HDAC inhibitor Givinostat targets DNA-binding of human CGGBP1

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Abstract

The antineoplastic agent Givinostat inhibits histone deacetylases. We present here our finding that the DNA-binding of human CGGBP1 is also inhibited by Givinostat. CGGBP1, a DNA-binding protein, is required for cancer cell proliferation. In our quest to exploit the potential anti-proliferative effects of CGGBP1 inhibition, we have developed a simple screening assay to identify chemical inhibitors of DNA-protein interactions. We have applied this screen for human CGGBP1 on a library of 1685 compounds and found that Givinostat is a direct inhibitor of CGGBP1-DNA interaction. The mechanism of action of Givinostat should thus extend beyond HDACs to include the inhibition of the myriad functions of CGGBP1 that depend on its binding to the DNA.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

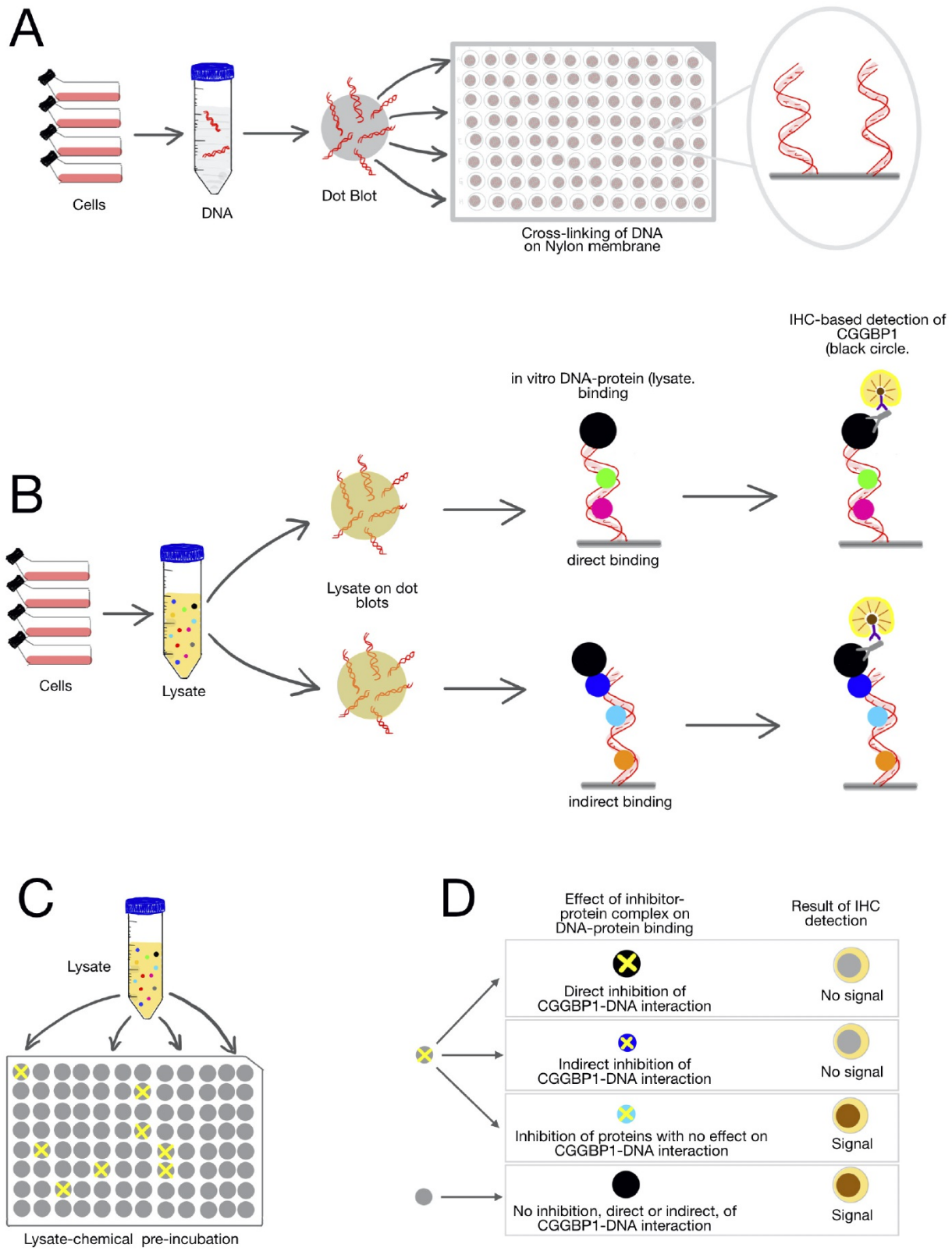


Figure 1

A schematic representation of the D ot- B lot and I mmuno D etection (DBID) assay. A:

