Targeting PRC2-regulated, super enhancer- driven transcription factors to treat malignant peripheral nerve sheath tumors

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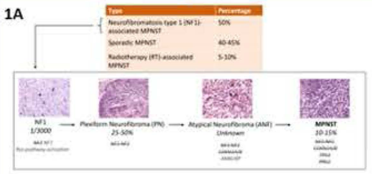
Abstract

Malignant peripheral nerve sheath tumor (MPNST) is an aggressive sarcoma known to be the leading cause of death in patients with neurofibromatosis type 1. About 80% of MPNSTs harbor somatic mutations of core components of polycomb repressive complex 2 (PRC2), such as EED and/or SUZ12. As a major complex responsible for maintenance of transcriptional repression, PRC2 deposits trimethylation at lysine 27 of histone H3 (H3K27me3). The loss of H3K27me3 due to defective PRC2 has been identified as a biomarker of MPNST. To understand the consequences of PRC2 loss in MPNST, we profiled epigenetic changes accompanying the loss of H3K27me3 and identified a group of super enhancer (SE)- driven transcription factors (TFs) that were activated due to the loss of H3K27me3. We hypothesize that these SE-driven TFs are essential to MPNST survival and can be targeted in treating MPNST.

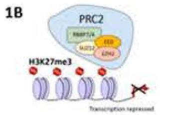
Background

1A

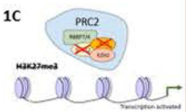


(1A) NF1-associated tumor goes through the malignant transformation accompanied with three stages of genetic alterations before developing into MPNSTs.

1B



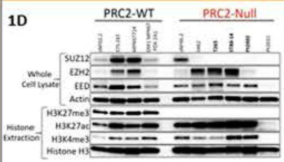
1C



(1B,C) Functional PRC2 deposits H3K27me3 that maintains gene repression while inactive PRC2 may results in gene transcription.

Methods

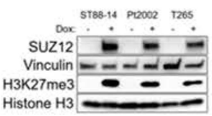
1D



(1D) We profiled PRC2 status in a panel of MPNST cell lines. The following three cell lines were used in this study: STS-26T, T265, and ST88-14.

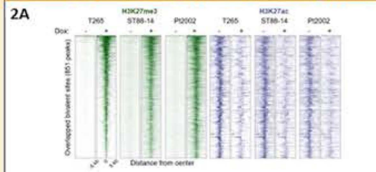
1E

(1E) In using doxycycline inducible MPNST cell lines to overexpress SUZ12 leading to restored PRC2 function, we observed a gain in H3K27me.



Transcriptional changes in inducible system

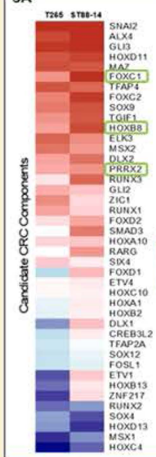
2A



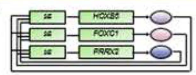
(2A) Upon PRC2 restoration in doxycycline treated inducible cell lines, ChIP-seq shows genome-wide gain of H3K27me3 accompanying loss of H3K27ac.

siRNA FlexiPlate reveals top SE-driven TFs

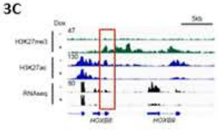
3A


T265
ST88-14
SNAI2
ALX4
GLI3
HOXD11
MAZ
FOXC1
TFAP4
FOXC2
SOX9
TGIF1
HOXB8
ELK3
MSX2
DLX2
PRRX2
RUNX3
GLI2
ZIC1
RUNX1
FOXD2
SMAD3
HOXA10
RARG
SIX4
FOXD1
ETV4
HOXC10
HOXA1
HOXB2
DLX1
CREB3L2
TFAP2A
SOX12
FOSL1
ETV1
HOXB13
ZNF217
RUNX2
SOX4
HOXD13
MSX1
HOXC4
Candidate CRC Components
%reduction
60
40
20
0
−20

