Small molecule microarray lysate screen identifies bromodomain ligands that target the MYC transcription regulatory network

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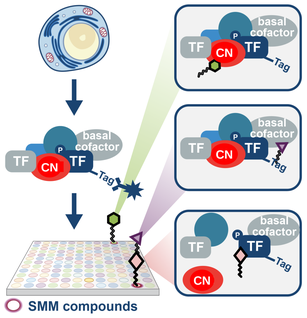
Abstract

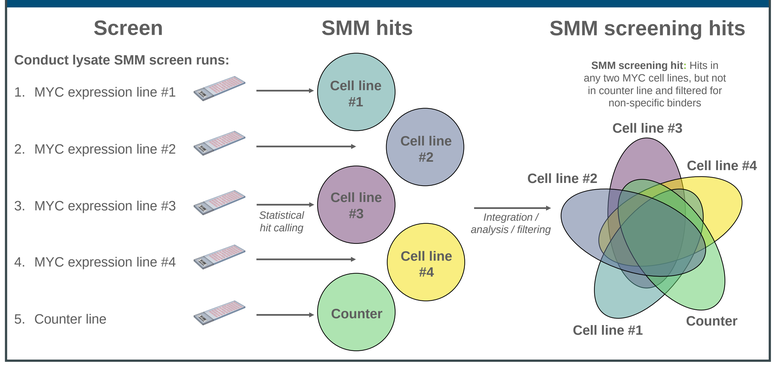
MYC is the most frequently amplified gene in human cancers and one of the most sought-after drug targets for cancer therapy. Its function as a transcription factor (TF) is essential for its oncogenic potential. However, development of small molecules that target oncogenic MYC function in cells has been intractable due to its lack of known ligand binding sites. To overcome these structural challenges, we leveraged Kronos Bio's small molecule microarray (SMM) screening platform to identify small molecules that can bind to MYC transcriptional complexes in cell lysates from cancer cells with deregulated MYC function. Unbiased transcriptional signature-based profiling identified multiple SMM hits that modulated the MYC transcription regulatory network (TRN) in MYC-dependent cancer cell lines. One of the SMM hits mimicked signature changes due to MYC loss of function, which were also similar to signature changes effected by BET bromodomain inhibitors. Biochemical, biophysical, and structural analyses found that the SMM hit could directly engage with BET bromodomains in purified systems. Cell-based proximity labeling demonstrated binding to BET proteins in live cells, and comparative pharmacology approaches suggested that the BET protein engagement was likely driving the observed gene expression changes. These results demonstrate the utility of the SMM platform in identifying ligands that modulate transcription factor TRNs through binding to critical cofactors.

SMM lysate screens identify binders to TF complexes

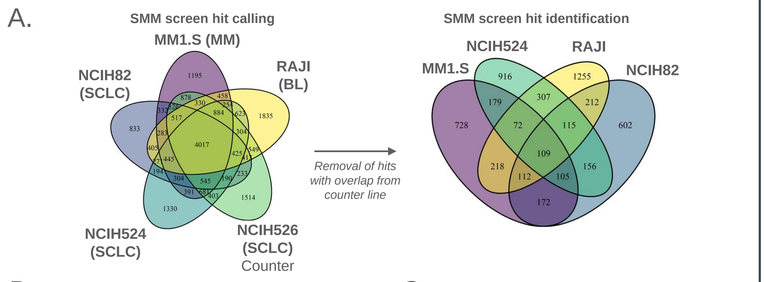


- Continuous platform development has improved screening throughput, quality and turnaround time
- 240,000 compound diversity library with lead-like properties
- Drives discovery of multi-modality binders:
  - Direct critical node (CN) binder
  - Basal cofactor binder
  - Protein-protein interaction (PPI) modulator
- Binders can be modulators or elaborated into bifunctional degraders
- Target deconvolution identifies mechanisms-of-action

SMM lysate screen process



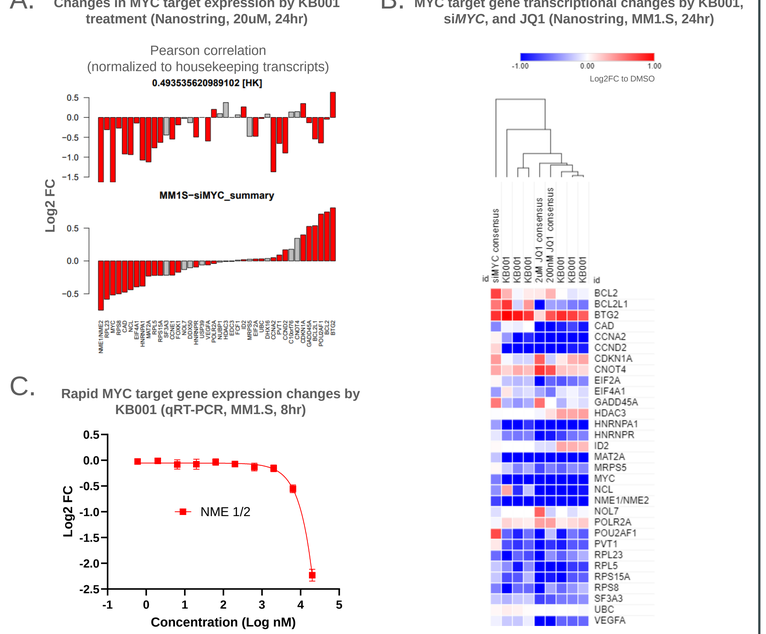
SMM screening identifies compounds that bind MYC complex proteins



Cell line	Overlap #
RAJI_MM1S_H82_H524	1
RAJI_H82_H524	2
RAJI_MM1S_H524	3
RAJI_H524	4
RAJI_MM1S_H82	5
RAJI_H82	6
RAJI_MM1S	7
H82_MM1S_H524	8
H82_H524	9
MM1S_H524	10
MM1S_H82	11

