNA analysis of plasma samples was also performed, and no genomic resistance mechanism other than HTs to SqCC was identified (although there is no evidence that serine/threonine kinase 11 (*STK11*)/*LKB1* was studied).

Some reports have stated that HTs in patients with lung cancer did not occur in cases where molecular targeted drugs were administered to patients with NSCLC with rare driver gene abnormalities, such as rearrangements during transfection (*RET*) and in the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) V600E.[66,67] However, the sample sizes of those studies were limited. Thus, the possibility of HTs in rare driver gene alterations in patients with lung cancer cannot be ruled out, until the resistance mechanism is studied on a large scale.

Mechanism of HTs and lineage plasticity in lung cancer

Histologic origin of lung cancer

The respiratory epithelium from the trachea to the alveoli is composed of basal, club/Clara, neuroendocrine, ciliated, goblet, and alveolar type I and type II cells (Figure 2). The development of lung cancer has been studied using genetically engineered mouse models.[68] Such studies have postulated that type II alveolar cells are the origin of lung adenocarcinoma,[69–72] while it has been suggested recently that club cells may be responsible for this cancer subtype.[73,74] Moreover, the origin of lung SqCC has been postulated to be basal cells based on the expression of p63, a marker of bronchial basal cells, and this theory is still supported as one of the origins of the disease.[75–77] However, carcinogenesis of SqCC from type II alveolar cells and club cells has also been widely reported in recent years.[78,79] Furthermore, because of its neuroendocrine neoplastic nature, SCLC was considered to be derived from neuroendocrine cells in the lung.[80–82] Even in recent years, the development of SCLC from neuroendocrine cells is still considered one of the most promising origins.[83,84] However, SCLC can arise from basal, club, and type II alveolar cells.[80,85] Thus, although generally, the cell of origin of lung cancer development was presumed by histological type, each lung cancer subtype has no single histological origin.[86]

Furthermore, recent years have brought attention to the fact that the histology of lung cancer is not constant, but plastic. Cancer cell plasticity is defined as the ability of a cell to modify its identity substantially and to take on a new phenotype that resembles a distinct developmental lineage more closely.[87] It is considered that some cancer cells possess plasticity and that further specific changes may cause dynamic changes, such as HTs. Herein, we review the mechanisms of HTs in patients with lung cancer, focusing on HTs to SCLC, which has been relatively well studied.

Genetic characteristics of SCLC

Inactivation of the *Rb1* gene was first discovered as a characteristic genetic abnormality in retinoblastoma.[88,89] Subsequently, an association with neuroendocrine tumors was suggested and it was reported to be more common in SCLC than NSCLC.[90,91] However, in a mouse model, the inactivation of *Rb1* alone did not cause

