Distinct Mutagenic Activity of APOBEC3G Cytidine Deaminase Identified in Bladder Cancer

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The APOBEC cytidine deaminase enzyme family is linked to mutational signatures identified in cancer. While previous work has provided insights into the role of APOBEC3A and APOBEC3B in mutational processes in cancer, understanding of the mutational signatures induced by other APOBEC family members is limited. In this issue of *Cancer Research*, Liu and colleagues investigated the role of APOBEC3G (A3G) in bladder cancer. The authors revealed that transgenic expression of *A3G* in a murine bladder cancer model promotes tumorigenesis and induces a unique mutational signature distinct from previously identified APOBEC signatures. Expression of this A3G-related mutational signature correlated with significantly worse survival in patients with urothelial bladder carcinoma, and *A3G* expression was identified in 21 different cancer types. These findings suggest that different APOBEC3 enzymes induce unique mutation signatures and play distinct roles in cancer evolution. More complete understanding of the function of each APOBEC3 enzyme will improve anticancer therapy.

See related article by Liu et al., p. 506

As next-generation sequencing costs decreased, characterization of the mutational processes that occur in cancer became possible. Nik-Zainal and colleagues were one of the first groups to explore the mutational signatures present in 21 patients with breast cancer, revealing five unique mutational signatures (1). Measuring intermutational distance in the genome uncovered mutation clustering termed *kataegis*, from the Greek word for thunderstorm. These regional clusters mostly occurred on the same parental chromosome and were present in 13 of 21 of the breast cancers sequenced. On the basis of previous work, the authors theorized that the underlying mechanism driving *kataegis* was most likely induced by the AID/APOBEC cytidine deaminase enzyme family. This study elegantly demonstrated that mutations fall into patterns that repeatedly occur in multiple breast cancer samples, but many questions remained, including the timing, underlying mechanisms, broader applicability across cancer types, and importance of APOBEC-associated mutational processes in tumor evolution.

A subsequent study by Alexandrov and colleagues began to address the question of whether many of the mutational signatures identified initially in breast cancer were present in other cancer types (2). The authors analyzed 7,042 primary tumors of 30 different cancer types. This study allowed for a more complete illustration of the landscape of mutational patterns that exist within and across different cancer types. Certain mutational patterns were linked to exogenous mutagenic exposures including smoking, more commonly observed in lung cancer, and UV exposure, more commonly observed in melanoma. In contrast, other mutational processes, such as the mutagenic signature linked to APOBEC family members, were observed in multiple cancer types including breast, cervical, chronic lymphoid leukemia, and lung cancer. With the incontrovertible evidence that the mutational signature linked to APOBEC cytidine deaminase enzymes was present across cancer types, the question of the specific family members, repair processes, and timing of APOBEC mutagenesis in cancer became more relevant.

With 11 cytidine deaminase enzymes encoded in the human genome, seven of which are APOBEC3 proteins, the specific APOBEC enzymes that induce APOBEC-associated mutation signatures became a critical question for understanding this mutagenic process (3). Preclinical cell line and xenograft models of breast cancer were utilized to uncover the endogenous mutagenic processes driving the APOBEC-linked mutational signatures in breast cancer (3). Nuclear localization of the cytidine deaminase enzyme APOBEC3B (A3B) was identified, and inducible overexpression and knockdown studies revealed A3B-dependent increases in mutation burden. In addition, xenograft studies using breast cancer cell lines with or without A3B depletion demonstrated a more durable response to the hormone therapy tamoxifen with reduced *A3B* expression (4). This phenotype was specifically linked to the cytidine deaminase activity of A3B using a deaminase inactive version of the enzyme (4). These initial studies were important forays into which specific APOBEC enzymes are responsible for the mutational signatures attributed to APOBEC activity, single-base substitution signatures 2 and 13 (SBS2 and SBS13).

More in-depth genomic studies using yeast models further elucidated APOBEC-associated mutational signatures. In one study, Chan and colleagues demonstrated that a distinct APOBEC3A (A3A) and A3B mutational signature was induced in a yeast reporter strain (5). Using these specific A3A and A3B mutational signatures, the authors uncovered A3A as the more prominent mutator when the prevalence of these distinct signatures was examined across cancer types (5). Yet questions remained about the interactions between APOBEC family members and the specific contexts and stage of cancer evolution when these signatures occur.

To more closely study which APOBEC family members are responsible for the APOBEC-associated mutational signatures and the mechanistic nature of this mutagenic process in cancer, a larger number of preclinical cancer models were needed. Two studies led by Petljak and colleagues used long-term sequencing experiments with cancer cells lines and xenograft models (6, 7). This elegant work revealed that APOBEC mutagenesis occurs in episodic bursts in contrast to other endogenous mutagenic processes and that reduction in A3A expression levels reduced APOBEC-associated mutation burden to a greater extent than reduction of A3B expression levels (6, 7). This ground-breaking work was critical for mechanistic insight into these mutational processes, yet the studies highlighted were not carried out using *in vivo*

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experimental models of cancer. *In vivo* models are needed for contextualization of the effects of APOBEC enzyme expression with regard to the stage of tumor progression and tumor immune microenvironment.

Venkatesan and colleagues provided insight into the function of APOBEC3 enzymes in an *in vivo* setting using EGFR-mutant p53-deficient mouse models of lung cancer with or without *A3B* transgenic expression (8). These murine models in combination with TRACERx patient data and cell line models revealed that *A3B* expression significantly increases DNA replication stress and chromosomal instability early in tumorigenesis, driving cancer evolution (8).

The studies highlighted so far in this commentary mostly focused on the two APOBEC3 enzymes in the field considered to be the likely drivers of the SBS2 and SBS13 APOBEC-associated mutation signatures, A3A and A3B. Work focused on characterizing the mutational signatures induced by other APOBEC family members in cancer is limited. Using a murine bladder cancer model, Liu and colleagues showed that transgenic *APOBEC3G* (*A3G*) expression induces a unique mutational signature (SBS.A3G) distinct from SBS2 and SBS13 that is present across multiple cancer types (9). *A3G* transgene expression in the bladder cancer model resulted in significantly worse mouse survival. *A3G* expression was identified in patients with 21 different cancer types, with diffuse large B-cell lymphoma, urothelial bladder carcinoma, and renal clear carcinoma being the top A3G-expressing tumor types. Mutations in an A3G context were significantly correlated with *A3G* mRNA expression in urothelial bladder carcinoma. Classifying patients with urothelial bladder carcinoma into either an SBS.A3G signature dominant group or an SBS2 signature dominant group revealed the SBS.A3G predominant group had significantly worse survival.

In vivo studies, like the one led by Liu and colleagues, are advantageous, as signatures previously not associated with APOBEC activity can be identified and the impact on cancer progression can be studied, illustrating the importance of murine models for scientific discovery. Future *in vivo* studies like this work will add layers of understanding to the function of APOBEC enzymes by allowing for alteration of the context under which APOBEC enzymes are expressed. With temporal and spatial control of APOBEC enzyme expression, induction of APOBEC family members under therapeutic selection or under specific immune microenvironmental conditions will aid in further characterization of the protumor and antitumor effects of APOBEC enzyme function and expression. The study by Liu and colleagues is a timely and important study that will contribute to improved cancer therapeutic strategies aimed at utilizing the APOBEC mutagenic process to inhibit tumor evolution.

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