Investigating the molecular role of Brd9 in synovial sarcoma

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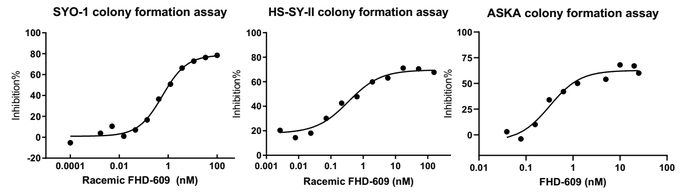
Abstract

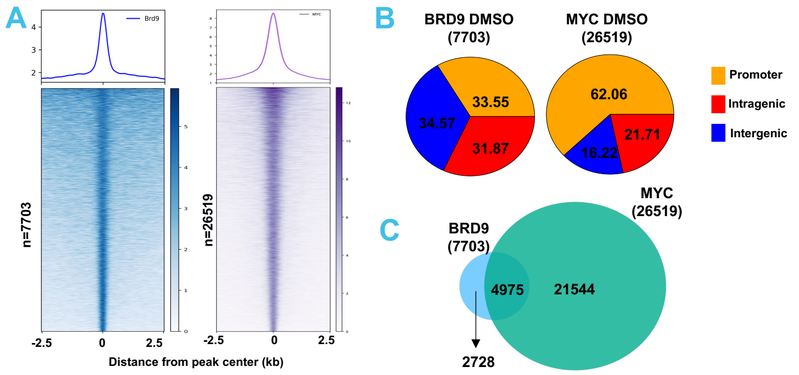
Bromodomain-containing protein 9 (BRD9) represents a potential selective vulnerability for tumors with specific alterations in the Brahma-associated factor (BAF) chromatin remodeling complex. One example of such a modification is the incorporation of the SS18-SSX fusion into BAF complexes. This alteration is a hallmark of synovial sarcoma and thought to drive tumorigenesis. As BRD9 is a subunit unique to ncBAF, tumors that depend on ncBAF for survival may be susceptible to BRD9 degradation. Accordingly, selective BRD9 degradation is a potential therapeutic approach to treat such diseases. Previous work has shown that loss of BRD9 causes cell and tumor proliferation defects (Brien et al. 2018, Michel et al. 2018). To explore the mechanism by which degradation of BRD9 causes cell and tumor proliferation defects in synovial sarcoma, we utilized a multi-omic approach using RNA-seq, ATAC-seq, and ChIP-seq data to understand the molecular impact of loss of BRD9 on relevant cell lines and animal model tumors. ChIP-seq revealed genome-wide colocalization of BRD9 and Myc on chromatin, and BRD9 degradation can lead to MYC loss at the majority of these co-bound regions. Genes commonly regulated in synovial cell lines treated with a BRD9 degrader *in vitro* and/or *in vivo* are enriched in MYC target genes, ribosome biogenesis genes and cell cycle genes. Furthermore, the genes commonly downregulated in synovial cell lines treated with a BRD9 degrader have notable MYC binding at their promoter regions. Therefore, we propose that these downregulated genes might be potential MYC targets, specifically in synovial sarcoma, and that downregulation leads to observed proliferation defects.

Background: FHD-609 inhibits proliferation in multiple synovial cell lines



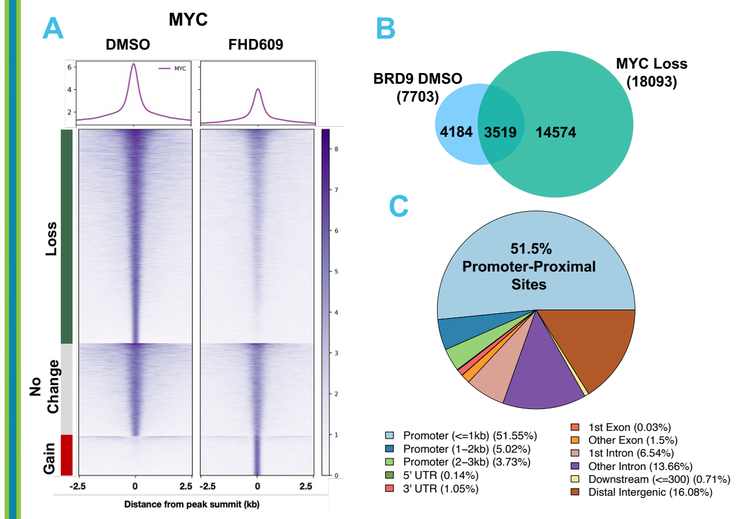
10-day colony formation assay showing strong proliferation inhibition in SYO1 (left), HSSYII (middle) and ASKA (right) treated with FHD-609.

