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Therapy-induced APOBEC3A Drives Evolution of Persistent Cancer Cells

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Case Study

Introduction

Cancer research is a complex and multifaceted field that requires the integration of various technological approaches to unravel its pathogenesis and identify potential therapeutic targets. Among these technologies, **whole-genome sequencing (WGS)** has emerged as a powerful tool that has demonstrated unique value in cancer research.

Published on August 23, 2023, in the prestigious journal ***Nature***, a study led by Dr Kazuo Isozaki, alongside teams from renowned oncology research centers, explored the intriguing evolution of resistance mutations in **non-small cell lung cancers (NSCLCs) treated with tyrosine kinase inhibitors (TKIs)** [1]. Utilizing comprehensive genomic profiling of patient tumors, this study offered new insights into the mechanisms of tumour evolution during treatment by identifying and mapping the mutational signatures linked to APOBEC enzymes, particularly enriched in patients with prolonged responses to targeted therapies. The research in this paper focused on the following key questions:

- **How do non-small cell lung cancers (NSCLCs) develop resistance to tyrosine kinase inhibitors (TKIs)?**
- **What role does APOBEC3A-induced mutagenesis play in the evolution of drug-resistant cancer cells?**
- **Are there specific mutational signatures associated with APOBEC activity in TKI-treated tumors?**
- **Can inhibiting APOBEC3A expression or activity prevent or delay the emergence of drug resistance in NSCLC?**

Background

Targeted therapies have improved the survival rate for cancer patients, but acquired resistance remains an unsolved clinical problem in targeted cancer therapies, greatly limiting their effectiveness. Although many drivers of acquired resistance have been identified, the potential molecular mechanisms by which tumours evolve during treatment are not fully understood. Understanding the potential molecular mechanisms of tumour evolution enables us to stay ahead of the disease and intervene at an earlier stage to enhance treatment effectiveness.

Tyrosine kinase inhibitors (TKIs) are a form of targeted therapy used to treat non-small cell lung cancer (NSCLC), although individuals frequently develop resistance mutations after receiving TKIs. Genomic profiling of patient cancers has linked APOBEC cytidine deaminases to tumour evolution; however, their involvement during therapy and the development of acquired drug resistance remains unknown.

This study examined a specific case of ALK fusion-positive NSCLC, where distinct clones with resistance mutations

evolved from an initial clone with an ALK E1210K mutation during crizotinib treatment. Using whole-exome sequencing, researchers observed a significant increase in the tumor mutation burden post-treatment compared to pre-treatment levels. A detailed mutational analysis revealed that many of these new mutations were associated with APOBEC cytidine deaminase activity, characterized by C>T and C>G substitutions at specific DNA motifs.

Research Pipeline and Methodology

The study utilized various cell lines, including PC9, H3255, H3122, and others, all obtained from the MGH Center for Molecular Therapeutics. These cell lines were authenticated using STR analysis. Patient-derived cell lines were established from biopsy or pleural effusion samples, with patients providing informed consent for research purposes. Cell lines were cultured in appropriate media, and resistant clones were developed by exposing parental cells to escalating concentrations of gefitinib or lorlatinib until resistance was established. Drug-tolerant persister (DTP) cells were generated by treating sensitive cells with TKIs for 14 days.

For **whole-genome sequencing (WGS) and whole-exome sequencing (WES)**, DNA was extracted from cell lines and tumor specimens. Sequencing libraries were prepared and sequenced by the Broad Institute Genomics Platform, **Novogene**, or New York Genome Center, using Illumina platforms. Sequencing data were aligned to the human genome, and mutations were called and analyzed using established pipelines.

RNA sequencing was performed to analyze gene expression and mRNA editing activity. Chromatin immunoprecipitation (ChIP)-PCR and ATAC-seq were conducted to study chromatin accessibility and transcription factor binding. Various assays, including digital PCR, comet assay, immunofluorescence, and western blotting, were used to assess gene expression, DNA damage, cell cycle profiling, and protein interactions. The overall research pipeline is provided in the following flowchart (Fig. 1).