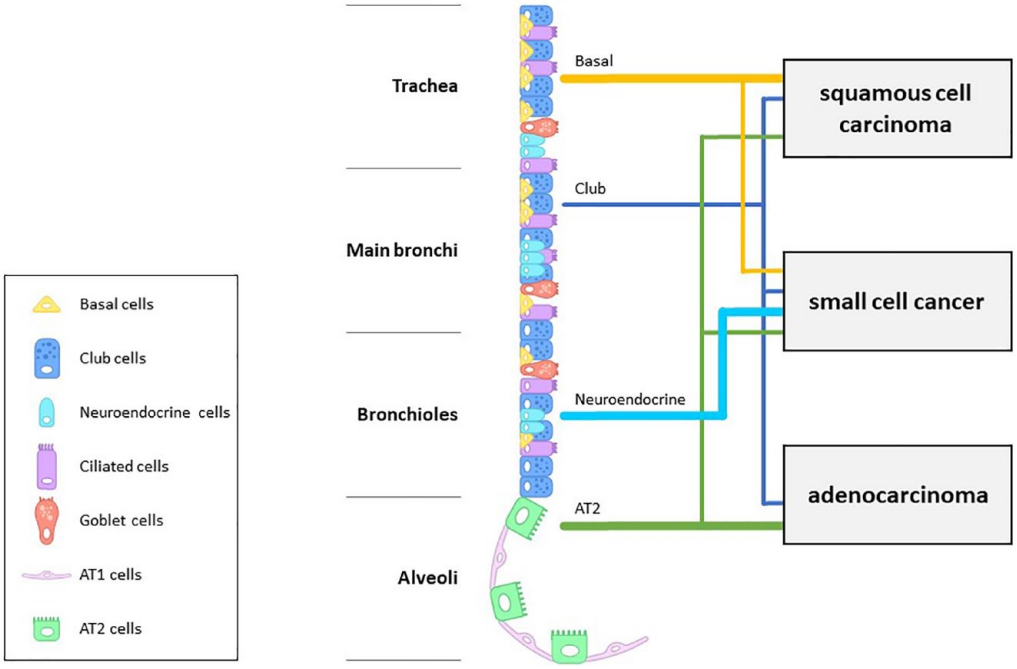

Figure 2. Respiratory epithelium and the origin of lung cancers. The airway epithelium is composed of basal, club/Clara, neuroendocrine, ciliated, goblet, and alveolar type I and type II cells. The main histologic origin of each lung cancer subtype is indicated by the bold lines; however, each histological subtype has various origins. AT1, alveolar type I cell; AT2, alveolar type II cell.

neuroendocrine tumors in the lungs.[92,93] In 2003, it was shown that loss of both *TP53* and *Rb1* in a wide range of lung epithelial cells in a mouse model could transform them into SCLC.[94] In 2011, the inactivation of *TP53* and *Rb1*, particularly in neuroendocrine cells and type II alveolar cells, was reported to induce small cell transformation.[80] The following year, the results of a large-scale whole-genome, transcriptome, exome, and copy number analysis of SCLC were reported.[95,96] As a result, mutations in *TP53* and *Rb1* were identified as highly frequent abnormalities in SCLC. These findings suggest that loss of function due to mutations in both *TP53* and *Rb1* plays a major role in the development of SCLC.

Mechanism of HT from NSCLC to SCLC

The acquired loss of function of *Rb1*, which occurs after the initiation of treatment for *EGFR*- and *TP53*-mutated NSCLC, was first hypothesized as the cause of SCLC transformation.[15] Since the loss of *TP53* and *Rb1* is vital to the carcinogenesis of *de novo* SCLC, the *Rb1* deletion was found in all 11 cases of the transformed SCLC and only in those with HTs to SCLC after resistance to EGFR-TKIs, but not in those whose cancer remained NSCLC with resistance to EGFR-TKIs.[97] Therefore, it was hypothesized that the additional alterations in *Rb1* during EGFR-TKI treatment would result in transformation and resistance against EGFR-TKIs. However, when the paired pre- and post-treatment tumor samples were analyzed, the alterations of *Rb1* and *TP53* were found to have existed before anticancer treatment.[98] Furthermore, the alterations of *Rb1* and *TP53* were reported to be present in patients who did not develop HTs to SCLC. In other cohort studies, although *EGFR*/*Rb1*/*TP53*-mutant lung cancers were present in 5% of *EGFR*-mutant lung cancers, only approximately 20% of these cases showed HTs to SCLC after EGFR-TKI treatment.[99] Moreover, *EGFR*-mutant lung cancers without baseline *TP53* and *Rb1* alterations were rarely transformed to SCLC. Taken together, the loss of *TP53* and *Rb1* might be a necessary, but not sufficient condition for HTs to SCLC.

