IN THE SPOTLIGHT

Bringing Oncohistones into the Fold

Jay F. Sarthy1,2 and Steven Henikoff1,3

Summary: Identification of cancer-associated mutations in core histone genes has proved challenging due to these genes’ highly conserved nature and presence in large arrays. Recent analyses of cancer genomes, including one in this issue of Cancer Discovery, show that mutations in the histone fold can affect nucleosome stability, providing a novel mechanism by which oncohistones contribute to tumorigenesis.

See related article by Bennett et al., p. 1438 (2).

At its most fundamental level, cancer is a disease of aberrant gene expression. The most intricate element of this highly orchestrated dance is the establishment and maintenance of chromatin. Nucleosomes are the fundamental subunit of chromatin and comprise a histone octamer tightly wrapped by 147 bp of DNA. Nucleosome-bound DNA is inherently refractory to transcription, providing an important layer of control in regulation of gene expression. As evidence of the importance of histones in gene regulation, “oncohistone” mutations in genes encoding H3 variants are mutated in a variety of rare malignancies including diffuse midline gliomas, chondroblastomas, and giant cell tumor of the bone (1). These mutations are thought to affect the deposition of post-translational modifications (PTM) on the H3 tail involved in transcriptional regulation, thereby resulting in aberrant gene expression.

Two recent studies (2, 3), including one published in this issue of Cancer Discovery (2), have added to the list of histone mutations in cancer and confirm an interesting mechanism that was posited by biochemical studies of oncohistones published last year (4). First, using a combination of publicly available datasets and a large single-institution database, Nacev and colleagues identified more than 4,000 missense mutations in histone-encoding genes in samples derived from approximately 3,000 patients (3). Although some of these mutations were likely passengers in malignancies with high mutational burden, examination of tumors with lower mutational burden revealed approximately 1,900 mutations that are likely to affect cancer biology. In their analysis, histone missense mutations occurred in 3.8% of samples, a conservative estimate that is similar to the prevalence of mutations in BRCA2, TET2, SMAD4, and NOTCH1.

Current oncohistone paradigms are centered on lysine-methylating substitutions in the H3 tail, resulting in dominant-negative oncohistones that inhibit lysine methyltransferases such as EZH2, the H3K27 methyltransferase (1). In this regard, the Nacev study does not disappoint. The authors find restriction of the well-studied H3.3K27M/I substitutions to high-grade gliomas, whereas its putatively rarer cousin H3.1K27M was seen in acute myeloid leukemia and melanoma. Reasons that H3.3K27M/I are restricted to gliomas whereas H3.1K27M occurs in other malignancies were not identified in this study but may be related to different underlying biology of H3 variants. Examination of other lysine to methionine/isoleucine substitutions found H3K4, H3K18, and H4K12 as candidate oncohistone mutations. Whether these K to M substitutions inhibit chromatin-modulating enzymes or simply result from AAG to ATG mutations with minimal biological impact is not known.

Arguably the most interesting finding of the analysis by Nacev and colleagues is that the majority of mutations in core histone genes occurred at sites that are unlikely to affect PTMs, including sites within the histone fold domain. These results were also observed in an independent study by Bennett and colleagues. Using The Cancer Genome Atlas (TCGA) dataset and rigorous bioinformatic analyses, Bennett and colleagues identify novel recurrent mutations in core histones in a variety of malignancies, including several common carcinomas. The H2B-E76K substitution was the most common mutation identified in their analysis. As this mutation is within the globular histone fold domain, it is not expected to affect post-translational modifications.

How might a mutation within the histone fold domain promote oncogenesis? A report by Arimura and colleagues (4) provided the first clues to this mystery. First, they determined the crystal structure of an H2B-E76K–containing nucleosome. Their analysis revealed that the E76K substitution eliminates a salt bridge between H2B-E76 with R92 on H4 (Fig. 1), which is the seventh most common H4 mutation identified in the TCGA dataset. Substitution of glutamic acid with lysine also causes electrostatic repulsion of H4 R92, profoundly disrupting the interaction between the H2A-H2B dimer and H3-H4. Using thermal stability assays, they showed that H2B-E76K–containing nucleosomes dissociate at lower temperatures than their wild-type counterparts, consistent with reduced stability predicted by the crystallographic data.

©2019 American Association for Cancer Research.
They also showed that H2B-E76K dimers do not bind H3-H4 without DNA present, further evidence of an unstable interaction between mutant H2A-H2B dimers and H3-H4.