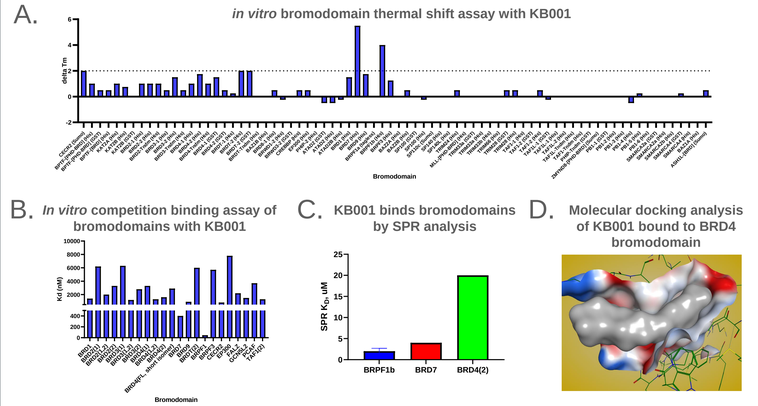
A. Venn diagrams of SMM screen hits (left: all cell lines, right: removal of overlap with counter line). SCLC: small cell lung cancer; MM: multiple myeloma; BL: Burkitt lymphoma. B. (Left) Table of each representative cell line overlap. (Right) Venn diagram of screen hits with assigned overlap number labeled. Red numbers correspond to overlap # from table to the left. C. Correlation plot showing the correlation of hit calls from technical replicates A and B of library subset slides (54 per screen) for each cell line screen. Cell line-specific hit rate may vary, yet all screens show excellent replicate correlation (Overall R^2 = 0.82).

Identification of KB001 that targets the MYC TRN in MYC-dependent cancers



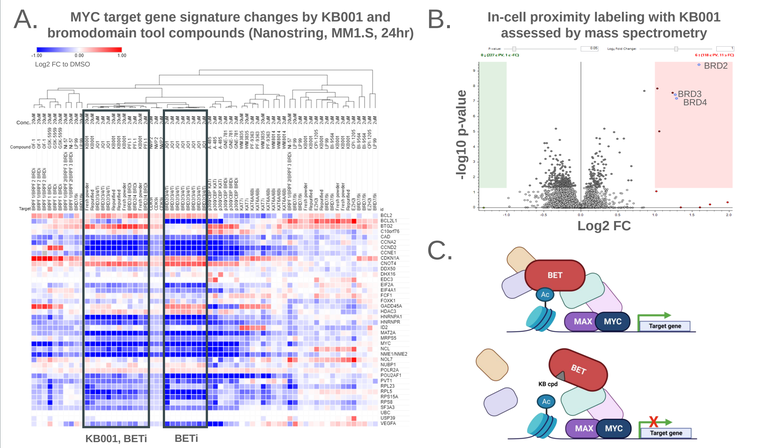
A. Waterfall plot of transcriptional changes induced by KB001 treatment at 20uM compared to si*MYC* in MM1.S cells at 24 hours as assessed by Nanostring using custom MYC-target gene codeset. Pearson correlation of KB treatment and si*MYC* after housekeeping gene normalization. Bars in red represent MYC-responsive genes and bars in gray represent housekeeping genes. B. Hierarchical clustering of transcriptional changes induced by KB001 treatment compared to si*MYC* and two concentrations of BRD2/3/4/T inhibitor, JQ1, as assessed by Nanostring in MM1.S cells at 24 hours. C. Relative mRNA expression of MYC target gene, NME1/2, in MM1.S cells at 8 hours in response to 10-pt serial dilution of KB001 as assessed by qRT-PCR.

MYC TRN modulator KB001 binds BRPF1b, BRD7 and BRD4 bromodomains *in vitro*



A. BromoMELT™ with 20uM KB001 (Reaction Biology Corp.). KB001 assessed for thermal shift of designated bromodomains. Y-axis represents delta of melting temperature with KB001. B. BromoSCAN® of KB001 (Eurofins). KB001 used in 11-point 3-fold serial dilution with 10uM top dose to assess Kd of KB001 with bromodomains. C. Assessment of Kd of KB001 with BRPF1b, BRD7, and BRD4 by SPR. D. Molecular docking analysis of KB001 into bromodomain, BD1, of BRD4 (PDB: 6CJ2).

MYC TRN modulator phenocopies BET inhibitors and binds BETs BRD2, BRD3 and BRD4 in cells



A. Hierarchical clustering of transcriptional changes induced by KB001 treatment and reference compounds measured in MM1.S cells at 24 hours by Nanostring using a custom MYC-target gene codeset. B. In-cell proximity labeling of KB001 in MM1.S cells assessed by mass spectrometry. Enriched proteins identified as putative targets of KB001. Proximity labeling derivative of KB001 used at 5uM along with competition with 100uM of parent KB001. C. Possible model of KB001 targeting the BET proteins and inhibition the MYC transcriptional network. Created with BioRender.com.

Summary

- **Small molecule microarray screening platform employed to identify small molecules that bind to MYC transcriptional complexes in cell lysates from MYC-dependent cancer cells.**
- **Small molecule successfully identified in SMM lysate screen that modulates the MYC TRN in cancer cells with deregulated MYC.**
- **MYC TRN perturbations after KB001 treatment mimic signature changes due to MYC loss of function and signature changes effected by BET bromodomain inhibitors**
- **KB001 binds directly to BET bromodomains in purified protein systems.**
- **Cell-based proximity labeling demonstrates KB001 bound BET proteins BRD2, BRD3 and BRD4 in live cells.**