Thus, a second hypothesis of the mechanisms of HTs in patients with lung cancer is that gene alterations occur before or after anticancer drug therapy in cancer cells with a baseline loss of function of *TP53* and *Rb1*. Both of the cohort studies of the paired pre- and post-treatment tumor sample analysis reported that

activation-induced cytidine deaminase (*AID*)/apolipoprotein B mRNA-editing enzyme (APOBEC) mutation signature was more enriched in *EGFR*/*Rb1*/*TP53*-mutant lung cancers which transformed to SCLC than in *EGFR*/*Rb1*/*TP53*-mutant lung cancers which did not transform to SCLC, suggesting that gene alterations other than *TP53* and *Rb1* mutations are required for HTs to SCLC.[98,99] The APOBEC family is a group of DNA/RNA editing enzymes that convert cytidine (C) to uridine (U) through deamination reactions.[100] APOBEC3-specific genome mutation (APOBEC3 signature) has been suggested to play an important role in the induction of genomic variations in many cancers.[101–103] Furthermore, the presence of specific genomic mutations, such as the APOBEC signature, in *EGFR*/*Rb1*/*TP53*-mutant lung cancers prior to HTs suggests that genomic alterations other than *TP53* and *Rb1* may be involved in HTs to SCLC.[98,99] Other genes have also been implicated as possible contributors. For example, in a mouse model of transformed human basal cells of benign prostate tissue, the addition of cellular myelocytomatosis oncogene (*c-MYC*) and B-cell lymphoma 2 (*Bcl2*) alterations to *TP53, AKT*, and *Rb1* alterations induced SCLC. However, *TP53, AKT*, and *Rb1* alterations without *c-MYC* and *Bcl2* alterations induced adenocarcinoma. Furthermore, in a mouse model of transformed normal human bronchial epithelial cells, all five genes (*TP53, AKT, Rb1, c-MYC*, and *Bcl2*) had to be altered for carcinogenesis to SCLC to occur.[104] Thus, not only *TP53* and *Rb1* loss, but also other genetic factors may be needed for HTs to SCLC. However, while involvement of *AKT* and *c-MYC* has been detected in some clinical specimens of *EGFR*-mutated NSCLC with HTs to SCLC, it was not as prevalent as *TP53* and *Rb1* alterations.[98,99] Therefore, the specific set of genetic abnormalities required for SCLC transformations has not yet been identified.

Recently, a third hypothesis of HTs in patients with lung cancer proposed that acquired transcriptional regulation occurs in cancer cells with loss of function of *Rb1* and *TP53*. Detailed genomic, epigenomic, transcriptomic, and proteomic characterization of combined lung adenocarcinoma/SCLC, pre-transformation lung adenocarcinoma, post-transformation SCLC, never-transformed lung adenocarcinoma, and *de novo* SCLC have been reported.[105] This study suggested that HTs in patients with lung cancer may be caused by transcriptional reprogramming rather than by acquired gene alterations. The authors identified an increased expression of genes involved in the polycomb repressive complex 2 (*PRC2*), phosphoinositide 3-kinases (*PI3K*)/*AKT*, and neurogenic locus notch homolog protein (NOTCH) pathways. It was also found that pre-transformed lung adenocarcinoma had an intermediate pattern between never-transformed lung adenocarcinoma and post-transformed SCLC in the methylation profiling. These findings suggested that HTs from NSCLC to SCLC may be caused by methylation-induced changes in the transcriptional regulation with a prior loss of the *TP53* or *Rb1* function. The current hypothesis is summarized in Figure 3.

Nevertheless, these hypotheses are problematic because these studies did not target only transformed SCLC and they included only a small number of patients. In addition, whether there are genetic differences between *de novo* SCLC and transformed SCLC remains unknown because the genomic analysis of transformed SCLC has only been performed in a small number of cases. In the few cases where the genomic analysis has been performed, no specific genetic abnormalities distinguishing the *de novo* SCLC from the transformed SCLC have been identified, aside from retained driver gene alterations. Therefore, in the future, more pre- and post-transformation clinical specimens of lung adenocarcinomas in which HTs to SCLC have occurred need to be collected and analyzed in detail.