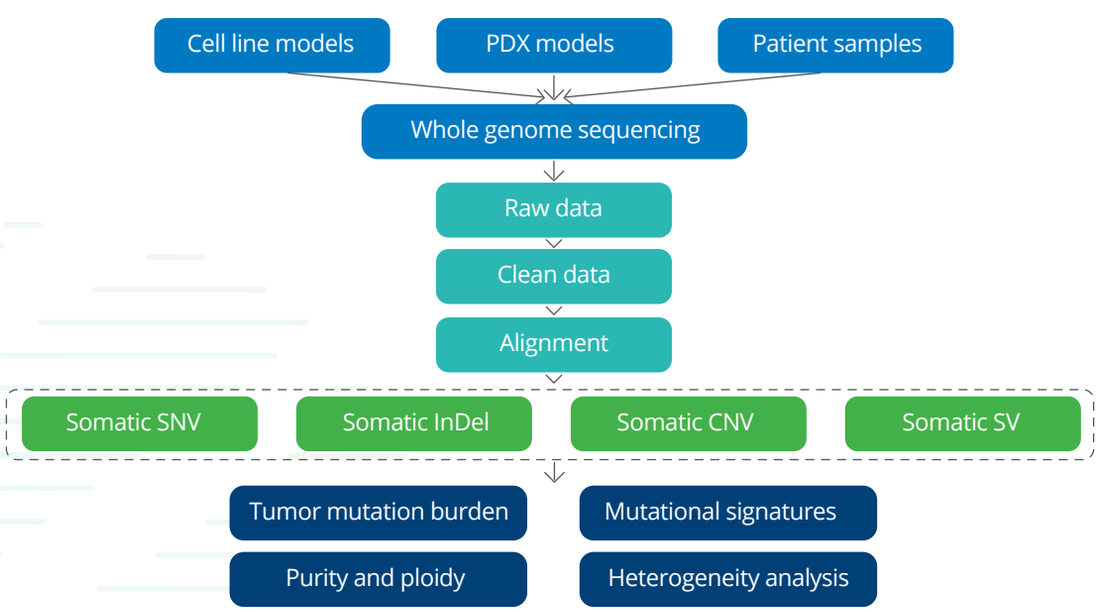
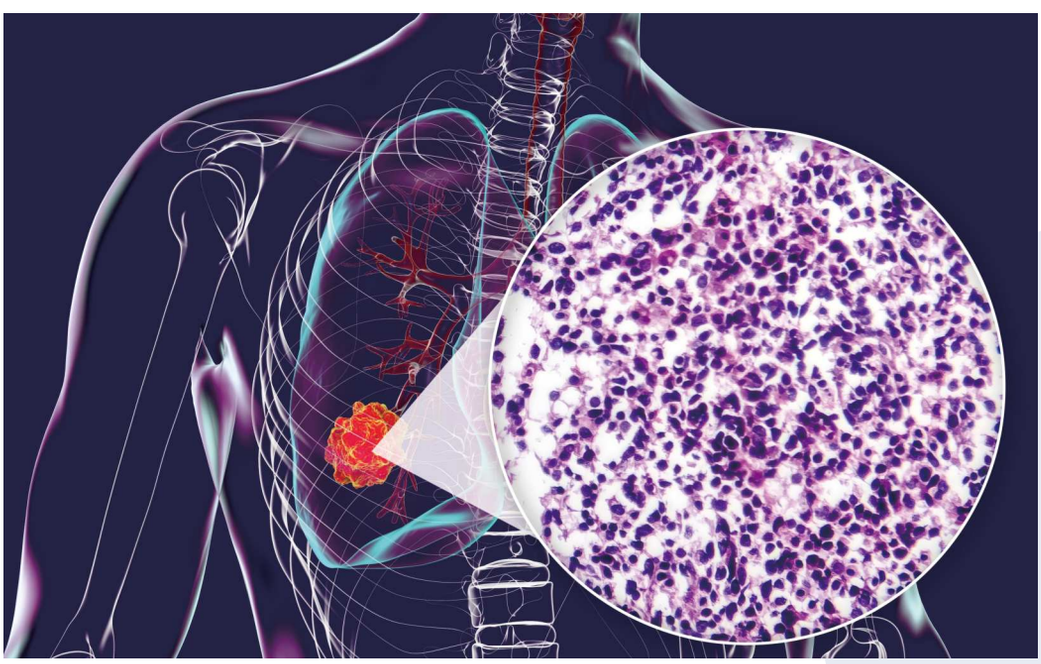


Fig. 1 Research pipeline

Results

The study found a marked increase in APOBEC mutations in resistant tumors post-TKI treatment. In the case of the ALK-positive NSCLC patient, these mutations appeared during sequential TKI therapies. Similar patterns were observed in EGFR-mutant NSCLC patients, where APOBEC mutations were significantly higher in post-treatment tumors compared to pre-treatment samples. In addition, the study demonstrated that targeted therapies could induce the expression of APOBEC3A, a key enzyme in the APOBEC family, leading to increased mutagenesis in cancer cells.

This induction was linked to the suppression of oncogenic signaling pathways, particularly those involving EGFR and ALK. By examining the evolutionary history of resistant clones, researchers found that late-evolving clones with high levels of APOBEC mutations showed greater genomic instability and structural variations compared to early-resistant clones. These results highlight the dynamic nature of tumor evolution under targeted therapy pressure and the pivotal role of APOBEC enzymes in this process.



