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Authors

Zhang, Weiguo
Karpen, Gary H
Zhang, Qing

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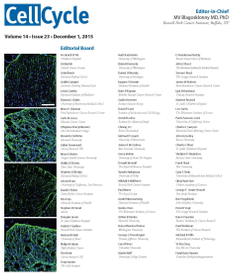

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EXTRA VIEW

Exploring the role of CENP-A Ser18 phosphorylation in CIN and Tumorigenesis

Weiguo Zhang[a], Gary H. Karpen[a], and Qing Zhang[b]

[a]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA; [b]Department of Pathology and Laboratory Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, USA

ABSTRACT

Chromosome instability (CIN) contributes to the development of many cancer. In this paper, we summarize our recent finding that a novel pathway by which FBW7 loss promotes Centromere Protein A (CENP-A) phosphorylation on Serine 18 through Cyclin E1/CDK2, therefore promoting CIN and tumorigenesis. Our finding demonstrates the importance of CENP-A post-translational modification on modulating centromere and mitotic functions in cancer.

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Chromosome instability (CIN) contributes to tumor heterogeneity, drug resistance and cancer progression, and high levels of CIN are associated with poor patient survival for many cancer types.[1,2] Major mechanisms proposed for CIN include oncogene-induced replication stress, telomere dysfunction, and aberrant mitosis.[1] FBW7 is a tumor suppressor protein frequently mutated in multiple cancer types, and belongs to the F-box protein family as part of the SCF ubiquitin E3 ligase complex.[3] Cyclin E1 is a well-characterized FBW7 substrate that regulates G1/S cell cycle entry. Aberrant accumulation of Cyclin E1 due to overexpression or FBW7 mutation leads to polyploidy[4], and is prevalent in many types of cancers. Existing evidence strongly suggests that Cyclin E1 misregulation results in genomic instability primarily through excessive replication origin firing and oncogene-induced replication stress.[5] However, we have recently identified a novel pathway where FBW7 modulates phosphorylation of an essential centromere protein CENtromere Protein A (CENP-A) through Cyclin E1/CDK2, presenting a new paradigm for how this tumor suppressor regulates CIN and tumorigenesis through centromere and mitotic functions.[6]

The centromere is the specialized chromatin locus that recruits the kinetochore, and is crucial for proper mitosis and genome maintenance. The centromere is enriched for CENP-A, an important histone H3 variant that is considered a key epigenetic mark for centromere identity and propagation.[7,8] Too little or too much of CENP-A can disrupt genome integrity. CENP-A depletion displaces the downstream components of the Constitutive Centromere-Associated Network (CCAN) and the KMN network (KNL1 complex, MIS12 complex, NDC80 complex) from centromeres and kinetochores, resulting in chromosome missegregation.[9-12] Moreover, ectopic localization of CENP-A to non-centromeric loci due to overexpression or targeted recruitment leads to fragmented chromosomes in *Drosophila melanogaster* and human cell lines.[13-16] At least in *Drosophila* this is through formation of neo-centromeres and ectopic kinetochores.[13-16] Therefore, faithful chromosome segregation requires tight regulation of CENP-A protein levels, to ensure proper CENP-A nucleosome assembly only at centromeres.[17] In tumors, overexpression of centromere and kinetochore genes is prevalent.[18,19] Importantly, centromere gene upregulation strongly correlates with CIN and poor prognosis in numerous human cancer types[18-23], and predicts enhanced cancer cell and patient sensitivity to genotoxic adjuvant therapies.[18] However, it is unclear whether centromere misregulation contributes to malignancy, or is merely a consequence of other changes during tumor progression.

Post-translational modifications of centromere proteins also influence centromere functions. These include phosphorylation of Ser16 and Ser18 residues within the CENP-A N terminal tail[24], as well as Ser68 in the histone fold domain.[25,26] Ser to Ala mutations at these sites lead to defective CENP-A deposition or mitotic defects, although there is some debate about how essential these modifications are in normal cells.[26,27] Nevertheless, CENP-A and other centromere and kinetochore protein genes are rarely mutated in large TCGA patient datasets[18], thus the clinical relevance of these modifications and the potential roles of CENP-A regulation in cancer progression remain poorly understood.

We found that increased Cyclin E1 levels promote CIN and tumor growth through centromere misregulation.[6] Specifically, loss of the tumor suppressor *FBW7* results in increased Cyclin E1/CDK2 activity, leading to hyper-phosphorylation of CENP-A at the N- terminal Ser18 site, and reduced CENP-A, CENP-B and HEC1 levels at centromeres. Mechanistically, our study demonstrated that Cyclin E1/CDK2 is necessary and sufficient

CONTACT Weiguo Zhang wzhang2@lbl.gov One Cyclotron Road, MS 977, Berkeley, CA 94720-8206, USA; Qing Zhang Qing_Zhang@med.unc.edu Room 21-246, 450 West Drive, UNC-Chapel Hill, NC 27599, USA.