Small-cell transformation of prostate cancer

Next, we discuss the small cell transformations of prostate cancer, which is helpful in considering SCLC transformations from NSCLC. HTs to small-cell carcinoma of the prostate have been known to occur after the administration of androgen receptor (AR) signaling inhibitors, such as abiraterone and enzalutamide for the treatment of metastatic castration-resistant prostate cancer.[106,107] Transformations of prostate cancer into small-cell carcinoma by AR inhibition have been reported and analyzed before that of *EGFR* mutation-positive NSCLC to SCLC. Thus, small-cell transformations of prostate cancer have been examined in more detail than that of lung cancer in some areas. In a recent large prospective study, this condition was named treatment-emergent small-cell neuroendocrine prostate cancer (t-SCNC), and its frequency was examined. The results showed that t-SCNC as a

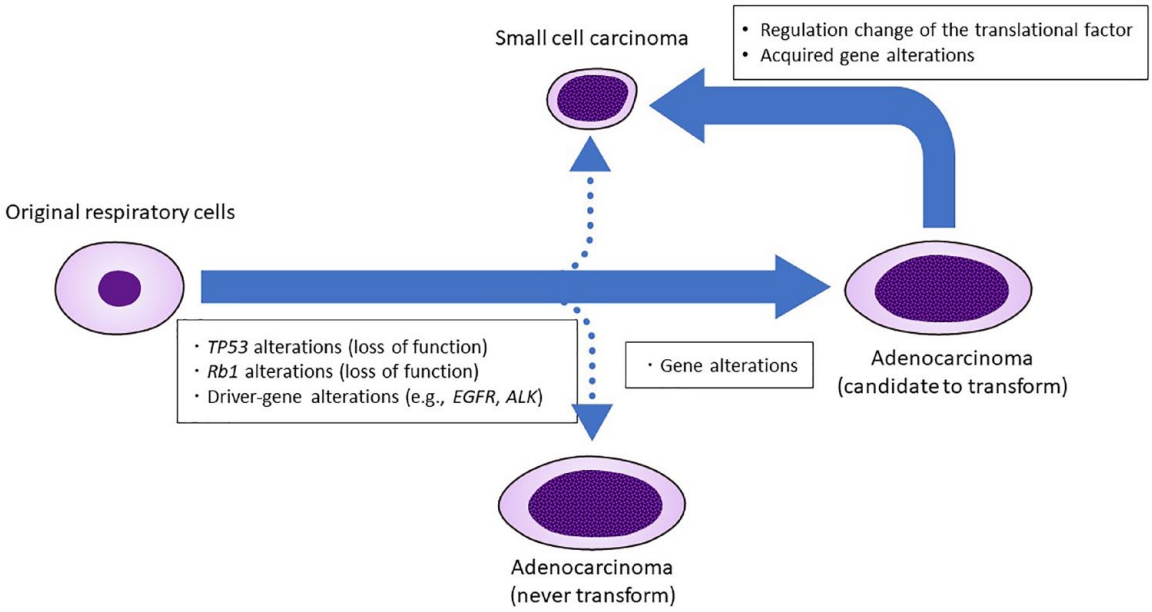

Figure 3. Current hypothesis of HTs to small-cell lung cancer from NSCLC. *TP53, Rb1*, and driver gene alterations are commonly observed before HTs, and some regulation change of translational factor or acquired gene alterations might be required for HTs.
ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; HT, histologic transformation; Rb1, retinoblastoma 1 gene; NSCLC, non-small-cell lung cancer; TP53, tumor suppressor protein p53.

