Middle-Down Characterization of the Cell Cycle Dependence of Histone H4 Post-Translational Modifications and Proteoforms

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Results and Discussion

Post-translational modifications (PTMs) of histones are important epigenetic regulatory mechanisms that are often dysregulated in cancer. We employ middle-down proteomics to investigate the PTMs and proteoforms of histone H4 during cell cycle progression. We use pH gradient weak cation exchange-hydrophilic interaction liquid chromatography (WCX-HILIC) for on-line liquid chromatography-mass spectrometry analysis to separate and analyze the proteoforms of histone H4. This provides enhanced separation of proteoforms, including positional isomers, and simplifies downstream data analysis. We use ultrahigh mass accuracy and resolution 21 T Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometry to unambiguously distinguish between acetylation and tri-methylation (Δm=0.036 Da). In total, we identify and quantify 233 proteoforms of histone H4 in two breast cancer cell lines. We observe significant increases in S1 phosphorylation during mitosis, implicating an important role in mitotic chromatin condensation. A decrease of K20 unmodified proteoforms is observed as the cell cycle progresses, corresponding to an increase of K20 mono- and di-methylation (see Figure 1). Acetylation at K5, K8, K12, and K16 declines as cells traverse from S phase to mitosis, suggesting cell cycle-dependence and an important role during chromatin replication and condensation. These new insights into the epigenetics of the cell cycle may provide new diagnostic and prognostic biomarkers.

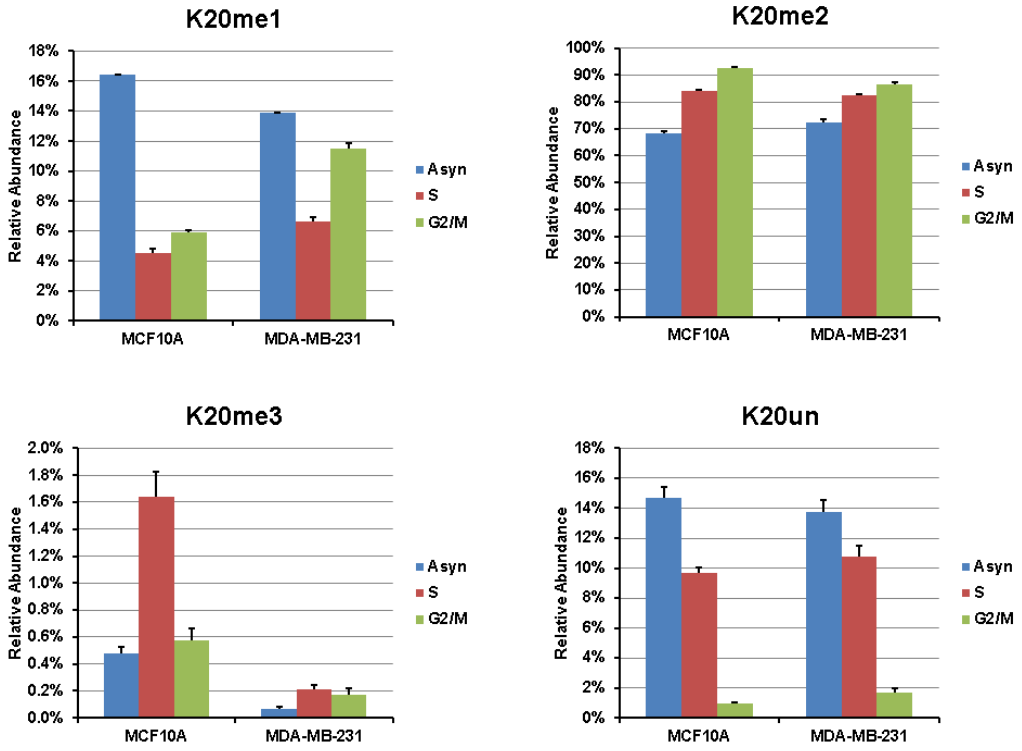

Fig. 1. Relative abundances of mono-, di- and tri-methylation and unmodified proteoforms on lysine 20 observed from cell lines MCF-10A and MDA-MB-231 at different cell cycle stages. Error bars represent S.E. from two biological replicates in S and G2/M phase for each cell line, two biological replicates of asynchronous cells for cell line MDA-MB-231, and one biological replicate for cell line MCF-10A. Each biological replicate contains one to three technical replicates depending on sample amount.

Acknowledgments

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References

[1] Jiang, T., *et al.,* Proteomics, **18** (11), 1700442 (2018).