An integrated top-down and bottom-up strategy for analysis of Bromodomain-containing protein 4 (BRD4) mediated histone post-translational modifications

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Introduction

BRD4 is a key transcriptional regulator in a variety of disease processes (e.g. inflammation, cancer), in part by recruiting histone modifying enzymes to specific genes. However, the full range of histone post-translational modifications (PTMs) regulated by BRD4 is incompletely understood. Herein, we utilized a combined bottom-up and top-down proteomics strategy, featuring 2DLC and novel bromodomain-specific inhibitors to study BRD4-mediated histone PTMs. This revealed previously uncharacterized, bromodomain-specific patterns of histone modification.

Background

BRD4, a member of the BET protein family, is a molecular scaffold which guides interacting proteins to acetylated histones via tandem bromodomains (BD1/BD2). BRD4 is implicated in the mechanisms of inflammation, fibrosis, and some cancers.[1] Disruption of BRD4's histone binding activity is a mechanism for several anti-cancer and anti-inflammation therapeutics.

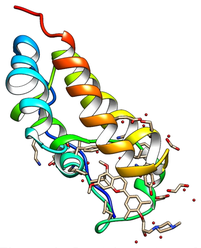

Figure 1. Crystal structure of BRD4-BD1 in complex with the BRD4 inhibitor ZL 0516[1]

Methods

- Immortalized hSAECs were treated with the following inhibitors:
  - ZL 0591 (BRD4-BD1)
  - ZL 0516 (BRD4-BD1/BD2)
  - (+) JQ1 (Pan-BET Inhibitor)
- Histones were purified and prepared for bottom up analysis as per Sidoli et. al.[2]
  - Histones were derivatized with propionic anhydride to block tryptic digestion at unmodified lysine residues.
- For top-down analysis, protein was desalted using Pierce PES 10K MWCO Protein Concentrators.
  - LC separation was conducted with a Waters nanoAcquity UPLC system.
- Data was analyzed using Skyline, Bruker DataAnalysis, and MSnbase.

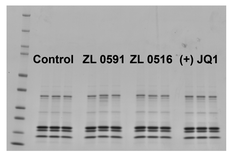

Figure 2. Coomassie stain of purified histones.

Bottom-Up Analysis of Histone Marks

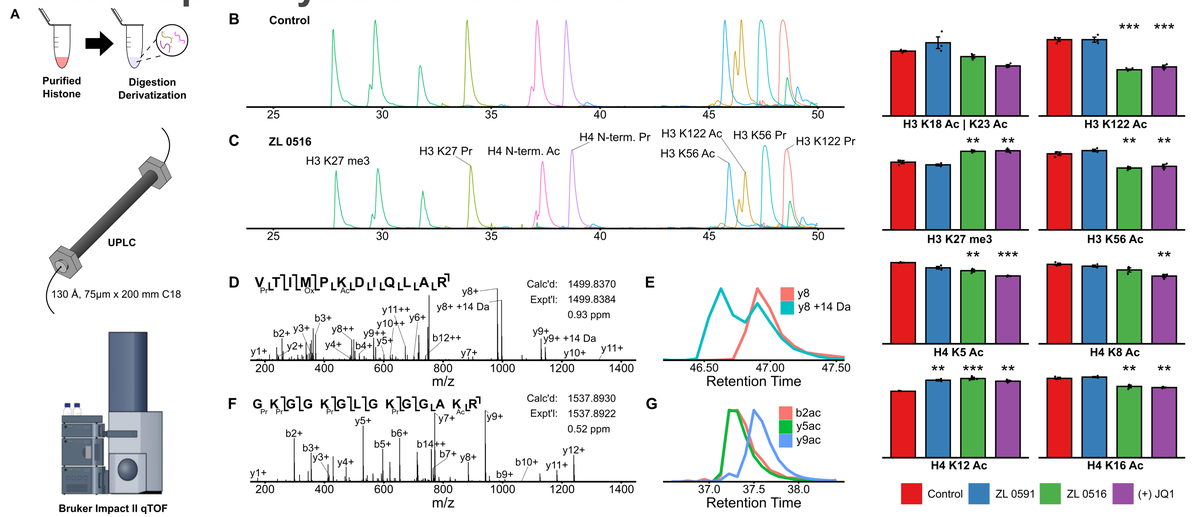

Figure 3. (**A**) Schematic of the bottom-up LCMS workflow for histone mark analysis. (**B**) Extracted Ion Chromatographs of key histone peptides in the control condition and in the ZL 0516 treated condition (**C**). 'ac' indicates acetylation, me3 indicates trimethylation, and 'pr' indicates propionyl derivatization. (**D**) Tandem mass spectra of the H3 K122ac peptide and isobaric species, and discriminatory fragment ion traces (**E**). (**F**) Tandem mass spectra of the monoacetylated H4 N-terminus peptide (K5ac/K8ac/k12ac/K16ac), and discriminatory fragment traces (**G**). (**H**) Quantitation of selected histone marks. Bar graphs represent the mean ± SE of n=3 biological replicates. $^{**}P_{adj}<0.01$, $^{***}P_{adj}<0.001$