resistance mechanism occurred in 17% of patients after the initiation of AR-targeted therapy.[31] Alterations of *TP53* and *Rb1* were detected in several cases. In a mouse model, defects of *TP53* and *Rb1* depressed epigenetic initiators, such as enhancers of the zeste homolog 2 (*Ezh2*) and sex-determining region Y-box 2 (*Sox2*), were identified, which were shown to be involved in resistance to antiandrogen therapy and lineage plasticity.[108] De-repression of the placental gene, paternally expressed gene 10 (*PEG10*) and involvement of transmembrane serine protease 2 (*TMPRSS2*)/endocrine/reproductive/gastrointestinal (*ERG*) fusion or transcription factors, such as the forkhead box protein A1 (*FOXA1*) and POU domain class 3 transcription factor 2 (*POU3F2*) have been reported to play roles in the small-cell transformation mechanisms of prostate cancer.[109–112] However, although the loss of function of *TP53* and *Rb1* is common between NSCLC and prostate cancer in the mechanism of HTs to SCLC, some of the above-mentioned genes, especially the transcription factors, have not been identified in HTs to SCLC from NSCLC. Further studies are needed to determine whether NSCLC and prostate cancer share common or distinct mechanisms of transformation to small-cell carcinoma.

Mechanism of squamous cell transformation

Knowledge on transformations to SqCC is limited. Notably, for the *de novo* SqCC, there have been no reported genetic abnormalities, such as *TP53* or *Rb1* in SCLC, which are present in many cases and can be key to investigating the mechanisms of transformation, which may hinder the establishment of models for HTs to SqCC. However, a few studies using *KRAS* G12D-positive adenocarcinoma mouse models, and paired tissues before and after treatment, have suggested that the loss of the *LKB1* function may play an important role in HTs to SqCC. The possibility that the loss of *LKB1* function contributes to the development of *de novo* SqCC has been noted in several reports.[113,114] In the *KRAS* G12D-positive adenocarcinoma mouse models, the deletion of *LKB1* may have resulted in transformations to SqCC.[65,115] Moreover, the administration of Adenovirus-Cre *via* an intranasal addition to $LKB1^{fl/fl}$ and phosphatase and tensin homolog $(PTEN)^{fl/fl}$ mice resulted in carcinogenesis of SqCC and the expression of the squamous markers keratin 5 (*KRT5*), *p63*, and *Sox2*, which transcriptionally resembled the human lung SqCC.[116] In addition, the loss of function of *LKB1* and *PTEN* activated *AKT* and the mechanistic target of rapamycin (mTOR) pathways,

contributing to cell proliferation and tumorigenesis. The possibility that the loss of *LKB1* or *PTEN* may have activated the mTOR pathway, leading to HTs to SqCC, has been suggested in reports of pre- and post-treatment clinical samples of transformed SqCC, and is a promising hypothesis.[117] However, HTs to SCLC without any obvious acquired alterations of the mTOR pathway has also been reported;[21] thus, the loss of *LKB1* and *PTEN* is not considered to be the sole mechanism of SqCC transformations. Importantly, loss of *LKB1* is detected in up to 2% of cases of *de novo* SqCC, which may make it difficult to establish a single model for HTs to SqCC.[118] Further analysis of paired pre- and post-transformation tumors may reveal multiple transformation mechanisms.

Therapeutic strategies after HTs

Survival after HTs in patients with EGFR *mutation-positive NSCLC*

Treatment strategies after HTs in patients with lung cancer are a major problem for both patients and clinicians. Existing data from a cohort study are limited to patients with *EGFR* mutation-positive NSCLC. The prognosis of HTs in patients with lung cancer, obtained from cohort studies is summarized in Table 2. An OS of 9–15 months from the time of HT diagnosis has been reported in previous literature.[10,119–121] Moreover, the OS and PFS of HTs to SCLC were comparable with those of *de novo* SCLC.[122,123] From the perspective of disease behavior, one previous report suggested that HT to SCLC in patients with *EGFR* mutation-positive NSCLC might be more aggressive than in patients without *EGFR*-mutation because the median time to HT for patients with *EGFR*-mutation was significantly shorter than that for patients without *EGFR*-mutation.[124] However, this might be biased by the recommendation of repeat biopsy in *EGFR*-mutant patients after a line of targeted therapy, whereas a repeat biopsy is not recommended in patients without *EGFR* mutation.[125] For patients with HTs to another subtype of NSCLC, the OS was reported to be 12.1 months.[10] However, the data of survival outcomes of patients with HTs to SqCC were limited compared with that of patients with HTs to SCLC. Moreover, no study has yet compared OS from the time of diagnosis between HT and non-HT cases; therefore, the clinical impact of HTs should be investigated in future studies.

