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When defense turns into attack

Antiviral cytidine deaminases linked to somatic mutagenesis in HPV-associated cancer

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The APOBEC3 cytidine deaminases play an important role in innate immunity but have also emerged as mediators of somatic mutations in human cancer. We recently reported a high incidence of APOBEC-mediated driver mutations in human papillomavirus-associated cancer, suggesting a key role for these enzymes in the development of such tumors.

Keywords: APOBEC, HPV, PIK3CA, cancer, mutagenesis

Abbreviations: APOBEC, apolipoprotein-B mRNA editing catalytic polypeptide like; BER, base excision repair; BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma; HPV, human papilloma virus; HNSC, head and neck squamous cell carcinoma

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The apolipoprotein-B mRNA editing catalytic polypeptide like (APOBEC) family of enzymes catalyze the deamination of cytosine bases, resulting in their conversion to uracil. In humans the APOBEC3 gene cluster on chromosome 22 encodes 7 enzymes (A3A, B, C, D/E, F, G, and H) capable of deaminating cytosines in single-stranded (ss) DNA. Much of our knowledge of A3 gene function has come from studies of A3G hypermutation of HIV cDNA, an innate immune defense. However, a number of recent studies have suggested that certain A3 enzymes can display an off-target activity leading to somatic mutations in cancer. Work by Stratton and colleagues analyzing the nature and sequence context of mutations to identify the mutational processes operating in different cancers uncovered a particular signature of long clusters of the point mutations TC > TT or TC > TG on the same strand, which they called "kataegis," the Greek for thunderstorm. These mutation showers are likely caused by activity of a subset of the A3 enzymes that preferentially target cytosines following a thymine,[1] with the resulting uracil then undergoing transversion to guanine or transition to thymine.

Around the same time, Harris and colleagues showed using functional studies in cell lines and data from clinical samples, that one such 'TC-specific' A3 (A3B), is frequently overexpressed in breast cancer (BRCA) and that it is likely responsible for many of the cytidine mutations seen in these tumors.[2] This discovery was followed by widespread screens for evidence of A3 activity in cancer exome sequences from The Cancer Genome Atlas (TCGA) project and others.[3,4] Several cancer types, notably cervical squamous cell carcinoma (CESC), bladder urothelial carcinoma (BLCA), and head and neck squamous cell carcinoma (HNSC), show a particularly strong enrichment for these signature mutations. Noting that the majority of CESCs are associated with human papillomavirus (HPV) and that A3 enzymes have previously been implicated in hyperediting of HPV DNA in premalignant cervical lesions,[5] we hypothesized that this mutational process may play a particularly important role in the development of HPV-associated cancers. We set out to test this by analyzing HNSC, a tumor type for which sequence data were available for sufficient numbers of both HPV^+ and HPV^- samples to permit a comparative analysis.[6]

We used a variety of metrics to compare the extent of A3-mediated mutagenesis in the HPV^+ and HPV^- HNSC samples, including extraction of mutational signatures and calculating the prevalence of TCW (TpCpA/T) > TTW and TCW

