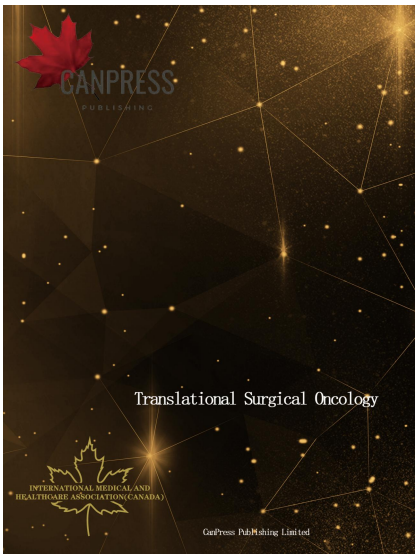
APOBEC3 deaminase drives acquired anticancer targeted therapy resistance



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Abstract

Despite the huge success of targeted therapy in the last two decades, their clinical application has been hindered by the emergence of drug resistance. Early investigation into the mutation patterns of cancer genomes have revealed the role of APOBEC in tumor mutagenesis. However, the role of APOBE in tumor evolution and the development of acquired therapy resistance remains unclear. Recently two research groups signified therapy-induced APOBEC as a key mutagenesis machinery involved in the formation of double-strand DNA breaks and enhancing genomic instability to promote tumor evolution. Most of these mutations are observed in driver oncogenes, making cancer cells independent of their conventional driver genes/pathways leading to acquired therapy resistance. Overall, the data indicate that induction of APOBEC in response to targeted therapy drives the evolution of resistant clones and the inhibition of APOBEC might serve as a new strategy for the prevention of acquired therapy resistance in cancer.

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Therapeutic resistance to targeted therapies is the key challenge in the treatment of patients with advanced cancer. Most patients with advanced cancer ultimately fail to respond to targeted therapy and develop therapy resistance[1]. Resistance to targeted therapies could arise from genetic mutations in the drug target, the reactivation of the targeted pathway, or the activation of alternate pathways that facilitate sustained proliferation[2]. Although various drivers of acquired drug resistance have been discovered, the underlying molecular mechanism fostering tumor heterogeneity and clonal evolution are inadequately studied. Two papers published recently in Nature and Cancer Cell linked therapy-induced cancer mutagenesis with the development of acquired drug resistance[3,4]. The two research groups independently unveiled an important role of apolipoprotein B messenger RNA editing catalytic polypeptide-like (APOBEC) family of enzymes in tumor evolution and therapy resistance.

APOBEC is a deaminase that catalyzes the hydrolytic deamination of cytidine to pro-mutagenic uridine (C → T or C → G) at TpC motifs in single-stranded (ss) DNA. APOBEC-associated mutation signatures have been identified in a wide variety of cancer types [5-7]. Among the candidates APOBEC cytidine deaminases, APOBEC3A (A3A) and APOBEC3B (A3B) have been mostly implicated in human cancer progression [8-10]. Recent findings have confirmed the sequence-specific deamination by A3A and A3B at TpC sites: A3A deaminates a pyrimidine at the -2 position (YTCA) whereas 3B deaminates at purine (RTCA). Expression of A3B has been shown to be upregulated in various cancers, however, A3A has over 100-fold greater mutation potential in vitro than A3B [11].

It has been previously known that non-small cell lung cancers (NSCLCs) develop drug-resistance mutations after treatment with tyrosine kinase inhibitors (TKIs) due to the evolution of drug-tolerant clones[12,13]. However, the mechanism driving the drug-induced mutations is not clear.Isozaki et al. [3]observed a substantial increase in A3A transcription in the EGFR-mutant NSCLC cell line following treatment with the EGFR TKI Osimertinib. An independent analysis of 21 NSCLC patient-derived cell lines having ALK, KRAS, and

EGFR driver mutations resulted in a 4-fold increase in A3A expression when treated with their corresponding drug targets. Markedly, the authors observed a strong association between C→U RNA editing and A3A mRNA levels in all NSCLC cell lines treated with or without TKI. These findings reveal that targeted treatment of oncogene-driven NSCLC leads to A3A upregulation. In addition, estimation of the RTCA versus YTCA character of resistant tumors from ALK and EGFR patients revealed a bias towards YTCA motifs, highlighting the leading role of A3A, but not A3B, in inducing APOBEC-associated mutations in NSCLC.