Efficacy of chemotherapy after HTs

The indicators of treatment efficacies such as PFS, OS, and OS after the lung cancer diagnosis are summarized in Table 2. Therapeutic strategies should be adjusted for SCLC after HTs to SCLC, since SCLC is highly malignant and

Table 2. Summary of the prognosis of patients with HGNEC or another subtype of NSCLC.

Histology	Author	Journal	Year	*N*	Treatment after HT diagnosis	PFS after HT diagnosis (Mo)	OS after HT diagnosis (Mo)	OS after lung cancer diagnosis (Mo)
HGNEC	Fujimoto *et al.*[10]	*Eur J Cancer*	2022	59	Chemo 51/ICI 12/ TKI 21	4.1	12.6	N.R.
	Marcoux *et al.*[119]	*J Clin Oncol*	2018	65	Chemo 63/ICI 17/ TKI 34	3.4	10.9	31.5
	W. Wang *et al.*[120]	*Lung Cancer*	2021	32	Chemo 30/ICI 3/ TKI 7	3.5	9.7	34.5
	S. Wang *et al.*[121]	*Thorac Cancer*	2021	29	Chemo 27/TKI 18	4.7	14.8	N.R.
	Ferrer *et al.*[124]	*J Thorac Oncol*	2018	48	Chemo 41/ICI 1/ TKI 2	N.R.	9	28
Another NSCLC	Fujimoto *et al.*[10]	*Eur J Cancer*	2022	15	Chemo 9/ICI 9/ TKI 5	6.4	12.1	N.R.

HGNEC: high-grade neuroendocrine carcinoma; HT: histologic transformation; ICI: immune checkpoint inhibitor; NSCLC: non-small-cell lung cancer; N.R.: not reported; PFS: progression-free survival; OS: overall survival; TKI: tyrosine kinase inhibitor.

develops rapidly.[15] In previous studies, most patients received cytotoxic chemotherapy. Among them, SCLC-based chemotherapy (etoposide plus platinum or irinotecan plus platinum) was used most frequently.[10,119] In a previous study, the PFS of etoposide plus platinum was 3.4 months. Considering that the PFS of etoposide plus platinum in *de novo* extensive-disease SCLC was 4–6 months, the treatment efficacy may be limited.[122,123] In this clinical setting, a repeat biopsy is highly recommended to rule out SCLC transformation because platinum-etoposide chemotherapy yields responses in patients with transformed SCLC, similar to *de novo* SCLC.[125]

Efficacy of ICIs after HTs

Previous studies have shown the limited efficacy of ICIs for patients who developed HT, although recent research has suggested that HTs are associated with the increased ability to induce an immune response.[105] Specifically, in our study, the total objective response rate and disease control rate of ICI therapy (PD-1/PD-L1 inhibitor monotherapy or platinum-pemetrexed combined with PD-1/PD-L1 inhibitors) were 0% and 17%, respectively, and the median PFS was 2.0 months.[10] Another large-scale report showed that no responses were observed after HTs in 17 patients who received ICIs, either as a single-agent PD-1 or PD-L1 inhibitor ($n = 9$) or as part of a combination ipilimumab–nivolumab regimen ($n = 8$).[119] None of the 17 patients derived clinical benefits from these therapies, as the longest time to progression was only 9 weeks.

