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A Histone Modifier's Ill-Gotten Copy Gains

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Little is known about the molecular machinery that contributes to site-specific copy number variations or how CNVs fit into the chronology of tumor progression. Black et al. (2013) now demonstrate that the overexpression of a histone demethylase induces transient copy gain of specific genomic loci known to harbor proto-oncogenes.

DNA replication is a tightly regulated and highly coordinated process that minimizes incorporation of mismatched nucleotides and ensures that each DNA strand is faithfully replicated exactly once per cell cycle. This vital task is shared between thousands of separate replicons to accommodate timely genome replication (Pope et al., 2013). Molecular analyses of replication demonstrate that not all replicons behave the same in regard to replication timing during S phase. Transcriptionally active, gene-rich domains replicate during the first half of S phase (early), whereas transcriptionally repressed, gene-poor loci replicate in the second half of S phase (late). Furthermore, alterations in replication timing accompany key stages of development (Hiratani et al., 2009).

In addition to diverse roles of histone modifications in the regulation of gene expression (Lee et al., 2010), there are close links between posttranslational histone modifications and the cell cycle (Schulze et al., 2009). Studies utilizing chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) suggest a role for covalent chromatin modifications in regulating early versus late replicating domains (Schwaiger et al., 2009). It has also been hypothesized that DNA replication is regulated at the chromatin level, where histone modifications recruit factors that impede DNA replication (Black et al., 2010). Chromatin regions lacking these histone modifications are more accessible to the replication machinery and are therefore more likely to be mistakenly rereplicated. In a previous study, Whetstine and colleagues demonstrated that the levels of KDM4A/JMJD2, a histone H3K9 and H3K36-specific demethylase, are regulated during S phase and that KDM4A overexpression increases chromatin accessibility while altering replication timing of specific genomic loci (Black et al., 2010). In this issue, they demonstrate that KDM4A overexpression induces copy number gains at specific loci, such as 1q12, which contain putative oncogenes (Black et al., 2013). Though KDM4A overexpression was previously reported in a small set of cancers (Mallette and Richard, 2012), Black and colleagues extend this list by analyzing the Cancer Genome Atlas (TCGA) for tumors containing increased KDM4A copy numbers and expression levels (Black et al., 2013). They identify ovarian cancer as being significantly enriched for KDM4A copy number amplification in 46% of the tumor samples. Although they were unable to detect copy gains by spectral karyotyping, upon reanalyzing previous KDM4A ChIP-chip data, they identify enrichments for cytogenetic bands such as 1q12 as a result of KDM4A overexpression. Fluorescent in situ hybridization (FISH) experiments in KDM4A-overexpressing cells confirm the copy number increase while ruling out whole-chromosome duplications. Importantly, this site-specific copy gain coamplifies with KDM4A in primary tumor samples.

To investigate the molecular mechanism linking KDM4A and copy number variation (CNV), Black et al., overexpress KDM4A point mutants and find that the Jumonji catalytic domain and both Tudor domains are required for CNV. To exclude the possibility that KDM4A functions through a histone-independent pathway, the authors coexpress histone H3 mutants with methionine in place of either lysine 9 or 36 (H3K9M, H3K36M). Histone mutants that interfere with H3K9/36 methylation recapitulate the KDM4A-dependent CNV phenotype, indicating that KDM4A histone-demethylase activity plays a direct role in this process. Additionally, KDM4A immunoprecipitation resulted in the identification of interacting proteins such as members of the minichromosome maintenance (MCM) complex and several DNA polymerase subunits. The authors speculate that KDM4A promotes unlicensed replication via bypassing the need for the origin of replication complex (ORC) formation via direct MCM and DNA polymerase recruitment (Figure 1).

Previously, Black et al. identified HP1γ as an antagonist of KDM4A-dependent S-phase progression (Black et al., 2010). Here, they further characterize this antagonism by showing that HP1γ overexpression disrupts KDM4A-dependent CNV. HP1γ contains a chromodomain that binds methylated histone H3K9. Therefore, HP1γ is able to block KDM4A demethylation, creating a "steric blockade" that prevents local DNA replication. Consistent with this hypothesis, overexpression of H3K9 methyltransferase Suv39h1/KMT1A is also able to suppress 1q12 copy gains.

