Genomic and Transcriptomic Analysis of Neuroendocrine Transformation in ALK-Rearranged Lung Adenocarcinoma After Treatments With Sequential ALK Inhibitors: A Brief Report

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ABSTRACT

Introduction: Neuroendocrine (NE) transformation has been reported in patients with ALK-rearranged NSCLC after ALK inhibition, but unlike EGFR-mutant NSCLC, the exact mechanism of NE transformation in ALK-rearranged NSCLC is poorly studied.

Methods: We collected the matched pre- and post-transformation samples from a patient with ALK-rearranged lung adenocarcinoma (LUAD) and performed targeted panel sequencing, whole exome sequencing, and bulk RNA sequencing.

Results: Multiple mutations were shared between the pretransformation and post-transformation samples. Neither RB1 nor TP53 mutation was detected, but CDKN2A deletion and CDK4 amplification were found instead. Mismatch repair-associated mutational signature was significantly enriched after transformation. Genes associated with Notch signaling and PI3K/AKT pathway were significantly up-regulated, whereas genes related to lymphocyte activation and NF-kB signaling were down-regulated. Signatures relating to homologous recombination, mismatch repair, and Notch signaling pathways were enriched, which were further validated in The Cancer Genome Atlas cohorts. Macrophages M2 were found to have prominently higher abundance in the tumor immune microenvironment after NE transformation.

Conclusions: The mechanism of NE transformation in ALK-rearranged LUAD may be different from that in EGFR-mutant LUAD.
selection pressure from targeted agents and eventually acquire drug resistance. Neuroendocrine (NE) transformation is a kind of resistance mechanism with poor prognosis and lack of effective therapeutic strategies. In published series to date, the molecular characteristics of NE transformation are mainly investigated in EGFR-mutant lung adenocarcinoma (LUAD). Nevertheless, as for ALK-rearranged LUAD, whether it shares the same mechanism or has a unique mechanism is still largely unknown because of a low incidence rate and a paucity of paired pretransformation and post-transformation clinical samples.\(^1,2\)

Here, we report a patient with metastatic ALK-rearranged LUAD with NE transformation after progressing on the third lines of ALK inhibitors. Further analyses of prettransformation and posttransformation samples from this case were performed at both genomic and transcriptomic levels, providing specific molecular basis of NE transformation in ALK-rearranged LUAD.

**Materials and Methods**

**Patient Information**

The patient was treated and evaluated at Guangdong Provincial People’s Hospital. Tissue and liquid samples of this case were collected. Written informed consent of tumor acquisition for research has been obtained before surgery and approved by internal review board from Guangdong Lung Cancer Institute and Guangdong Provincial People’s Hospital (Guangzhou, People’s Republic of China, Institutional Review Board–approved protocol number GDREC2016175H).

**Sequencing Analysis**

Target panel sequencing, whole exome sequencing (WES), and bulk RNA sequencing were performed in LUAD samples and transformed samples of the case. The details can be found in the Supplementary Materials and Methods.