Treatment efficacy of TKIs

Data are limited on the efficacy of TKIs after HTs in patients with lung cancer, although continuing TKI therapy after the development of HT has been used in some cases. In a retrospective study, the PFS after HTs of patients receiving chemotherapy with EGFR-TKIs was significantly longer than that of patients receiving chemotherapy without EGFR-TKIs (5.2 *versus* 3.0 months, $p = 0.0014$).[121] However, the OS benefit has not been demonstrated; thus, the efficacy of continuing TKI therapy has not reached consensus. Another study has implied the beneficial efficacy of multi-kinase inhibitor therapy after HTs in patients with lung cancer.[120] Anlotinib is an orally administered multi-kinase inhibitor that targets *VEGFR*, fibroblast growth factor receptor, platelet-derived growth factor receptors, and *c-kit*. Owing to its antiangiogenic effect, this agent is considered to be a potential new treatment option for SCLC.[126] In a multicenter, retrospective study conducted in China, anlotinib showed good efficacy in patients with HTs to SCLC (median PFS was 4.3 months in the anlotinib group *versus* 0.7 months in the placebo group, HR = 0.19, $p < 0.0001$). From these studies, the use of TKIs after HTs in patients with lung cancer may be considered as a treatment option.

Future strategies for patients who experience HTs

Currently, there are no published prospective trials for patients with HTs. After the accumulation of data from the IMpower 133 and the CAPSIAN study, the platinum + etoposide + anti-PD-L1 inhibitor regimen has been recognized as standard care for patients with extensive-disease SCLC.[127,128] In addition, experimental module 7 of the Orchard clinical trial (NCT03944772) is investigating etoposide + durvalumab + carboplatin or cisplatin for patients who develop HTs.[129] Another promising strategy is the use of olaparib, a poly-ADP ribose polymerase (*PARP*) inhibitor. One approach to enhance the clinical activity of ICIs is to modulate the DNA damage response.[130] *PARP1* is highly expressed in SCLC, and PARP inhibitors have shown antitumor activity in preclinical models and in patients with SCLC. A recent study using a multiomics approach identified a greater activation of immune-related pathways in the transformed SCLC compared with the *de novo* SCLC.[105] Therefore, olaparib is expected to be a useful treatment option for patients with *EGFR*-positive NSCLC that have undergone HTs to SCLC. Currently, a phase II trial of durvalumab and olaparib for patients with *EGFR*-mutated transformed SCLC is ongoing (NCT04538378). Furthermore, in a recent study, *de novo* SCLC cases were categorized into four subtypes (SCLC-A, N, P, and Y), based on the most predominant expression of the four transcription factors that are characteristic of SCLC. Achaete-scute homolog 1 (*ASCL1*) is predominant in SCLC-A, neurogenic differentiation 1 (*NEUROD1*) in SCLC-N, POU domain class 2 transcription factor 3 (*POU2F3*) in SCLC-P, and yes-associated protein 1 (*YAP1*) in SCLC-Y.[131] Treatment strategies for each molecular subtype, such as *Bcl2* inhibitors for SCLC-A and the Aurora inhibitors for SCLC-N are warranted.[97,132]

No comprehensive data have yet been compiled regarding the four subtypes of SCLC after HT. Specific treatment strategies for patients with HTs to SCLC are also warranted.

Future strategies for patients who are at high risk of HTs

Treatment strategies for patients at high risk of HTs have also been investigated. As noted previously, the loss of the *TP53* and *Rb1* function is not a sufficient condition for HTs to SCLC; however, it is present in most cases of HTs to SCLC. Moreover, in previous research, patients with triple-mutant (*EGFR*/*RB1*/*TP53*) lung cancer had a shorter time to initial EGFR-TKI discontinuation than *EGFR*/*TP53*- and *EGFR*-only mutant cancers.[133] In patients with *EGFR*/*RB1*/*TP53*-mutant lung cancer, the persistent cell population after EGFR-TKI treatment may include a subclone that is at high risk of HTs to SCLC, and may benefit from the combination of EGFR-TKIs and a neuroendocrine-based chemotherapy regimen.[99] Thus, the continuing use of osimertinib followed by a platinum + etoposide insertion for patients at high risk of HTs to SCLC is currently being investigated in an ongoing phase I clinical trial (NCT03567642).