Finally, to determine whether 1q12 copy gains are stably inherited by daughter cells, single-cell clones from KDM4A-overexpressing cell lines were generated. Strikingly, when performing 1q12 FISH, only 17% of cells contain the 1q12 copy gain, indicating that KDM4A-dependent copy gains are not stably inherited by daughter cells. By arresting KDM4A-overexpressing cells at either G1/S or G2, it was determined that copy

gains are generated during S phase but disappear by G2 via an undetermined mechanism. This suggests that the copy gains are not incorporated into the genome but, rather, exist as extrachromosomal DNA. Intriguingly, the 1q12/21 regions contain several proto-oncogenes, including Mcl1 and Bcl9, and this region is frequently amplified in lung cancer and multiple myeloma. In addition, 1q12/21 copy gains are associated with drug resistance in ovarian cell lines and multiple myeloma, underscoring the significance of understanding the relationship between KDM4A and 1q12/21 coamplification in human health.

Chromosome copy number alterations are thought to be important for acquired cellular plasticity by allowing a precancerous cell to amplify genes that are required for proliferation, angiogenesis, and evasion of cell death. The discovery of KDM4A overexpression as a driver of CNV has uncovered a potential mechanistic link between local chromatin structure/composition and DNA rereplication with copy number variation. Histone H3K9 trimethylation, the substrate for KDM4A, is associated with both active and repressed gene states. A role for histone H3K9 methylation and HP1-γ in this process is very exciting; however, it is unclear whether KDM4A's role in cancer pathogenesis is due to its function at heterochromatin or through the transcriptional elongation regulatory function associated with H3K9 trimethylation. Among the three isoforms of HP1 (α, β, and γ), HP1- γ is found on transcriptionally active regions (Smith and Shilatifard, 2007). Because KDM4A demethylates histone H3K36, a chromatin mark associated with the elongating and active form of RNA polymerase II (Smith and Shilatifard, 2007), a role for the misregulation of transcription elongation control in cancer pathogenesis through CNV as a result of KDM4A overexpression should also be considered. Indeed, aberrant transcriptional elongation checkpoint control has been proposed as a key regulatory mechanism for leukemic pathogenesis through chromosomal translocations and other forms of cancer (Smith and Shilatifard, 2013).