Since tumor evolution could result from mitotic errors or error-prone repair of double-strand DNA breaks (DSBs) resulting in chromosomal instability and genomic heterogeneity [14]. The authors speculated that TKI-induced A3A activity could have elicited chromosomal aberrations resulting in tumor evolution and TKI-induced therapy resistance. To verify this, Isozaki et al. tested whether TKI induces DNA damage in drug-tolerant persister cells (DTP) cells and surprisingly found that DTP cells displayed an increased expression of γH2AX, a marker of DNA damage response, in the G2/M phase. DTP cells accumulated DSBs when treated with TKI and these DSBs were diminished following knockout (KO) of A3A. Therefore, these data signified the role of TKI-induced A3A in promoting DNA damage and provided a molecular basis for the chromosomal alterations observed in TKI-treated cells.

Another study by Li et al.[4] also observed genomic irregularities as the crucial factor associated with AR therapy resistance in prostate cancer (PCa). Using advanced algorithms, they identified SYNCRIP (Synaptotagmin Binding Cytoplasmic RNA Interacting Protein) as a significant candidate associated with AR therapy resistance. Patients with loss of SYNCRIP have a higher risk of therapy resistance and develop AR therapy resistance earlier than the patients with wild-type SYNCRIP. Lower levels of the full-length SYNCRIP were found in androgen-independent PCa cell lines compared to the androgen-dependent cell lines. The authors further demonstrated that loss of SYNCRIP conferred considerable resistance to enzalutamide across different PCa cell lines and in vivo xenograft models.

SYNCRIP is an inhibitory protein that binds to the APOBEC1 to block its RNA editing activity[15]. Li et al. observed increased expression of DNA damage response markers, γH2AX and 53BP1, in resistant SYNCRIP-deficient tumors [4]. Since the ectopic level of several APOBEC proteins has been known to cause DNA damage and introduce C to T/G/A DNA mutations, the authors speculated that SYNCRIP-deficiency may alleviate the inhibition of APOBEC. They subsequently demonstrated that the APOBEC-driven mutations in the SYNCRIP-deficient cells were considerably dominant compared to the wild-type cells, indicating that APOBEC is involved in eliciting DNA mutations in SYNCRIP-deficient cells.

Interestingly, Li et al. found that SYNCRIP directly interacts with APOBEC A3B but not A3A. The expression of A3B was constantly upregulated in four independent patient-derived explants (PDEs) upon treatment with enzalutamide, indicating A3B as the driving factor in conferring resistance in these PDEs. A3B inhibitors considerably reduced the growth of enzalutamide-resistant patient-derived organoids (PDOs), supporting the clinical significance of A3B in steering AR therapy resistance. The In vitro SYNCRIP deficiency led to a significantly higher frequency of RTCW mutations (~ 55-fold) compared to YTCW mutations (~ 17-fold), emphasizing the dominant role of A3B in targeted therapy-resistance in PCa in contrast to the finding of Isozaki et al. in lung cancer, which shows a dominant role of A3A.

Isozaki et al. performed ATAC–seq on TKI treated PC9 cells to identify enhanced chromatin accessibility regions upstream of the A3A transcriptional start site. They further evaluated the ENCODE database and revealed several transcription factors, including NF-κB, that bind these enhancer regions. The role of NF-κB in TKI-induced A3A upregulation was further validated through RNAi, ChIP and inhibition of IKKβ, an upstream kinase activating p50 nuclear translocation[3].