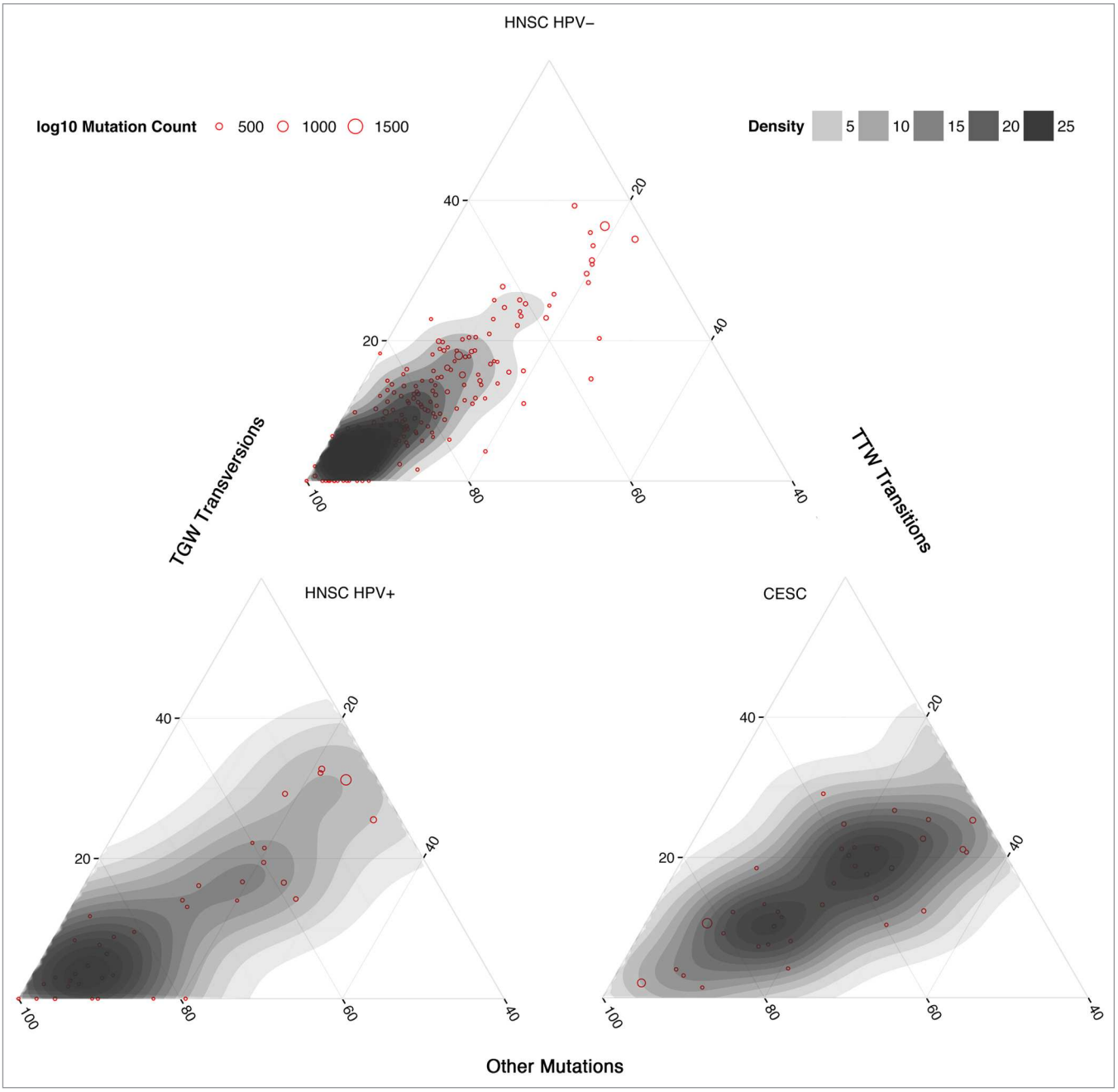

Figure 1. The transition transversion mutational balance. Ternary plots displaying the percentage balance between TCW > TTW transitions, TCW > TGW transversions, and all other point mutations in HPV$^-$ HNSC, HPV$^+$ HNSC, and CESC. HNSC, head and neck squamous cell carcinoma; CESC, cervical squamous cell carcinoma.

> TGW mutations in each sample.[4,7] Each metric by which we could assess A3-mediated mutation showed enrichment with HPV positivity.

Compelling evidence for the involvement of A3 enzymes in the pathogenesis of HPV$^+$ tumors came from examining the genes that are mutated at TCW sites. We found that specific hotspot mutations in the *PIK3CA* proto-oncogene are of the TCW type and that *PIK3CA* is exclusively mutated at these sites in tumors displaying strong exome-wide enrichment for TCW mutations (HPV$^+$ HNSC, CESC, and BLCA).

A number of questions arise from our findings: Does HPV infection or viral oncogene expression actively drive A3 hypermutation of cellular DNA, or is there simply a dearth of other mutational processes in these tumors? What is causing the relatively high level of A3 mutations in BLCA? HPV has been linked with BLCA but a recent comprehensive analysis of viral transcripts in tumor samples suggests that HPV is present in only a very small fraction of BLCA.[8] Whether transient infections might occasionally initiate tumorigenesis by activating APOBECs is a fascinating question.