Figure 1: BRD9 and MYC colocalize in the synovial genome



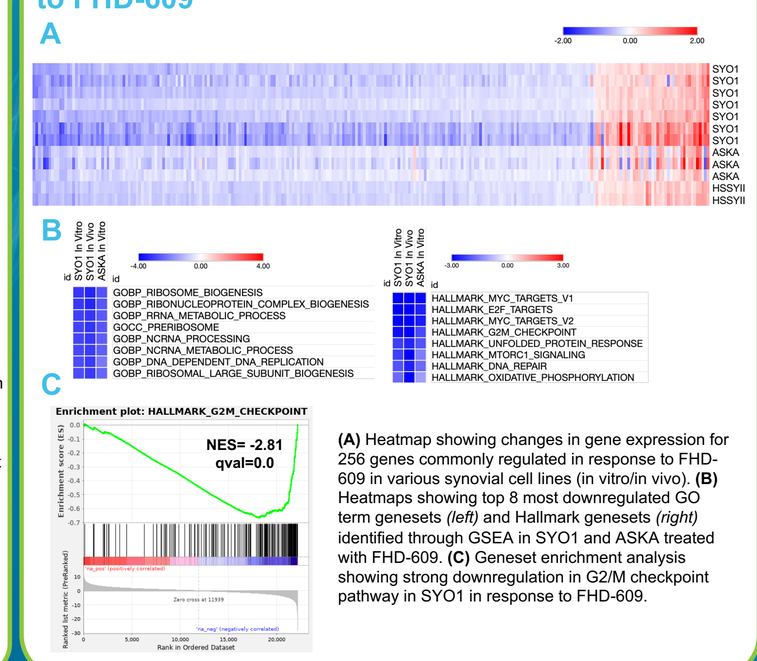
(A) Tornado and anchor plots showing the result of normalized ChIP-seq data for BRD9 *(left)* and MYC *(right)* in SYO1. The anchor plots at the top row show the average signal for BRD9 or MYC binding sites (+/- 2.5 kb flanking) in the DMSO. The tornado plot show the ChIP-seq signal intensity sorted from the highest to the lowest in the DMSO samples over BRD9 or MYC binding regions. **(B)** Pie charts showing genomic distribution of regions bound by BRD9 *(left)* and MYC *(right)*. **(C)** Venn diagram showing overlap between regions bound by Brd9 (7703) and MYC (26519) in SYO1. 65% of BRD9 peaks colocalize with MYC in the synovial genome. **(D)** Pie chart showing genomic distribution of regions co-bound by BRD9/MYC. **(E)** IGV genome browser view of an example gene: RPS26 has strong MYC and BRD9 binding in the promoter region.

Figure 2: BRD9 degradation leads to MYC loss from chromatin at BRD9-MYC co-bound regions



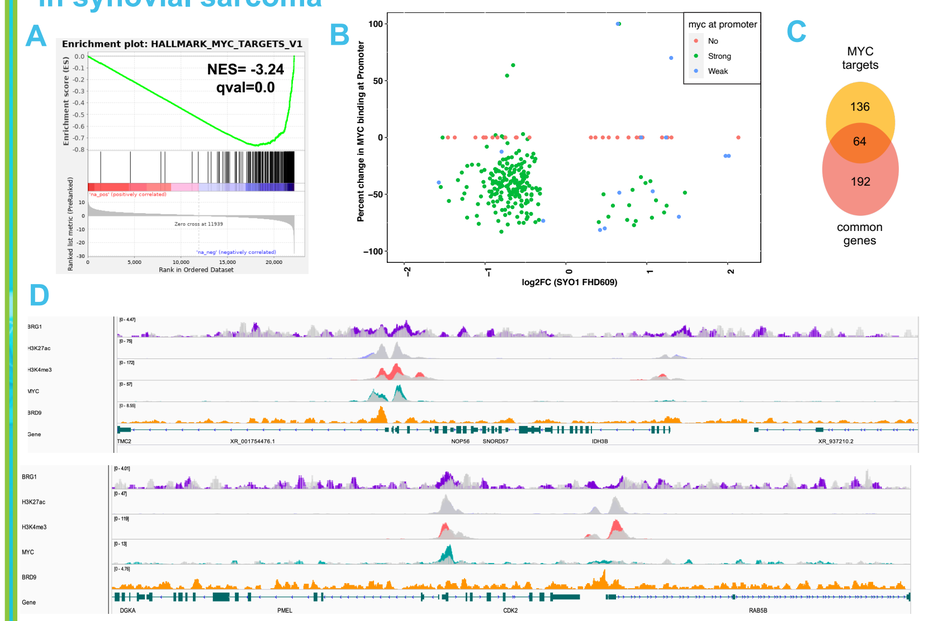
(A) Tornado and anchor plots showing the result of normalized ChIP-seq data in SYO1. The anchor plots at the top row show the average signal for MYC binding sites (+/- 2.5 kb flanking) in the DMSO *(left)* and FHD-609 *(right)*. The tornado plots show the ChIP-seq signal intensity sorted from the highest to the lowest in the DMSO samples over MYC binding regions. **(B)** Venn diagram showing overlap between regions bound by Brd9 and regions lost MYC binding upon FHD-609 treatment. 70% of BRD9-MYC co-bound regions lose MYC binding in response to FHD-609. **(C)** Pie chart showing genomic distribution of regions overlapping in (B). 52% of these regions are promoter-proximal sites.