3B



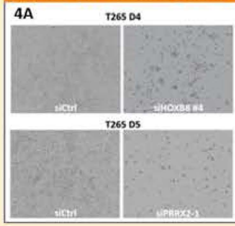
3C



(3A) A customized small interfering RNA (siRNA) screen using FlexiPlate to knockdown each candidate core transcriptional regulatory circuitries (CRC) TF was performed to investigate the dependency of MPNST cell lines to these identified SE-driven TFs. (3B) Three TFs resulted from best hits while (3C) gain of H3K27me3 and loss of H3K27Ac near their gene promoters demonstrate PRC2 regulation.

siRNA show distinct changes in morphology

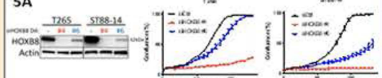
4A



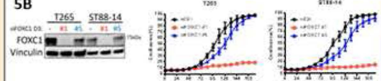
(4A) HOXB8, FOXC1, and PRRX2 knockdown (KD) was achieved through siRNA reverse transfection to validate its function and effect on MPNST cell survival. Here are examples of morphological differences in HOXB8 KD and PRRX2 KD cells.

SE-driven TFs are critical for MPNST viability

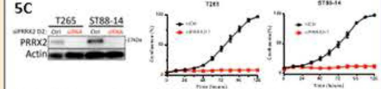
5A



5B



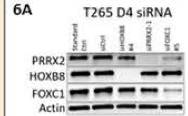
5C



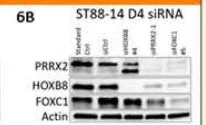
(5A,B,C) Western blot confirmed HOXB8, FOXC1, and PRRX2 KD and MPNST cell growth was monitored for a week using the IncuCyte.

Inter-regulation between SE-driven CRC

6A



6B

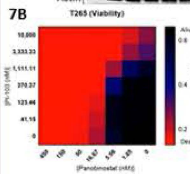


(6A,B) Western blot demonstrated inter-regulation between these SE-driven CRC TFs through siRNA KD in T265 and ST88-14.

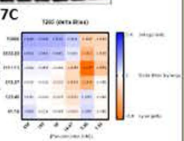
Targeting SE-driven CRC TFs

7A

7B



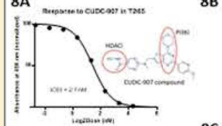
7C



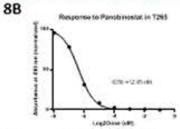
(7A) Because phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR pathway is known to be activated in NF1 tumors and histone deacetylase inhibitor (HDACi) can target CRC members, we compared single agent drug treatment using PI-103 (PI3Ki) or Panobinostat (HDACi). Panobinostat decreased protein of selected SE-driven CRC. Heat maps indicate (7B) T265 cell viability and (7C) synergy via delta bliss scores in combination treatment of PI-103 and Panobinostat.

CUDC-907 is more potent in treating MPNST

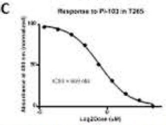
8A



8B



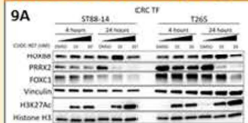
8C



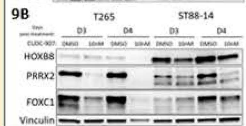
(8A) As a dual PI3K and HDAC inhibitor, CUDC-907 exhibited lowest IC50 in T265, indicating enhanced MPNST sensitivity. (8B, C) IC50 shown here were from single agent treatment using PI-103 or Panobinostat in T265.

CUDC-907 selectively targets SE-driven CRC

9A



9B



(9A) Protein of SE-driven CRC TFs in T265 and ST88-14 were significantly decreased by CUDC-907 treatment starting at 24 hours of treatment. Activation mark H3K27Ac increased in a dose-dependent manner upon treatment. (9B) Complete protein depletion of these TFs occur on day 4 of CUDC-907 treatment.

9C

(9C) Western blot confirmed loss of phosphorylated ERK1/2 by CUDC-907 treatment as early as 1 hour.

Conclusions

- Knockdown of PRRX2, FOXC1, and HOXB8 each inhibited cell growth in PRC2-Null MPNST cell lines→ these PRC2-regulated SE-driven TFs play essential role in cell survival and viability of MPNST cell lines
- siRNA of PRRX2, FOXC1, and HOXB8 show their interconnected regulation on each other
- Dual PI3K and HDAC inhibitor CUDC-907 treatment exhibited a potent killing effect in MPNST and selectively depleted these PRC2-dependent SE-driven TFs

Acknowledgements

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References

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