View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Dundee Online Publications

University of Dundee

Target validation

Ciulli, Alessio

Published in:
Nature Chemical Biology

DOI:
10.1038/nchembio.2154

Publication date:
2016

Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):
Ciulli, A. (2016). Target validation: switching domains. Nature Chemical Biology, 12(9), 659-660. DOI: 10.1038/nchembio.2154

General rights
Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 26. Feb. 2017

DOI: 10.1038/nchembio.2154

Target Validation

News & Views

Switching domains

Chemical probes and drugs often bind to functional domains on disease-relevant proteins. A study suggests a chemical genetic approach to establish on-target effects by swapping the targeted domain, affords resistance to pharmacological inhibition while retaining functionality.

Alessio Ciulli

In target-driven discovery world, a founded paradigm of chemical intervention involves developing small molecules that bind individual domains to inhibit protein function. Understanding the true "on" and "off" target contributions to pharmacological activity is one of the pillars of target validation[1], but can be difficult to realize when the protein in question shares homologous domains with other proteins. This is especially true of epigenetic protein families that mediate transcriptional signaling through the catalytic or scaffolding function of their structural domains[2], many of which are difficult to target selectively with small molecules because of their conserved binding sites[3]. In this issue, Hohmann *et al.*[4] describe an elegant approach that involves swapping the targeted domain with one from a structurally homologous family member that does not bind the probe, to confer probe resistance and validate the relevant cellular target.

Epigenetic effectors are multi-domain proteins that catalyze installment, removal or recognition of posttranslational modifications of amino acids on histones amongst other substrates. Many epigenetic proteins function as part of large complexes that bind to promoters, enhancers and super-enhancer regions of the genome, remodeling chromatin and regulating transcription of genes that can sustain growth and proliferation of cancer cells[5]. Amongst these are the chromatin remodelers switch/sucrose non-fermentable (SWI/SNF), multisubunit enzymes that utilize the energy derived from ATP hydrolysis to open up chromatin and activate transcription[6]. Identifying the most relevant target protein in a given disease context is an important first step.

In the study by Hohmann *et al.* Brd9, which is a subunit of a mammalian SWI/SNF complex (also known as BAF)[6], was found to be required for the growth of acute myeloid leukemia (AML) cells. Brd9 supports leukemic maintenance by sustaining *Myc* expression via its essential bromodomain - the reader domain of lysine acetylation. These observations provided convincing genetic rationale for chemical probe discovery effort, and nanomolar-potency inhibitors targeting the Brd9 bromodomain were developed by fragment-based drug design, achieving >1,000 fold selectivity against the main potential off-target bromodomain of the BET protein Brd4[7]. Inhibitor treatment led to the release, albeit partial, of the entire BAF complex from chromatin, and phenocopied the cellular effect and transcriptional response of Brd9 knockdown (Fig. 1a). Crucially, an engineered Brd9 variant in which the bromodomain of Brd9 was substituted by the first bromodomain of Brd4 was devised. This "domain-swap" allele *BRD9-BET* proved insensitive to chemical inhibition by chemical probe BI-7273 but could substitute the wild-type function,

providing a powerful genetic tool to validate Brd9 as the relevant cellular target, and demonstrating the superior on-target effect of BI-7273 over other Brd9 probes[4] (**Fig. 1b**).

The most compelling finding of this study for chemical biology is the replacement of entire functional domains as a successful strategy to generate inhibitor-resistant alleles. This new approach extends on the use of naturally emerging or engineered drug-resistant mutations, which have proven of broad utility in the protein kinase space but have yet to be established with epigenetic targets. With success, chemical probes have been rendered exquisitely selective by chemical genetic approaches such as the "bump and hole"[8], however for this to be generally applicable a pan-selective tool compound is required and single-point mutations have to be designed that do not significantly alter protein function, leaving the need for the mutant to be complemented by adequate synthetic modifications of the probe itself. The discovery that the *BRD9-BET* allele retained sufficient functionality in AML despite the evolutionary distance was unexpected, in part because it is known that bromodomains have different recognition specificities to histone peptides across the family[2]. This observation suggests context- and loci- dependent specificities of chromatin occupancy of epigenetic proteins than might not be revealed using isolated domains and truncated histone peptides as substrates. The new findings bear also implications for targeting cancer epigenetics. The biological activities of the reported probes provide important benchmark for non-BET bromodomain inhibitors, which have previously proved unexceptional in cells. For example PFI-3, a chemical probe for the bromodomain of Brg1 (also known as *Smarca4*, another BAF subunit), failed to display anti-tumoral activity in cancer cells sensitive to Brg1 knockdown[9]. This highlights that distinct domain interactions may have different susceptibilities to pharmacological target modulation, despite being present within the same complex.