One striking feature of TCW mutations that we observed is their close relationship with the number of non-TCW point mutations. Furano and colleagues recently demonstrated that ssDNA that is exposed during repair of mismatches can be mutated by TC-specific A3 enzymes.[9] This process occurs when base excision repair (BER) becomes coupled to a non-canonical mismatch repair (MMR) process, a pathway integral to activation-induced cytidine deaminase (AID)-dependent somatic hypermutation in lymphocytes. In such cases, repair of mismatches may expose extended stretches of ssDNA to A3 activity, offering a possible explanation for the strong correlation between TCW and other mutations.

Alternatively, the relative abundance of transitions and transversions at TCW sites can reveal the pathway by which deaminated cytosines are processed in a given tumor type.[10] Evidence from DNA repair models in yeast suggest that C > G transversions result largely from BER whereas C > T transitions may result from error prone DNA translesion synthesis during replication. When TCW > TGW, TCW > TTW and all other point mutations in HPV^+ HNSC and CESC and HPV^- HNSC are represented on ternary plots (**Fig. 1**) the increased fraction of C > T and C > G mutations at vulnerable sites in the HPV^+ tumors is clear. Furthermore, in the HPV^- tumors, TCW mutations are more often of the C > G type, suggesting that BER may be more active in these cells, whereas both HPV^+ HNSC and CESC are particularly enriched for transitions, potentially indicating replication across uracil without repair in HPV^+ tumors (**Fig. 1**; ref. 6).

In conclusion, although studies from multiple groups point to a role for A3 enzymes in the generation of somatic mutations in cancer, our work suggests that this process is particularly important in the development of HPV-associated tumors. Whether HPV infection induces A3 activity, or whether these tumors share common features with other cancers that are enriched for A3-driven mutations, such as defects in certain DNA repair pathways for instance, is currently unclear. Determining the answers to such questions will hopefully give insight into which patients are most likely to develop cancer following HPV infection and potentially open up new avenues for DNA cytotoxic therapy.

Acknowledgments

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References

1. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale A-L, et al.; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer. Nature 2013; 500:415-21; PMID:23945592; http://dx.doi.org/10.1038/nature12477
2. Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, Refsland EW, Kotandeniya D, Tretyakova N, Nikas JB, et al. APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 2013; 494:366-70; PMID:23389445; http://dx.doi.org/10.1038/nature11881
3. Burns MB, Temiz NA, Harris RS. Evidence for APOBEC3B mutagenesis in multiple human cancers. Nat Genet 2013; 45:977-83; PMID:23852168; http://dx.doi.org/10.1038/ng.2701
4. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, Kiezun A, Kryukov GV, Carter SL, Saksena G, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 2013; 45:970-6; PMID:23852170; http://dx.doi.org/10.1038/ng.2702
5. Vartanian J-P, Guétard D, Henry M, Wain-Hobson S. Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. Science 2008; 320:230-3; PMID:18403710; http://dx.doi.org/10.1126/science.1153201
6. Henderson S, Chakravarthy A, Su X, Boshoff C, Fenton TR. APOBEC-mediated cytosine deamination links PIK3CA helical domain mutations to human papillomavirus-driven tumor development. Cell Rep 2014; 7:1833-41; PMID:24910434; http://dx.doi.org/10.1016/j.celrep.2014.05.012
7. Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. Cell Rep 2013; 3:246-59; PMID:23318258; http://dx.doi.org/10.1016/j.celrep.2012.12.008
8. Tang K-W, Alaei-Mahabadi B, Samuelsson T, Lindh M, Larsson E. The landscape of viral expression and host gene fusion and adaptation in human cancer. Nat Commun 2013; 4:2513; PMID:24085110; http://dx.doi.org/10.1038/ncomms3513
9. Chen J, Miller BF, Furano AV. Repair of naturally occurring mismatches can induce mutations in flanking DNA. Elife 2014; 3:e02001; PMID:24843013; http://dx.doi.org/10.7554/eLife.02001
10. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, Jones D, Hinton J, Marshall J, Stebbings LA, et al.; Breast Cancer Working Group of the International Cancer Genome Consortium. Mutational processes molding the genomes of 21 breast cancers. Cell 2012; 149:979-93; PMID:22608084; http://dx.doi.org/10.1016/j.cell.2012.04.024