**Results**

A 58-year-old female never smoker was diagnosed with having stage IVB lung cancer with bilateral lung, bone, and liver metastases in May 2018 (Fig. 1). Biopsy result of the left supraclavicular lymph node revealed adenocarcinoma, and the specimen (T1) was positive for ALK by immunohistochemistry (IHC) (D5F3 clone). Comprehensive genomic profiling using next-generation sequencing (NGS) revealed noncanonical ALK fusion with SYNE1 (Fig. 2A). She received first-line crizotinib and had a partial response lasting only 6 months. Disease progression with lung nodules was identified by computed tomography. She underwent lung biopsy, and the specimen (T2) had the original ALK rearrangement but no crizotinib-resistant mutation. Crizotinib was continued, but new symptoms of dizziness and headache occurred after 2 months, and magnetic resonance emission tomography...
Figure 2. Genomic characterization of NE transformation. (A) ALK-SYNE1 fusion was detected by NGS. (B) The mutation landscape defined by WES and the Venn diagram revealing the number of shared mutations in T1, T2, and T3. (C) Copy number variations on cancer genes. (D) Predicted neoantigen burden. (E) Clonal evolution of resistance to ALK inhibitors in the case. (F) Mutational spectra based on 96 trinucleotide contexts and relative contributions of each signature are plotted. HR, homologous recombination; indel, insertion and deletion; MMR, mismatch repair; NE, neuroendocrine; NGS, next-generation sequencing; SB, strong binder; SNV, single nucleotide variant; WB, weak binder; WES, whole exome sequencing.
imaging result revealed new brain metastases. She refused to change the treatment and continued crizotinib until further disease progression 3 months after. Lumbar puncture was done and cerebrospinal fluid (CSF) was collected at that time. No tumor cell was found in the CSF, but ALK-SYNE1 fusion was detected in CSF cell-free DNA by NGS (Supplementary Fig. 1B). Nevertheless, the screening SAF-189s clinical trial for this patient failed because the patient’s electrocardiogram result revealed QTcF that exceeded 470 msec. She was then treated with SAF-189s in compassionate use of investigational drugs and achieved a progress-free survival of nearly 8 months. After the tumor progressed, she switched to alectinib without rebiopsy. Alectinib resulted in stable disease for approximately 5 months before tumor progression of all known lesions and marked increase of tumor biomarker NSE (Supplementary Fig. 1C). Rebiopsy of the right supraclavicular lymph node revealed SCLC combined with large cell NE carcinoma (approximately 30% and 70%, respectively). ALK protein was retained and programmed death-ligand 1 result was negative by IHC (Supplementary Fig. 1A). The specimen (T3) retained the original ALK rearrangement and harbored new mutations that were not present at the initial sample by NGS. She started on carboplatin/etoposide and continued alectinib. Partial response was achieved intracranially and extracranially, but she became comatose 8 months after. Magnetic resonance imaging result confirmed severe tumor progression in the brain. Lumbar puncture was performed again, and suspected cancer cells were found in the CSF. Further NGS revealed the original ALK rearrangement and some new mutations in the CSF (Supplementary Fig. 1B). Finally, she was switched to lorlatinib for 1 month but her condition deteriorated rapidly. She died eventually nearly 11 months after the detection of NE transformation.

**Genomic Landscape of Pre- and Post-NE Transformation**

For in-depth characterization of NE transformation in this patient, we performed WES of specimens T1, T2, and T3. Multiple shared mutations were identified in all specimens, confirming they were clonally related. Nevertheless, T2 shared much less mutations than T1 and T3 possibly owing to interlesional heterogeneity, and similar result was observed in copy number variation (Fig. 2B and C). Neither RB1 nor TP53 mutation was detected before or after transformation, but CDK4 amplification, CDKN2A deletion, and CDKN2B deletion were found in T3 (Fig. 2C), implicating CDK4 might be a potential therapeutic target. In addition, tumor neoantigen burden was notably higher after NE transformation (Fig. 2D). Clonal analysis suggested a founder clone harboring ALK-SYNE1 rearrangement before diagnosis. No acquired ALK mutation but a subclone with RHOD and RHPN2 mutations was developed after sequential ALK tyrosine kinase inhibitor treatments, and parental ALK-SYNE1 clone was still present during transformation (Fig. 2E). There was no obvious change in activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) hypermutation signature (signature 2), whereas mismatch repair (MMR)-associated mutational signature (signature 15) was significantly enriched after the transformation (Fig. 2F).

**Transcriptomic Landscape of Pre- and Post-NE Transformation**

We then analyzed gene expression of specimens T1 and T3 using RNA sequencing and determined differentially expressed genes between T1 and T3. Genes associated with Notch signaling and PI3K/AKT pathway were significantly up-regulated in T3, whereas genes related to lymphocyte activation and NF-κB signaling were up-regulated in T1 (Fig. 3A). Gene set enrichment analysis reveals prominent enrichment of signatures relating to the homologous recombination, MMR and Notch signaling pathways in T3 (Fig. 3B), which were validated in The Cancer Genome Atlas cohorts (Supplementary Fig. 1D). In addition, the biological processes enriched in T3 were DNA replication, cell cycle, and neuron projection development. In contrast, T1 was significantly involved in adaptive immune response, lymphocyte activation, and complement activation (Fig. 3C).