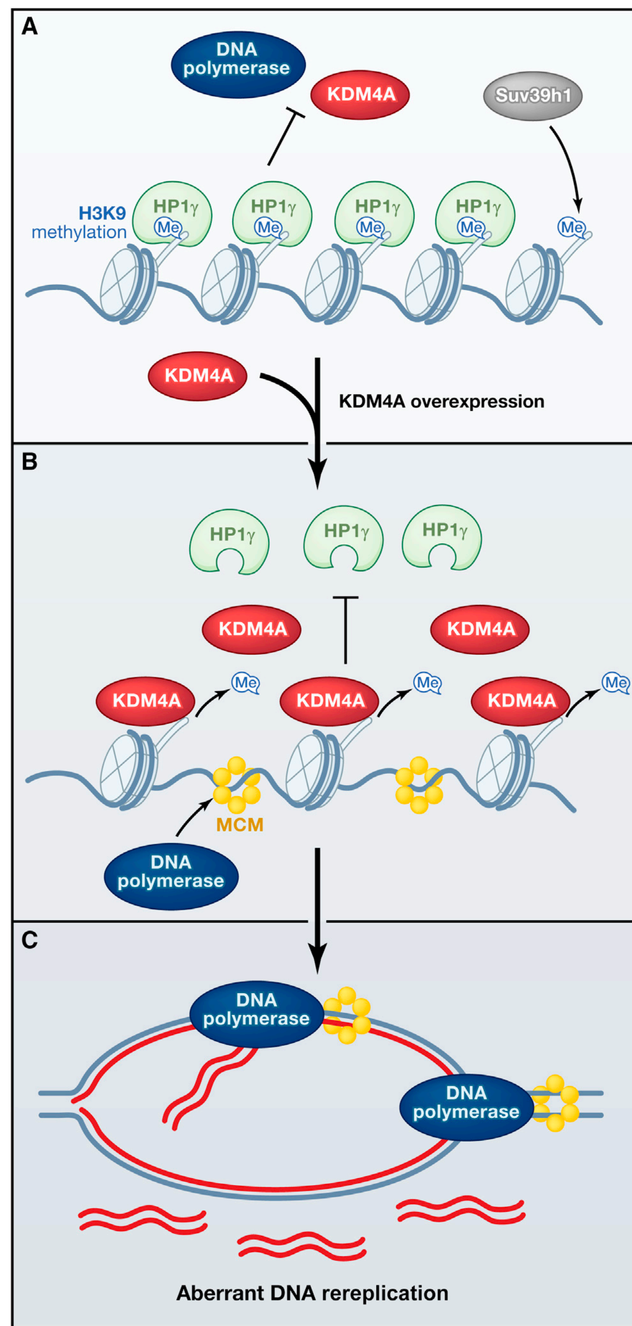

Figure 1. KDM4A Overexpression Promotes Local DNA Rereplication
(A) HP1γ binds to methylated H3K9, blocking access to KDM4A and the DNA replication machinery.
(B) To properly coordinate replication timing, KDM4A demethylates H3K9, promoting local DNA accessibility.
(C) When overexpressed, KDM4A demethylase activity maintains different regions of chromatin in an uncondensed state, promoting aberrant DNA rereplication (red lines). Black et al. hypothesize that the extrachromosomal DNA copies result from head-to-tail polymerase collisions.

Although it is currently unknown how KDM4A is initially amplified in tumors, one can imagine how KDM4A overexpression might eventually result in 1q12/21 copy gains becoming stably incorporated into the genome. It is not clear whether the continual passaging of KDM4A-expressing cells eventually leads to stable 1q12/21 copy gain inheritance. If so, what is the statistical likelihood of such an event? How does this likelihood change when DNA damage is induced? Do these cells have a competitive advantage over cells having only transient copy gains? Most importantly, does 1q12/21 amplification, independent of KDM4A overexpression, result in cancer pathogenesis, or are other unidentified genomic regions responsible for the postulated role of KDM4A in cancer? Moreover, how does KDM4A specificity for the 1q12/21 region arise? Is H3K9M/K36M-induced CNV restricted to 1q12/21, or are there more broadly distributed replication defects? This outstanding study by Black and colleagues raises many stimulating questions and may cause a shift in our thinking and understanding of the role of chromatin-modifying/demodifying enzymes—as they apply to not only transcriptional regulation, but also DNA

replication and genome stability. The very exciting identification of KDM4A overexpression and its association with ovarian cancer clearly has provided the field with yet another chromatin-modifying enzyme that can be used as a possible therapeutic target.

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Just a Trim, Please: Refining ER Degradation through Deubiquitination

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ER-associated degradation clears the secretory pathway of misfolded proteins and mediates the regulated degradation of some ER resident proteins. Only a minor increase in the interaction between a protein and a ubiquitin ligase is sufficient to signal substrate degradation. Zhang et al. have identified deubiquitination as a signal amplifier.

The decision to destroy misfolded proteins in the cell is not made lightly, as there is always the hope that proteins having transitional conformations may simply be en route to their native structures. This is especially true in the secretory pathway because soluble misfolded substrates are recognized in the lumen of the endoplasmic reticulum (ER) and then must be exported into the cytoplasm, where they are destroyed via the ubiquitin-proteasome pathway. This process is known as ER-associated degradation (ERAD). The ERAD of integral membrane proteins presents a special challenge, as membrane-spanning domains must be liberated from the lipid bilayer before the protein is threaded into the 26S proteasome. Genetic and in vitro analyses have delineated the varied pathways taken during the degradation of membrane proteins, with the spotlight directed at E3 ligases that append ubiquitin onto a proteasome-targeted substrate. In this issue of *Cell*, Hegde and colleagues redirect the spotlight toward an opposing reaction, the processive removal of the polyubiquitin chain, which amplifies subtle differences in E3-client interactions to generate a polyubiquitin chain that is sufficient for proteasome-mediated degradation (Zhang et al., 2013).

Mammals encode > 600 E3s, so one might envision that each E3 recognizes a misfolded conformation adopted by a subset of the proteome (Varshavsky, 2012). In turn, each protein might be identified by a select group of E3s. Indeed, functional redundancy among E3-client interactions is frequently observed. However, due to complexities inherent in the folding pathway, a protein displays a range of misfolded conformations. Moreover, previous studies uncovered relatively minor differences in the recognition of an ERAD substrate versus its wild-type counterpart by an E3 ubiquitin ligase (Gardner et al., 2001; Ishikura et al., 2010; Meacham et al., 2001). How are these differences magnified to ensure that folding-competent proteins do not fall victim to the ubiquitin-proteasome system or do so rarely?