Similar \textit{in vitro} studies were performed by Bennett and colleagues with both H2B-E76K and another cancer-specific mutation, H2B-E76Q (2). In nucleosome assembly assays with recombinant H2B-E76K, they showed that H2B-E76K–containing nucleosomes are less stable, with a larger fraction of H2B-E76K being found in H2A-H2B dimers instead of histone octamers. Similar results were observed with H2B-E76Q. The authors then turned to yeast to further characterize the H2B-E76K oncohistone. First, they noted that yeast strains carrying H2B-E79K (equivalent to E76K in humans) grew more slowly at restrictive temperatures than their wild-type counterparts. They also showed that H2B-E79K strains contain chromatin that is more sensitive to Micrococcal Nuclease (MNase) digestion, and that these strains struggle to repress \textit{PHOS} expression in phosphate-free media, a condition in which wild-type strains readily silence this gene. Taken together, these two studies provide convincing evidence that H2B-E79K–containing nucleosomes disrupt nucleosome stability and have physiologic consequences for the cell.

Both groups performed similar mammalian experiments to explore the role of H2B-E76K in oncogenesis. Using FRAP assays, they each showed that H2B-E76K recovers much more quickly after photobleaching, consistent with more rapid turnover. Bennett and colleagues explored the transcriptomic consequences of H2B-E76K in untransformed cells using RNA sequencing and found that genes involved in differentiation, proliferation, migration, and cellular signaling were upregulated, whereas biosynthetic processes appeared to be downregulated. They also showed that these differences are unlikely to be related to modulation of histone PTMs, as global methylation levels of the better-characterized modifications including H3K4, K9, K27, and K36 were unaffected by expression of H2B-E76K. Why certain cellular processes appeared to be sensitive to H2B-E76K expression but others were not affected is not known.

Most oncohistones identified to date do not meet the criteria for being oncogenes, but instead work with obligate secondary mutations to promote oncogenesis. Bennett and colleagues explored this concept by expressing H2B-E76K and the co-occurring oncoprotein PI3KCA-H1047R in MCF10 cells. Compared with cells expressing PI3KCA-H1047R and wild-type H2B, cells expressing the double mutants grew more colonies in a soft agar assay, indicating higher malignant potential, consistent with the finding by Arimura and colleagues that H2B-E76K promotes growth on soft agar when expressed in NIH3T3 cells. Finally, Bennett and colleagues show that H2B-E76K–expressing cells also have increased chromatin accessibility using MNase digestion and assay for transposable-accessible chromatin using sequencing (ATAC-seq). These studies provide further support for the hypothesis that mutations in the globular domain of H2B promote oncogenesis through alteration of chromatin accessibility in the context of secondary mutations that drive growth and proliferation.

Although the complementary studies by Bennett and colleagues and Arimura and colleagues greatly expand our understanding of the role of oncohistones and nucleosome dynamics in tumorigenesis, much remains unknown about histone mutations in cancer. For example, these analyses clearly show that some oncohistones display disease-specific associations and require certain secondary mutations, such as the aforementioned H3.3K27M in high-grade gliomas. Why these associations are present is not known but may be related to cell of origin, such as a glioma cell of origin that may be sensitive to subtle changes in PRC2 activity (5).

The precise contributions of unstable nucleosomes to cancer have yet to be fully elucidated. Cancer is increasingly being appreciated as a disease driven by metabolic, transcriptional, and genomic heterogeneity (6). The impact of unstable nucleosomes on heterogeneity is not known, but it is attractive to speculate that oncohistones such as H2B-E76K may promote increased heterogeneity by relaxing regulatory mechanisms imposed by nucleosomes, potentially allowing transcription factors (TF) to engage sites that are normally protected by nucleosomes. Alternatively, oncohistone expression may allow for activation of a transcriptional program, such as a differentiation- and proliferation-promoting pathway, but due to increased nucleosome instability, networks that are normally terminated by these programs may inappropriately persist, such as those mediated by stem-like TFs.

Finally, as the reports by Nacev and colleagues and Bennett and colleagues show, oncohistones are more common and occur in a broader range of malignancies than previously appreciated. It will be important to correlate these mutations with clinical data to assess their correlations with disease subtypes and prognoses. In addition, it is possible that oncohistone-driven malignancies share therapeutic vulnerabilities across cancers, the signatures of which may be ascertained from clinical data. Now that oncohistones have been brought “into the fold,” therapies targeting the biology underlying these drivers of malignancy will undoubtedly become the subject of future investigation.

\textbf{Disclosure of Potential Conflicts of Interest}

No potential conflicts of interest were disclosed.

Published first October 1, 2019.
REFERENCES

Bringing Oncohistones into the Fold
Jay F. Sarthy and Steven Henikoff

Updated version
Access the most recent version of this article at:
http://cancerdiscovery.aacrjournals.org/content/9/10/1346

Cited articles
This article cites 6 articles, 2 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/9/10/1346.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerdiscovery.aacrjournals.org/content/9/10/1346.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.