Given both differential expression genes and enriched pathways were closely linked to immune response, we further performed immune deconvolution of the transcriptomic data using CIBERSORT. T1 possessed higher infiltrated levels of T cells CD8 and NK cells activated, whereas T3 had the most prominent infiltration of macrophages M2 which was further validated in The Cancer Genome Atlas cohorts (Fig. 3D and E), indicating tumor immune microenvironment changed from active to suppressive during the process of transformation.

**Discussion**

NE transformation has been reported in several patients with ALK-rearranged NSCLC after progression on ALK inhibitors, but to our knowledge, this is the first study to take advantage of the matched pre-transformation and post-transformation samples to gain insight into the molecular landscape at both DNA and RNA levels in ALK-rearranged lung cancer.

Previous studies revealed that RB1 and TP53 loss is necessary but not sufficient to induce lineage plasticity
There are 12 reported ALK-driven cases with NE transformation, three of whom received NGS of RB1 and TP53 and one received IHC analysis of RB1 and TP53. Nevertheless, only one case has concomitant RB1 and TP53 mutations. In the present case, neither RB1 nor TP53 mutation was detected before or after transformation, but loss of CDKN2A was found instead, which was also observed in Coleman’s case. CDKN2A, as a CDK4/6 inhibitor, plays a pivotal role in cell cycle and participates in the RB pathway. Notably, CDKN2A loss and RB1 loss are mutually exclusive in most cancers, including lung cancer, suggesting the control of CDK4/6 activity and RB status seem to define a single element of the pathway. Therefore, CDK4 inhibition might be a potential therapeutic target of NE transformation. The clinical trial evaluating the efficacy of CDK4/6 inhibitors in chemorefractory, Rb wild-type extensive SCLC is still ongoing (NCT04010357). In addition, AID/APOBEC-associated mutational process was hyperactivated during SCLC transformation in EGFR-mutant LUAD. Nevertheless, MMR-associated mutational signature but not AID/APOBEC mutation signature seems to play a major role in NE transformation for this case.

Consistent with previous publications, high expression of genes in cell cycle and DNA repair was found after the transformation. Furthermore, up-regulation of homologous recombination and MMR signaling in post-transformation sample is in agreement with the highly proliferative capacity of SCLC. Notably, it is considered that inhibition of the Notch pathway is a prerequisite for SCLC transformation, as related genes were downregulated when comparing T-LUAD with control LUAD and comparing T-SCLC with de novo SCLC. Nevertheless, in this study, Notch signaling was significantly activated on transformation. The above-mentioned
discrepancy may be explained by the following possibilities: (1) Notch signaling can be both tumor suppressive and pro-tumorigenic in SCLC; (2) Notch signaling is in repressed state during the transformation process compared with untransformed LUAD and de novo SCLC, but it is up-regulated at the late stage of the transformation process compared with that in the early stage. M2 macrophages, as an immunosuppressive subtype of tumor-associated macrophages enriched significantly, which at least partially accounted for the immune-suppressive microenvironment after the transformation.

In summary, our case indicated that the mechanism of NE transformation in ALK-rearranged LUAD may be different from that in EGFR-mutant LUAD.

CRediT Authorship Contribution

Jie Huang: Investigation, Data curation, Writing—original draft preparation, Visualization.
Shi-Ling Zhang: Investigation, Data curation, Visualization.
Chaozheng Zhou: Visualization, Formal analysis.
Weiyu Huang: Pathological diagnosis, Visualization.
Peng Luo: Formal analysis.
Hua-Jun Chen: Validation.
Jin-Ji Yang: Conceptualization, Methodology, Writing—review and editing.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the JTO Clinical and Research Reports at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2022.100338.

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