Genomic DNA was isolated from HEK293T cells. Sonicated DNA fragments (mean length of 1kb) were blotted onto positively charged nylon membranes and crosslinked by vacuum heating at 80°C. (B) HEK293T cells were lysed and the cleared lysates were used as a source of protein for in vitro DNA binding. The assay depicted here is designed to detect binding of CGGBP1 (black circle) to DNA, although this protocol can be generically applied for any protein of interest. CGGBP1 can bind to DNA either directly (top panel) or indirectly (through linker proteins, depicted in blue circle in the bottom panel). The subsequent immunochemistry-based detection reports a brown signal for the direct as well as indirect CGGBP1-DNA complexes alike. The immunochemistry employs a primary antibody against the protein of interest, a biotinylated secondary antibody, streptavidin-HRP conjugates and DAB as the chromogenic substrate and is semi-quantitative in nature. C: Pre-incubation of the cell lysate with inhibitors allows the small molecule compounds to bind to their cognate target proteins in the lysate. Only some of these compounds potentially 4 inhibit the DNA-binding of their target proteins (exemplified with a yellow cross). D: The different possible outcomes of the DBID assay for inhibition of CGGBP1-DNA interactions are depicted. The direct inhibition of CGGBP1 (black circle with a cross) as well as the inhibition of a linker protein (blue circle with a cross) required for CGGBP1-DNA binding are expected to result in “No signal”. Inhibitors that do not have any direct or indirect effect on CGGBP1-DNA interaction show “Signal”.

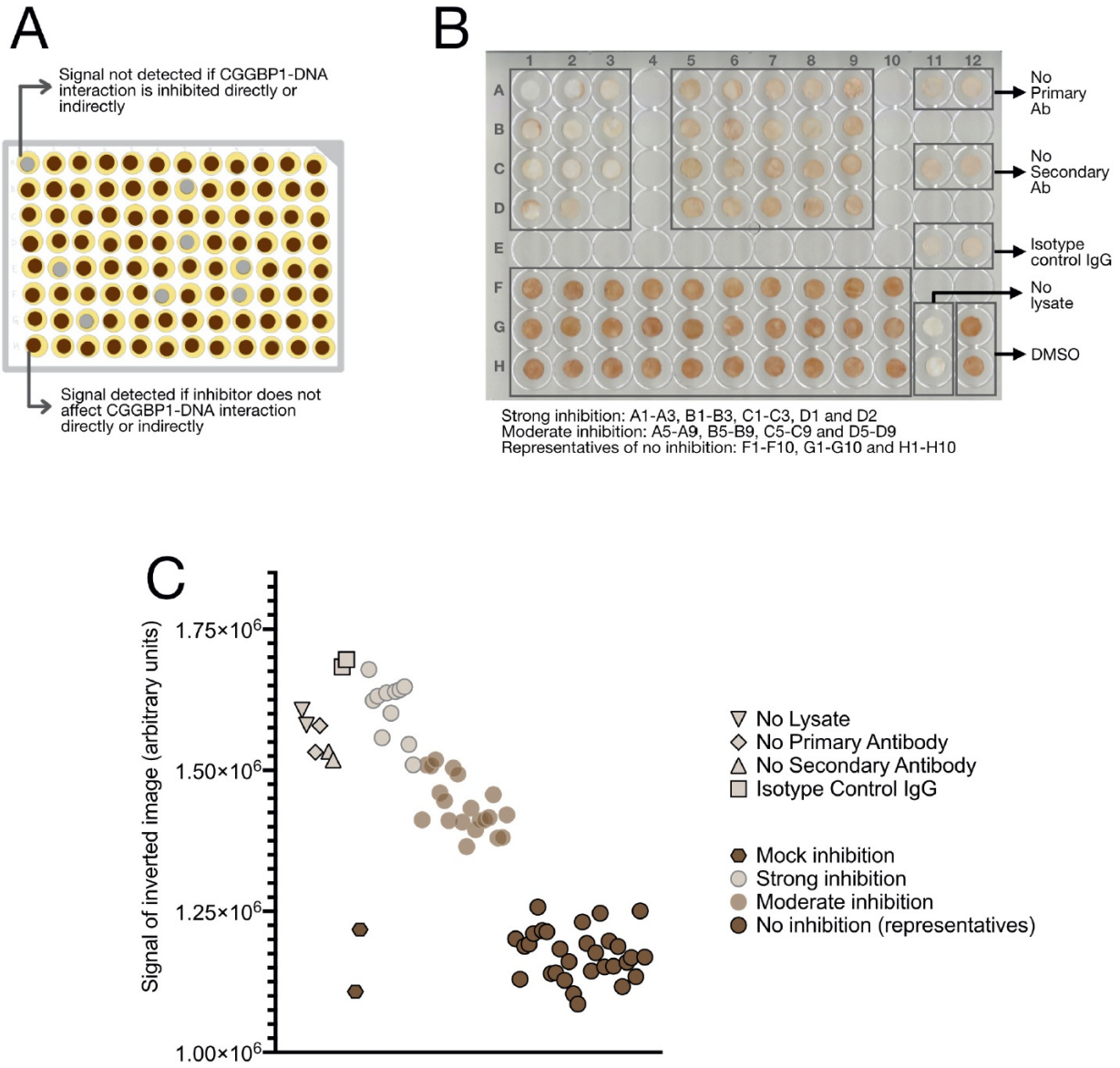
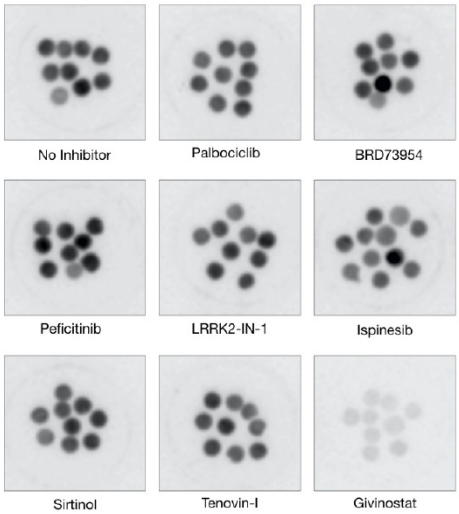