Figure 3: Ribosome biogenesis and cell cycle pathways are enriched amongst commonly regulated genes in synovial sarcoma in response to FHD-609



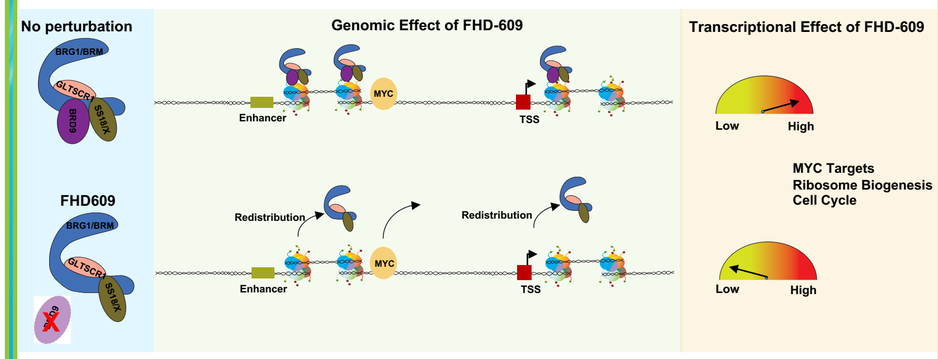
(A) Heatmap showing changes in gene expression for 256 genes commonly regulated in response to FHD-609 in various synovial cell lines (in vitro/in vivo). **(B)** Heatmaps showing top 8 most downregulated GO term genesets *(left)* and Hallmark genesets *(right)* identified through GSEA in SYO1 and ASKA treated with FHD-609. **(C)** Geneset enrichment analysis showing strong downregulation in G2/M checkpoint pathway in SYO1 in response to FHD-609.

Figure 4: Commonly regulated genes are potential MYC targets in synovial sarcoma



(A) Geneset enrichment analysis showing strong downregulation in MYC targets in SYO1 in response to FHD-609. **(B)** Scatterplot showing correlation between changes in gene expression for 256 genes commonly regulated in response to FHD-609 and percent change in MYC binding at the promoter regions of those genes in SYO1. Genes are annotated based on MYC binding (strong, weak or no) on their promoter regions. **(C)** Venn diagram showing overlap between the 256 commonly regulated genes and Hallmark MYC targets. **(D)** IGV genome browser view of a ribosomal gene cluster area: NOP56, SNORD86 and SNORD57 have strong BRD9 and MYC binding on the upstream of their promoter region. FHD-609 treatment leads to strong loss in H3K4me3 (active promoter mark) along with mild loss in MYC binding (H3K4me3 DMSO=red, MYC DMSO=green, BRD9 DMSO=orange, FHD-609=grey).

Proposed Mechanism of FHD-609 in Synovial Sarcoma



- BRD9 and MYC colocalize genome-wide in the synovial genome and BRD9 degradation leads to MYC loss from the chromatin at these colocalized regions.
- Genes commonly regulated in synovial cell lines in response to BRD9 degradation are MYC target genes, ribosome biogenesis and cell cycle genes.
- Promoter regions of these commonly regulated genes are occupied by strong MYC binding.

References / Acknowledgements

1. Michel et al., Nat Cell Biol, 2018 doi: 10.1038/s41556-018-0221-1
2. Brien et al., Elife, 2018 doi: 0.7554/eLife.41305