In considering the treatment strategy for patients with HTs to SCLC, early detection is critical. Although a tissue biopsy is crucial for the diagnosis of HTs in patients with lung cancer, it can be difficult and invasive.[134] In a previous report, elevated tumor markers, such as neuron-specific enolase and pro-gastrin-releasing peptide were reported at the time of HTs to SCLC; however, tumor markers are not precise indicators of HTs.[135,136] Liquid biopsy is a recently developed, non-invasive technique used in cancer treatment. It has demonstrated an ability to detect, characterize, and monitor cancers using a serum sample. In a previous study, a tissue and plasma analysis of *EGFR, TP53*, and *RB1* contributed to the early detection of HTs to SCLC.[99,134,137] Moreover, liquid biopsy techniques have been used in the previously mentioned clinical trial (NCT0 3567642). Furthermore, the use of a serum digital droplet PCR (ddPCR) might be helpful in identifying high-risk patients. In a previous study, a ddPCR was conducted before osimertinib administration for previously treated NSCLC with HTs to SCLC and showed a low ratio of T790M/activating mutation.[138] This study suggested that a tissue biopsy should be considered to exclude the occurrence of HTs to SCLC in cases of a low ratio of T790M/activating mutation in the blood sample. However, the number of patients included in this analysis was small; therefore, the significance of T790M mutations in HTs in patients with lung cancer should be studied in a large cohort.

Conclusions

HT is a major resistance mechanism in patients with lung cancer, not only for EGFR-TKI but also for other molecular targeted agents and ICI therapy. The most prevalent histology after HT is SCLC; however, some patients may experience HTs to another NSCLC. Recently, some reports analyzed paired pre- and post-treatment transformed-tumor samples, and several hypotheses were developed regarding the mechanism of HTs in patients with lung cancer. However, the number of cases in which such analyses have been performed remains small and the mechanisms remain unclear. Moreover, other data such as patient characteristics and treatment efficacy are scarce. Although the current standard treatment for transformed lung cancer is cytotoxic chemotherapy, the treatment efficacy has been reported to be limited. Further research is warranted for this group of patients.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publication

Not applicable.

Author contribution(s)

Yuki Sato: Data curation; Visualization; Writing – original draft; Writing – review & editing.

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Acknowledgements

The authors would like to thank Yoko Miyake (Clinical Research Center, Kobe City Medical Center General Hospital, Kobe, Japan) for figure editing. This study was supported by internal

funding from Internal Medicine III, Wakayama Medical University.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by internal funding from Internal Medicine III, Wakayama Medical University.

Competing interests

Dr. Sato reported receiving personal fees from AstraZeneca, Chugai Pharmaceutical, MSD, Ono Pharmaceutical, Novartis, Pfizer, Taiho Pharmaceutical, Nippon Kayaku, Bristol Myers Squibb, Eli Lilly, Takeda, and Kyowa Kirin outside the submitted work.

Dr. Saito reported receiving personal fees from Ono Pharmaceutical, Chugai Pharmaceutical, AstraZeneca, Novartis Pharma, and Taiho Pharmaceutical outside the submitted work.

Dr. Fujimoto reported receiving grants from Boehringer Ingelheim and AstraZeneca; personal fees from Astra-Zeneca K.K., Ono Pharmaceutical Co., Ltd., Bristol Myers Squibb Co., Ltd., Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., MSD K.K., Boehringer Ingelheim Japan Inc., Eli Lilly Japan K.K., and Novartis Pharma K.K. outside the submitted work. Dr. Fujimoto also served on an advisory board for AstraZeneca and Chugai Pharmaceutical.

Availability of data and materials

Not applicable.

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