Whole-Genome Sequencing (WGS) on Experimental Cell Line Models

The application of Tyrosine kinase inhibitors (TKIs) in experimental cell line models demonstrated that these treatments facilitate the accumulation of APOBEC3A (A3A) mutations. This process significantly contributes to the evolution and diversification of cancer cell clones, illustrating how TKIs might inadvertently promote genetic adaptations in cancer cells.

Further investigation using whole-genome sequencing of cell line models of TKI resistance confirmed that late-evolving resistant clones, derived from drug-tolerant persister cells, exhibited higher numbers of mutations, including clusters indicative of APOBEC mutagenesis. These findings were consistent across multiple models and patient samples, suggesting that APOBEC mutagenesis plays a critical role in the development of TKI resistance.

RNA sequencing and ATAC-seq on Cells with TKI Treatment

RNA-seq and ATAC-seq analyses revealed that the mutations accumulated in TKI-treated lung cancer cells stem from A3A activity induced by the treatment. Further investigations identified that this induction of A3A occurs through the activation of the transcription factor NF-κB1, suggesting a specific molecular pathway through which TKIs influence cellular genetics.

Whole-Genome Sequencing (WGS) on PDX Mouse Models

In patient-derived xenograft (PDX) mouse models treated with TKIs, A3A induction was observed to promote DNA damage. This finding provides a clear mechanistic insight into why chromosomal aberrations are prevalent in TKI-treated cells, underpinning the genetic instability that characterizes these cancer therapies.

The Characterization of A3A Overexpression Cell Lines

Cell lines engineered to overexpress A3A showed an increased survival rate of persistent resistance cells under TKI treatment, leading to the emergence of resistant clones. This result highlights the critical role of A3A in enabling cancer cells to withstand therapeutic pressures and evolve resistance mechanisms.

WGS or WES on NSCLC Patients Who Acquired TKI Resistance

Whole-genome sequencing (WGS) or whole-exome sequencing (WES) performed on NSCLC patients who developed resistance to TKIs revealed that post-treatment samples frequently exhibited increased frequencies of APOBEC mutations, along with signs of A3A induction. This supports the conclusion that A3A-mediated mutagenesis is a significant factor in the evolution of tumours under the pressure of targeted therapies, confirming its pivotal role in clinical scenarios.

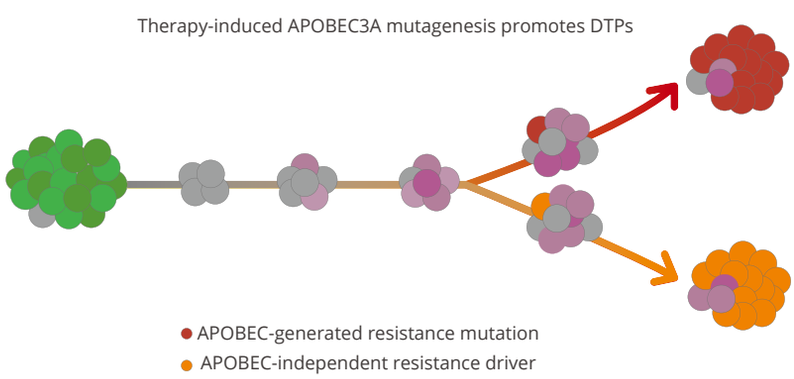


Fig. 2 Conceptual illustration of therapy-induced APOBEC mutagenesis, adapted from [1]

Conclusion

This case study investigates the role of APOBEC enzymes in the development of resistance to targeted therapies in non-small cell lung cancers (NSCLCs).

- The research focused on ALK fusion-positive and EGFR-mutant NSCLC patients who underwent sequential TKI treatments.
- The majority of NSCLC patients treated with TKIs showed an enrichment of APOBEC mutational signatures, particularly those who underwent extended and multiple lines of therapy.
- The study suggests that other APOBEC family members, such as A3B, might also contribute to the evolution of resistant tumors.
- The study also found context-specific differences in mutational patterns resulting from either A3A or A3B, with most post-treatment samples showing mutations in the YTCA context and stem-loop hairpin motifs, which are favored by A3A.
- Additionally, many A3A-associated RNA editing events were synonymous substitutions, not altering protein sequences, indicating that the role of APOBEC3A in drug resistance is complex and not solely dependent on specific driver mutations.

In summary, by examining clinical and experimental models, the study highlights how NSCLC tumors treated with tyrosine kinase inhibitors (TKIs) evolve to acquire resistance through APOBEC-mediated mutagenesis. The study further explored the potential therapeutic implications, suggesting that inhibiting A3A might prevent, or delay acquired resistance to lung cancer therapies. While efforts to develop catalytic APOBEC inhibitors are ongoing, targeting the expression of A3A through key transcription factors like NFκB could offer an alternative strategy.

References

[1] H. Isozaki *et al.*, "Therapy-induced APOBEC3A drives evolution of persistent cancer cells," *Nature*, 2023, doi: 10.1038/s41586-023-06303-1.

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