In Pca, a completely different molecular basis was noticed for the APOBEC-driven resistance. Li et al. hypothesized that the accumulation of mutations in some oncogenes leads to transcriptional alterations and activation of oncogenic downstream signaling pathways, making PCa cells independent of AR. To identify these resistance-driver oncogenes, the authors compared the whole exome sequencing (WES) results of the wild-type and SYNCRIP-deficient PCa cells and discovered that mutations were mostly enriched in the genes that are known to be mutated in cancer. By integrating the mutational frequencies of the APOBEC-mutated genes with the change in expression of their downstream targets, 16 genes were ascribed as potential resistance drivers. These drivers displayed an increased frequency of APOBEC mutations and significantly increased downstream signaling in enzalutamide-resistant SYNCRIP-deficient cells. In addition, 44 downstream effector genes showed increased expression that might contribute to therapy resistance. To discover which of these 16 mutated genes and their 44 downstream effectors are key drivers involved in conferring resistance, the authors individually deleted all these 60 genes to restore the sensitization to enzalutamide. Notably, the KO of eight potential genes including FOXA1, EP300, AR, BRD7, CBX8, HDAC5, HSF4, and STAT3, significantly reduced the growth of SYNCRIP-deficient PCa cells treated with enzalutamide. Evolutionary trajectory revealed that activated FOXA1 signaling plays a dominant role in driving AR therapy resistance. Since these mutations were identified in in vitro cultured cells, it would be interesting to know whether these gene mutations are also present in enzalutamide resistant prostate cancer patients and whether they can be targeted for the therapy. In line with this, EP300 bromodomain inhibitors have been promising in treating castration resistant PCa in

preclinical models[16,17]. Using single-cell RNA-sequencing, the authors observed elevated intratumoral heterogeneity in SYNCRIP-deficient tumor cells after both acute and prolonged treatment with enzalutamide. This implies that APOBEC-driven mutagenesis drives tumor heterogeneity and fosters the development of new resistant cells.

To study the consequences of APOBEC-induced mutations on the evolution of drug resistance, Hideko et al. demonstrated that A3A plays an important role in the emergence of DTP colonies following TKI treatment. In addition, the authors performed a cell pool assay comprising equal numbers of control and A3A KO cells and demonstrated that most of the DTPs emerged from the control clones whereas A3A KO clones were nearly wiped out following TKI treatment. Thus, TKI-induced A3A improves the survival of DTPs eventually resulting in the emergence of resistant clones. Similar results were observed by Li et al. in their evolutionary trajectory study of wild-type and SYNCRIP-deficient clusters. In contrast to the SYNCRIP-deficient cells, the evolution of resistance in the wild-type clusters was independent of both intertumoral heterogeneity and mutational burden. This emphasized that APOBEC-driven mutagenesis is the driver of intertumoral heterogeneity and therapy resistance in SYNCRIP-deficient PCa cells.

Overall, these two papers provide compelling evidence that patients treated with prolonged anti-cancer targeted therapies demonstrate increased APOBEC mutational signatures. Therapy-induced APOBEC expression or loss of SYNCRIP-associated APOBEC activation promotes the formation of double-strand DNA breaks and increased mutations. This leads to increased genomic instability, resulting in tumor heterogeneity and clonal evolution (Figure 1). Most of these mutations are observed in crucial driver oncogenes, activating downstream signaling pathways and thereby conferring acquired therapy resistance. In addition, the acquisition of small clusters of APOBEC mutations in targeted therapy-treated cells and xenograft mouse tumors proves the role of APOBEC in tumor evolution. These results suggest that the suppression of APOBEC is sufficient to reduce tumor mutational burden, providing novel insight into the development of therapeutic strategies targeting APOBEC.

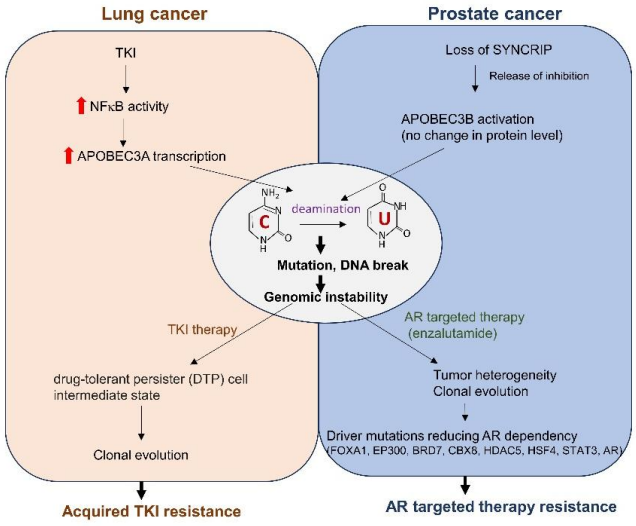

Figure1: Comparison of APOBEC-driven mutagenesis in developing therapy resistance in lung and prostate cancers.

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