Targeting histone demethylase KDM6B for treatment of neuroblastoma

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INTRODUCTION

- Neuroblastoma is the cause of 15% of all cancer-related childhood deaths[1]
- Current treatments such as chemotherapy and radiation have many side effects
- KDM6B (an H3K27me3/me2 demethylase) is an epigenetic factor that may play a role in neuroblastoma
- KDM6B forms a complex with NOTCH1 and leads to MYC expression in T-cell acute lymphoblastic leukemia[2, 3]
- MYC causes many things necessary or beneficial for certain cancers but is hard to target
- The role of KDM6B in neuroblastoma is still largely unknown
- Therefore, the knockdown of KDM6B could prove to be a potent treatment for neuroblastoma

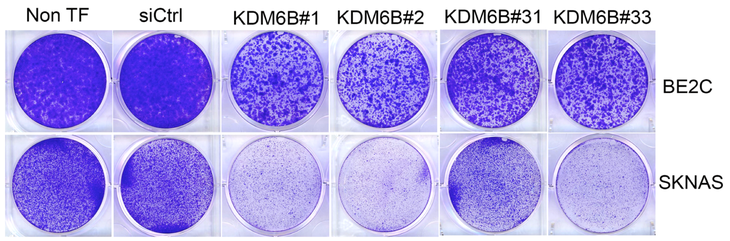


Figure 1. Loss of KDM6B reduces cell viability. 4 days after KDM6B knockdown in BE2C and SKNAS cell lines, cells were stained with crystal violet.

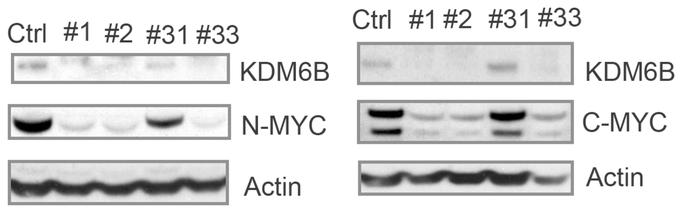
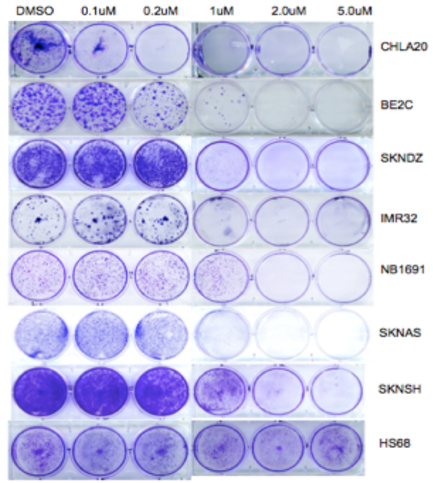
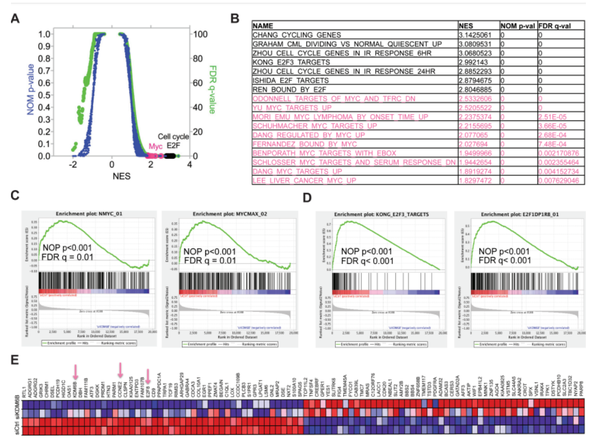


Figure 2. Loss of KDM6B results in the reduction of MYC. siRNA knockdown of KDM6B in BE2C and SKNAS cell lines. Western blot assessment of MYC with indicated antibodies.



Figures 3. GSK-J4, a KDM6 inhibitor, drastically reduces cancer cell viability. Normal human fibroblast HS68 and various cancer cell lines were treated with various concentrations of GSK-J4 for 7 days. Crystal violet staining was performed to show cell colony formation. Other samples were treated and collected 2 days later, normalized, and run in Western Blots to determine the effects of the drug on the expression of various proteins.



NAME	NES	NOM p-val	FDR q-val
CHANG_CYCLING_GENES	3.1425061	0	0
GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_UP	3.0809531	0	0
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_6HR	3.0680523	0	0
KONG_E2F3_TARGETS	2.992143	0	0
ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR	2.8852093	0	0
ISHIDA_E2F_TARGETS	2.8794675	0	0
REN_BOUND_BY_E2F	2.8046885	0	0
DOENNELL TARGETS OF MYC AND TFRC DN	2.5332606	0	0
YU_MYC_TARGETS_UP	2.5205522	0	0
MORI_EMU_MYC_LYMPHOMA_BY_ONSET_TIME_UP	2.2275374	0	2.51E-05
SCHUHMACHER_MYC_TARGETS_UP	2.2155605	0	1.68E-05
DANG_REGULATED_BY_MYC_UP	2.077065	0	7.68E-04
FERNANDEZ_BOUND_BY_MYC	2.027694	0	7.49E-04
BENPORATH_MYC_TARGETS_WITH_EBOX	1.9499966	0	0.002170876
SCHLOSSER_MYC_TARGETS_AND_SERUM_RESPONSE_DN	1.9442654	0	0.002354454
DANG_MYC_TARGETS_UP	1.8910274	0	0.004152734
LEE_LIVER_CANCER_MYC_UP	1.8297472	0	0.007629046

Figure 4.
A. Quantitative comparison of all chemical and genetic perturbation gene sets (n=3403) from the MSigDB by GSEA for increased (left) and reduced (right) expression of global genes caused by KDM6B knockdown. Data are presented as a scatterplot of normalized P value/false discovery q value versus normalized enrichment score (NES) for each evaluated gene set. The dotted square gene sets indicate locations of Myc/E2F pathway gene sets.
B. Selected GSEA datasets show that genes affected by depletion of KDM6B are regulated by the Myc/E2F pathway.
C. 2 examples of MYC pathways enriched by KDM6B targets.
D. 2 examples of MYC pathways enriched by E2F targets.
E. Heat map shows top 100 genes affected by KDM6B knockdown.

Cell Line	IC50	GI50	LC50
CHLA20	0.43	0.06667	0.41667
BE2C	0.65	0.45	1.9
SKNDZ	1.98	1.6975	2.3175
IMR32	0.615	0.045	0.715
NB1691	1.375	0.86	2.27
SKNAS	2.05	1.645	2.635
SKNSH	0.82	0.67	0.95
HS68	6.74	0.05	12.42

Figure 5. Some NB cell lines are more responsive to GSK-J4 than others. Cells were drugged with different concentrations of GSK-J4, and a Prestoblue analysis was performed 4 days later to determine IC50, GI50, and LC50 values.

CONCLUSIONS

- The inhibition of KDM6B leads to a loss of neuroblastoma cell viability.
- Key proteins are affected by the knockdown of KDM6B, notably MYC, E2F8, and CCNE2.
- GSK-J4 kills neuroblastoma cells by inhibiting KDM6B.
- More studies need to be done to determine the pathways involved and *in vivo* effects of KDM6B knockdown and GSK-J4 treatment.

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References

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3. Burchfield JS, Li Q, Wang HY, et al. JMJD3 as an epigenetic regulator in development and disease. International Journal of Biochemistry & Cell Biology 2015;67:148-157.