How general could the domain-exchange approach be? The data presented[4] on the methyltransferase EZH2, a prominent cancer target also found within a multiprotein histone-modifying complex[6], and its SET domain-targeting probe GSK126, encourages optimism and a broadly applicable appeal, at least in the context of epigenetic domains. CRISPR-Cas9 screens are increasingly being developed to reveal cancer dependencies of epigenetic domains[10], and it might be possible in many cases to identify paralogous domains that once swapped can be expressed in a functionally silent manner, without exhibiting dominant-negative effects on the endogenous protein. Ultimately, genetic engineering of homozygous domain swap knock-ins could surpass the need for transducing and selecting for exogenous alleles, not only improving target validation of existing probes but also opening new doors to the development of target-sensitive phenotypic screens in disease-relevant models. So switch domain, and validate your target!

Alessio Ciulli is at the School of Life Sciences, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, UK. E-mail: a.ciulli@dundee.ac.uk

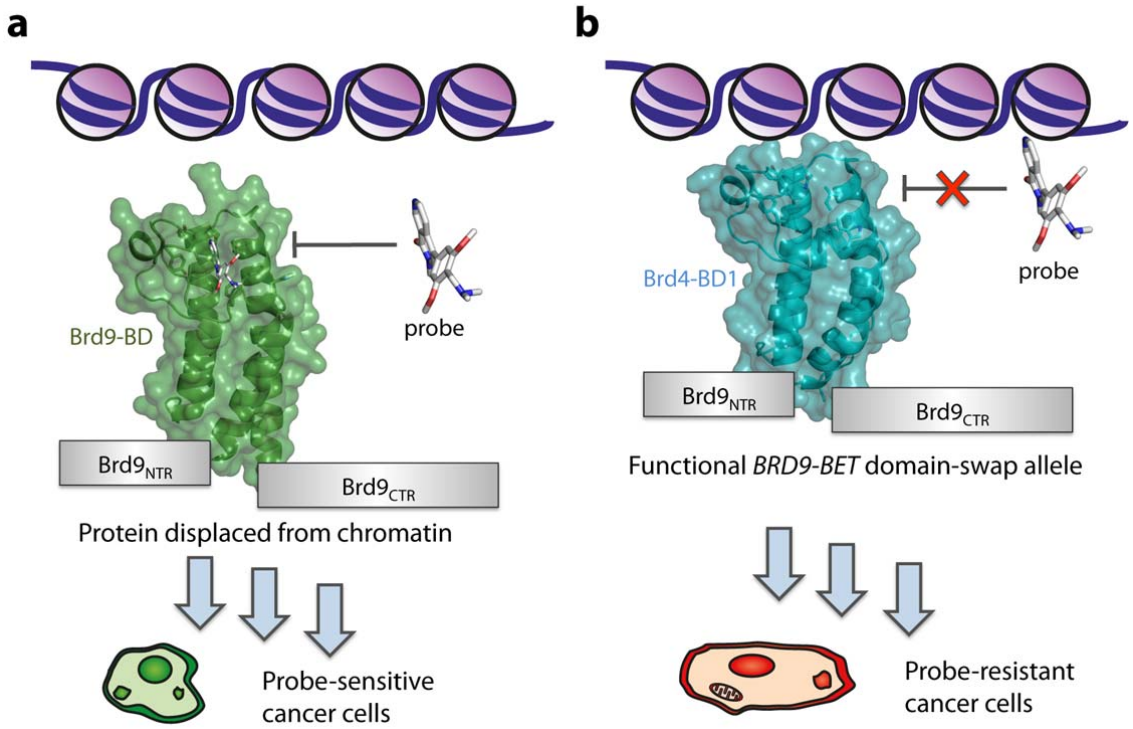

Figure 1.

Domain-swap to assess chemical probe on-target effects. (a) Probe binds to the target domain (BD, bromodomain), leading to pharmacological effect in cancer cells. (b) Swapping that target domain for one from a different protein that does not bind the probe leads to probe-resistant cells, establishing the probe's on-target effects. In each case the remainder of the target protein remains unaltered (NTR, N-terminal region; CTR, C-terminal region).

References

1. Bunnage, M. E., Chekler, E. L. P. & Jones, L. H. *Nat Chem Biol* **9,** 195–199 (2013).
2. Arrowsmith, C. H., Bountra, C., Fish, P. V., Lee, K. & Schapira, M. *Nat Rev Drug Discov* **11,** 384–400 (2012).
3. Runcie, A. R., Chan, K.-H., Zengerle, M. & Ciulli, A. *Curr Opin Chem Biol* (2016). doi:10.1016/j.cbpa.2016.06.031
4. Hohmann, A. F. *et al. Nat Chem Biol* (2016). doi:10.1038/nchembio.2115
5. Dawson, M. A. & Kouzarides, T. *Cell* **150,** 12–27 (2012).
6. Kadoch, C., Copeland, R. A. & Keilhack, H. *Biochemistry* **55,** 1600–1614 (2016).
7. Martin, L. J. *et al. J Med Chem* **59,** 4462–4475 (2016).
8. Baud, M. G. J. *et al. Science* **346,** 638–641 (2014).
9. Vangamudi, B. *et al. Cancer Res* **75,** 3865–3878 (2015).
10. Shi, J. *et al. Nat Biotechnol* **33,** 661–667 (2015).