Abstract

The ubiquitin-like, containing PHD and ring finger domains 1 (UHRF1) reader protein has been characterized as a "universal biomarker" of cancer, as its overexpression has been associated with DNA hypermethylation and tumorigenesis.[1,2] The dual-domain reader protein consists of a tandem tutor domain (TTD) and a plant homeodomain (PHD) connected by a short linker.[2] On the histone 3 tail, the PHD domain binds to unmodified arginine at position 2, and the TTD domain binds to trimethylated lysine (Kme3) at position 9 (H3K9me3) through an aromatic cage configuration that is highly conserved among reader proteins.[3,4] Binding in aromatic cages is typically driven by cation-π interactions, but recent reports unexpectedly show that the dual-domain UHRF1 protein binds to *tert*-butyl norleucine (tBuNle), a neutral isostere of Kme3, with equal affinity.[4,5,6,7] To investigate the driving forces of binding in this system, electrostatic tunability studies were conducted using Genetic Code Expansion (GCE) to mutate one of the residues forming the aromatic cage with para-substituted phenylalanine derivatives varying in electrostatic potential.[9,10] Isothermal titration calorimetry (ITC) was then used to measure changes in binding affinity and enthalpies of binding between each of the dual-domain UHRF1 variants and the two H3 tail peptides containing Kme3 or tBuNle at position 9 of histone 3. Our results show no correlation between free energy of binding and electrostatic potential, hydrophobicity, or polarizability of the aromatic portion of the phenylalanine derivatives, indicating that interactions within the aromatic cage of the TTD domain do not drive binding. To conclusively determine whether the PHD domain is responsible for binding, future experiments mutating residues within the PHD domain to disrupt acidic contacts with arginine 2 on histone 3 must be performed.

References

1. Ashraf etal. The epigenetic integrator UHRF1: on the road to become a universal biomarker for cancer. *Oncotarget*. **2017**, *8*, 51946.

2. Sidhu H.; Capalash N. UHRF1: The key regulator of epigenetics and molecular target for cancer therapeutics. *Tumor Biology*. **2017**, *39* (2). DOI: 10.1177/1010428317692205

3. Arita K.; Isogai S.; Oda T.; Unoki M.; Sugita K.; Sekiyama N.; Kuwata K.; Hamamoto R.; Tochio H.; Sato M.; Ariyoshi M.; Shirakawa M. Recognition of modification status on a histone H3 tail by linked histone reader modules of the epigenetic regulator UHRF1. *Proc Natl Acad Sci U S A.* **2012**, *109* (32), 12950-5. DOI: 10.1073/pnas.1203701109.

4. Musselman C. A.; Lalonde M. E.; Côté J.; Kutateladze T.G. Perceiving the epigenetic landscape through histone readers. *Nat Struct Mol Biol*. **2012**, *19* (12), 1218-27. DOI: 10.1038/nsmb.2436.

5. Hughes, Robert M et al. Recognition of trimethyllysine by a chromodomain is not driven by the hydrophobic effect. *Proceedings of the National Academy of Sciences of the United States of America*. **2007**, *104*(27), 11184-8. doi:10.1073/pnas.0610850104

6. Albanese, Katherine I, and Marcey L Waters. Contributions of methionine to recognition of trimethyllysine in aromatic cage of PHD domains: implications of polarizability, hydrophobicity, and charge on binding. *Chem. Science*. **2021**, *12*(25), 8900-8908. doi:10.1039/d1sc02175c

7. C. R. Travis, K. I. Albanese, K. M. Kean, H. C. Henriksen, M. L. Waters, unpublished results.

8. Kathryn C. Schultz, Lubica Supekova, Youngha Ryu, Jianming Xie, Roshan Perera, and Peter G. Schultz. *Journal of the American Chemical Society*. **2006,** *128* (43), 13984-13985.

9. Douglas D. Young, Travis S. Young, Michael Jahnz, Insha Ahmad, Glen Spraggon, and Peter G. Schultz. *Biochemistry*. **2011,** *50* (11), 1894-1900. DOI: 10.1021/bi101929e

10. Mackenzie W. Krone, Christopher R. Travis, Ga Young Lee, Hannah J. Eckvahl, K. N. Houk, and Marcey L. Waters. *Journal of the American Chemical Society* **2020** *142* (40), 17048-17056. DOI: 10.1021/jacs.0c06568

11. Stefanie A. Baril, Amber L. Koenig, Mackenzie W. Krone, Katherine I. Albanese, Cyndi Qixin He, Ga Young Lee, Kendall N. Houk, Marcey L. Waters, and Eric M. Brustad *Journal of the American Chemical Society* **2017** *139* (48), 17253-17256. DOI: 10.1021/jacs.7b09223