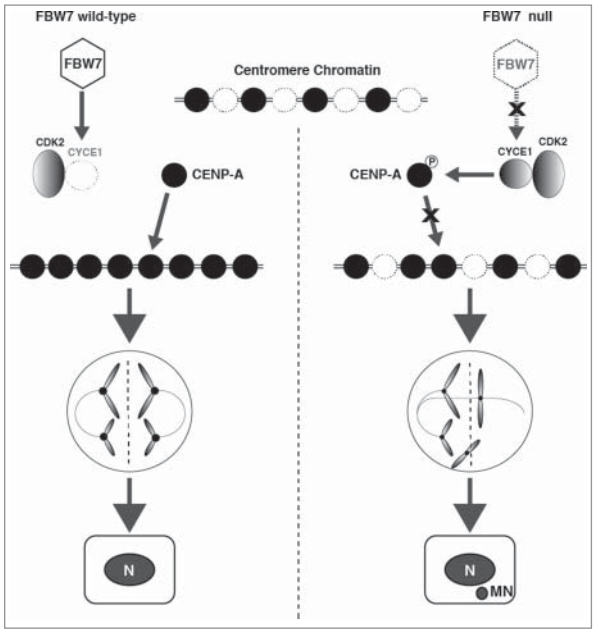

Figure 1. A schematic model of FBW7 defects leading to Cyclin E1 overexpression, CENP-A Ser18 hyper-phosphorylation and chromosomal instability. In FBW7 wild-type cells, CENP-A is successfully deposited at centromeres in late mitosis and early G1 cell cycle phases in the absence of ectopic Cyclin E1/CDK2 activity. In contrast, FBW7 null cells accumulate Cyclin E1, and Cyclin E1 itself is frequently amplified in many cancers. Excessive Cyclin E1/CDK2 activity promotes aberrant CENP-A phosphorylation at the Ser18 residue, reduced CENP-A deposition at the centromere, lagging chromosomes and bridges in mitosis, and micronuclei (MN) formation associated with tumor progression.

for CENP-A Ser18 phosphorylation both *in vitro* and in cultured cells. Further, excessive CENP-A Ser18 phosphorylation enhances CIN, including chromosome missegregation and micronucleus formation, and promotes anchorage-independent growth and tumor progression (Fig. 1). Strong evidence for the relevance of their findings to human clinical cancers was demonstrated using a disease relevant *FBW7* mutation, human clinical cancer tissues and a xenograft mouse model. Moreover, our results suggest that Ser18 hyper-phosphorylation due to increased Cyclin E1 activity and/or FBW7 loss reduces efficient CENP-A deposition at centromeres. Mechanistically, these results suggest that the HJURP chaperone and assembly factor does not interact with CENP-A S18D (Ser to Asp) mutant as efficiently as with WT proteins. Together with the data from clinical human breast cancer samples, we identify an important new function for aberrant Cyclin E1/CDK2 activation in cancer, distinguishable from its well-established role in the G1/S transition.[6] Previous research on Cyclin E1 in oncogenesis primarily focused on its role in replication initiation, which supports oncogene-induced replication stress.[5] However, the results from us suggest that an additional mechanism acting through centromere misregulation also occurs in a significant proportion of human cancers where *FBW7* is lost or Cyclin E1 is overexpressed.[6]

The exact mechanism responsible for reduced interaction between HJURP and CENP-A upon CENP-A phosphorylation on Ser18 is currently unclear. Previous structural and molecular studies indicated direct interactions between the HJURP N-terminal region and the CENP-A histone fold domain.[28,29] In our study, the failure of a phospho-mimetic CENP-A to be efficiently recruited to LacO arrays by LacI-HJURP, and decreased *in vitro* binding of HJURP in CENP-A S18D mutants, imply a reduced ability to form a competitive pre-nucleosomal complex or less competitive chromatin incorporation.[6] Perhaps CENP-A Ser18 phosphorylation perturbs the stable interaction between HJURP N terminal Scm3 domain and CENP-A histone fold domain. Additionally, it is possible that centromeric CENP-A phosphorylated at Ser18 reduces efficient HJURP recruitment for new CENP-A loading at endogenous centromeres.

Regardless, these results suggest that the CENP-A N terminal tail, and specifically levels of Ser18 phosphorylation, modulate proper centromeric CENP-A nucleosome assembly and centromere function. Previous domain replacement experiments using histone H3 N terminal tail suggested that CENP-A N terminal tail is not absolutely essential for cell viability.[30] However, the possibility that phosphorylation modulates centromere function in a cancer context could not be ruled out in those experiments. The results shown in our research are consistent with many findings in the field showing that the CENP-A N terminal tail regulates centromere functions in multiple species[31,32], including proper CENP-B function in human cells[30,33], epigenetic stability of centromeres in fission yeast[34], CENP-A protein stability in budding yeast and *Drosophila*[31,32], and meiosis and organismal fertility in *Arabidopsis*.[35] Interestingly, ectopic overexpression of a CENP-A S18A mutation led to modest but statistically insignificant chromosome missegregation in HeLa cells where most CENP-A is phosphorylated[24]; while in our publication, CENP-A S18D or forced hyper-phosphorylation in DLD1 cells induces micronuclei.[6] The mechanistic details of this pathway await further investigation to better understand its role in carcinogenesis, and for future applications to therapeutic intervention.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed

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