Figure 2

DBID screening of a small molecule chemical library of 1685 compounds identifies inhibitors of CGGBP1-DNA interaction. A: The primary DBID assay was performed in multiple 96-well plates. The lysate was individually pre-incubated with the compounds (one compound per well). After transferring the lysate-compound complexes (as shown in figure 1C) to dot blots (as shown in figure 1A), immunochemical detection was performed using a cocktail of Rabbit anti-CGGBP1 primary antibody. The schematic represents the signals obtained for a 96-well plate. B: The dot blots of the entire library screen for CGGBP1 were manually categorized into 11 strong inhibitors and 20 moderate inhibitors. The actual images of these

two groups of dot blots are shown here along with the positive and negative controls as indicated. The identities of the inhibitors are as follows: Strong inhibitors [A1-Givinostat (ITF2357), A2-LRRK2-IN-1, A3-Peficitinib (ASP015K, JNJ-54781532), B1-Ispinesib (SB-715992), B2-TWS119, B3-Domperidone, C1-Gallamine Triethiodide, C2-Moxifloxacin HCl, C3-Sirtinol D1-NAD+, D2-Palbociclib (PD-0332991) HCl], Moderate inhibitors [A5-VX-661, A6-BRD73954, A7-NCT-501, A8-Tenovin-1, A9-Prasugrel, B5-U0126-EtOH, B6-Foretinib (GSK1363089), B7-JNJ-7706621, B8-CHIR-99021 (CT99021), B9-Asenapine maleate, C5-Ethylparaben, C6-LY2874455, C7-Golgicide A, C8-PD173955, C9-Mirin, D5-Ramelteon, D6-Cilnidipine, D7-Dopamine HCl , D8-VR23, D9-AZD3759]. Majority of the compounds in the library did not show any inhibition of CGGBP1-DNA interaction. Thirty representative dot blots of the non-inhibitors are shown in well numbers F1-F10, G1-G10, H1-H10. C: The signals for the dot blots shown in B are quantified by densitometry. The graph shows the signals of the inverted images for each dot blot.