LC for Top-Down Analysis

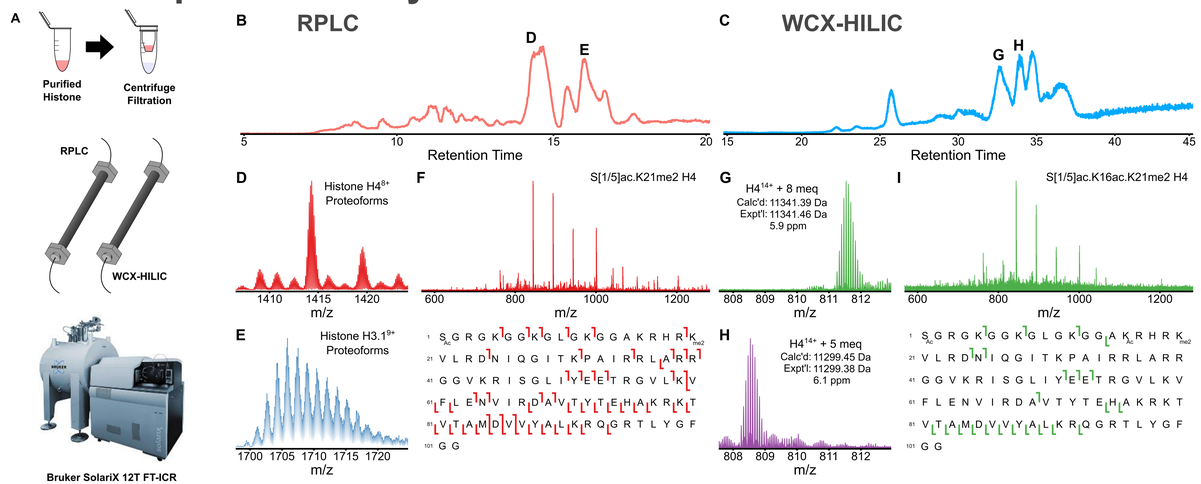

Figure 4. (**A**) Schematic of the top-down LCMS workflow for intact histone analysis. (**B**) Total Ion Chromatogram of RPLC and Weak Cation Exchange-HILIC (WCX-HILIC) (**C**) separations. (**D**) Mass spectra of intact Histone H4 and Histone H3.1 (**E**) proteoforms acquired during RPLC separation. (**F**) Tandem mass spectra of the S1ac.K21me2 H4 precursor. (**G**) Mass spectra of diacetylated and mono-acetylated (**H**) Histone H4 proteoforms separated by WCX-HILIC. (**I**) Tandem mass spectra of the $H4^{9+}$ + 8 methyl equivalent (meq) precursor.

Discussion

- BRD4 interacts with several histone acetyltransferases (HATs), and itself has putative HAT activity.
  - Lysine acetyltransferase 5 (KAT5)
  - p300
- H3 K122ac is proposed to drive localized gene activation by disrupting histone/DNA interactions.
- BRD4-mediated acetylation likely serves as an additional transcriptional priming mechanism.
- Histone acetylation may also recruit key transcription factors and scaffold proteins, including BRD4 itself.

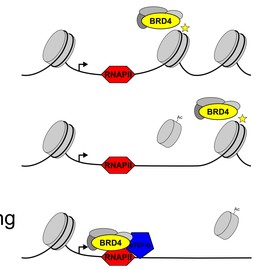

Figure 5. BRD4-dependent histone acetylation facilitates transcriptional elongation by evicting nucleosomes.

Conclusions & Future Directions

- BRD4 mediates acetylation of the Histone H4 N-terminus
- BRD4 mediates histone acetylation in a bromodomain-specific manner.
  - ZL 0591 (BD1 inhibitor) did not reduce the abundance of acetylation at H3 K56, H3 K122, or H4 K16.
  - ZL 0516 (BD1/BD2 inhibitor) had essentially identical effects as the pan-BET inhibitor (+) JQ1.
- Future studies will be conducted in a cell culture model of innate airway inflammation to determine if BRD4-mediated inflammation induces specific histone marks.
  - Polyinosinic-polycytidilic acid (PolyIC) is a specific activator of the innate immune pathway via Toll-like Receptor 3.
  - RPLC/WCX-HILIC 2DLC for histone family and acetylation-state resolved top-down mass spectrometry.

Acknowledgements

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References

1.Liu, Z. et. al. *J Med Chem.* **2020**.

2.Sidoli, S. et. al. *J Vis Exp*. **2016.**