A



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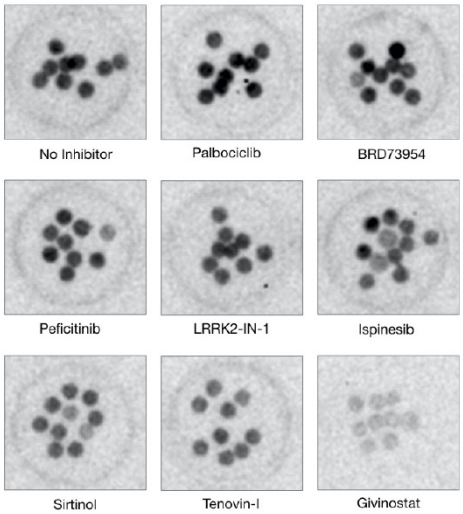
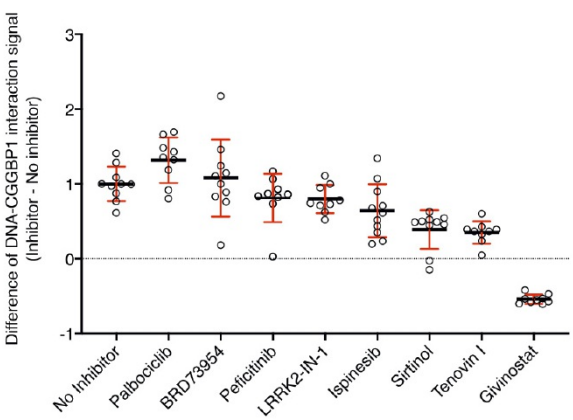


C

Inhibitor	Fold change in binding	Standard deviation
No Inhibitor	1.00	0.24
Palbociclib	1.32	0.30
BRD73954	1.08	0.51
Peficitinib	0.81	0.32
LRRK2-IN-1	0.80	0.19
Ispinesib	0.64	0.35
Sirtinol	0.39	0.26
Tenovin I	0.35	0.15
Givinostat	-0.54	0.06

D



E

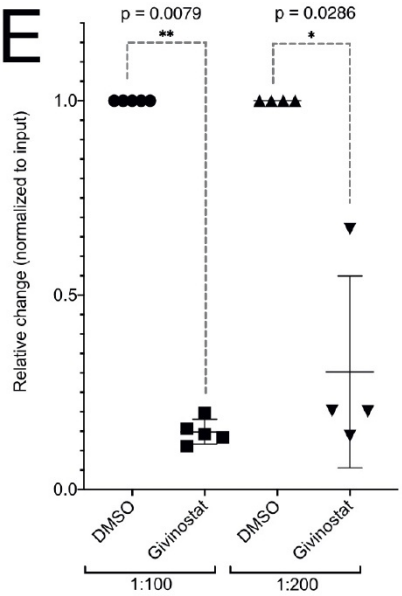


Figure 3

ivinostat acts as a direct inhibitor of CGGBP1-DNA interaction in vitro . The secondary DBID screen was performed on a panel of inhibitors identified in the primary screen using FLAG-tagged recombinant CGGBP1 (rCGGBP1). A: rCGGBP1 pre-incubated with compound diluent only (No inhibitor) or the indicated compounds were subjected to DBID by using anti-FLAG antibody. Of the eight different compounds, only Givinostat showed direct inhibition. B: The assay described in A was repeated with anti-CGGBP1 antibody and again Givinostat showed a direct inhibition of DNA-CGGBP1 interaction convincingly. In A and B the multiple dot blots per compound are technical replicates. C and D: Quantification of the signals obtained from the dot blots of secondary screening (B) are tabulated (C) and plotted (D). E: Relative quantification of the amount of Alu DNA immunoprecipitated with rCGGBP1 with or without inhibition with Givinostat was quantified using quantitative PCR and double delta Ct method. The input DNA was used as control and the immunoprecipitated Alu DNA template was used at two different dilutions (1:100 and 1:200). Pre-incubation of rCGGBP1 with Givinostat strongly inhibits the binding of rCGGBP1 directly to pure Alu DNA in vitro . Compared to the mock inhibition using DMSO, Givinostat caused a 70-80% reduction in rCGGBP1-Alu DNA interaction.

Supplementary Files

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Additional file_Patel_et_al.pdf