A Novel Role for the Histone Methyltransferase SETD2 during Mitosis

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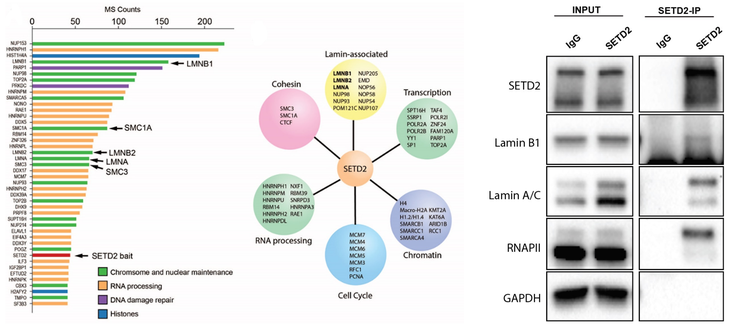
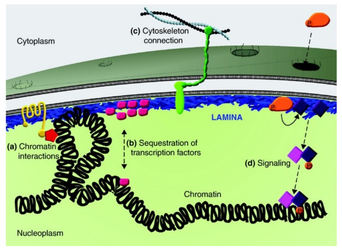
SETD2 is a histone methyltransferase responsible for trimethylation of lysine 36 of histone H3. This serves as a chemical tag promotes a number of processes, including DNA repair and preventing aberrant transcription. The SETD2 gene is commonly mutated in a variety of different cancers, most notably ccRCC, a type of kidney cancer. Here, SETD2 is reported to be the third most commonly mutated gene with a prevalence between 15 and 20 % among patients.[1] However, the mechanism by which SETD2 loss leads to cancer is poorly understood. Our lab's efforts have focused on illuminating the normal of SETD2 in human kidney cells to better understand how its loss leads to ccRCC and other cancers.

SETD2 Interacts with Nuclear Lamins



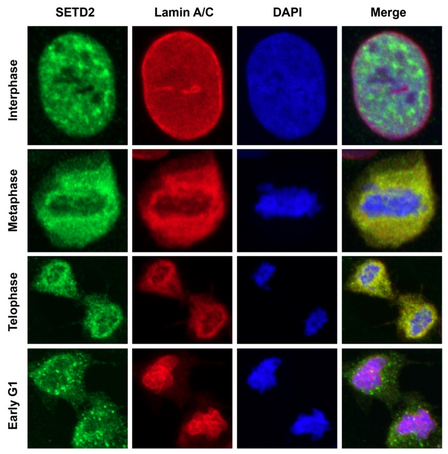
(Left. Mass spectrometry results from Bio ID of SETD2 showing its protein interactors. Colors represent different classes of proteins. (**Right**. Immunoprecipitation of endogenous SETD2 followed by western blot analysis, showing that SETD2 interacts with a number of nuclear lamina-associated proteins.

Lamins are the constituents of the nuclear lamina, a complex meshwork of proteins on the inner side of the nuclear membrane that plays a critical role in stabilizing the nucleus and safeguarding the genome.

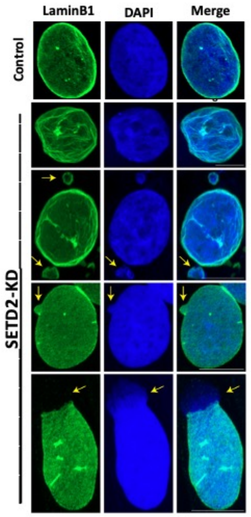


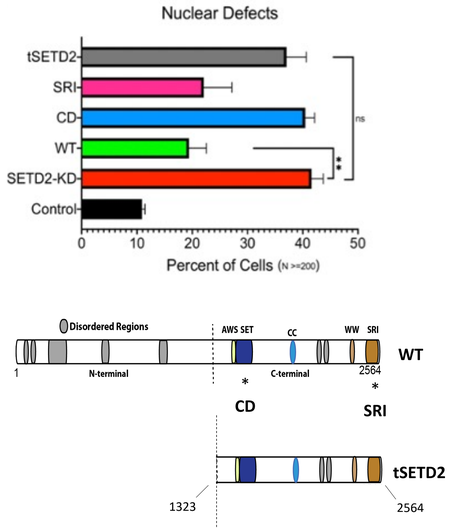
Dittmer, T.A., Misteli, T. (2011)

Right. Immunofluorescence of SETD2 and Lamins showing that these proteins colocalize during mitosis. These results suggest a novel, H3 methylation independent role for SETD2 during mitosis.



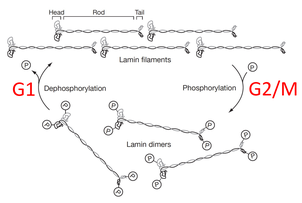
SETD2's N-terminus is Necessary for Proper Nuclear Membrane Morphology



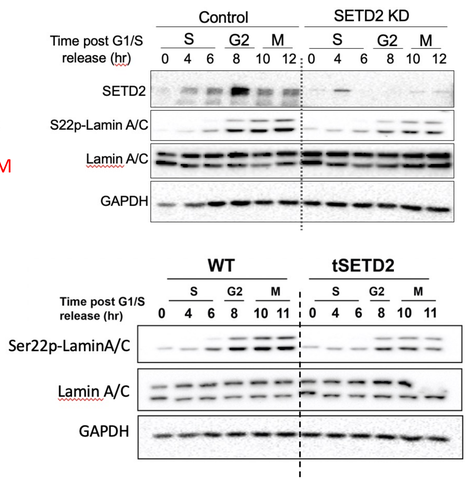


(Left. Immunofluorescence of Lamin B1 in control and SETD2-KD cells, showing that knockdown of SETD2 results in severe nuclear membrane defects. **(Right.** Quantification of nuclear membrane defects in cells expressing different versions of SETD2. These defects are rescued by cells expressing an exogenous wild type form of SETD2, but not a truncated form (tSETD2).

SETD2's N-terminus is Necessary for Lamin Phosphorylation During Mitosis

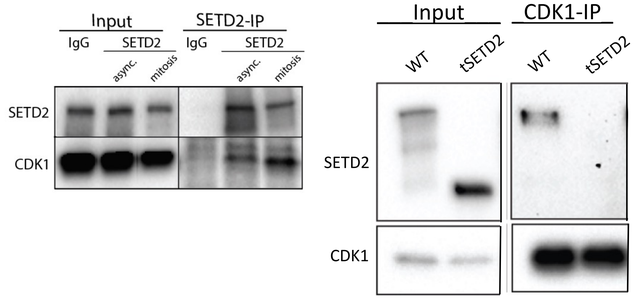


Paschal & Kelley 2013



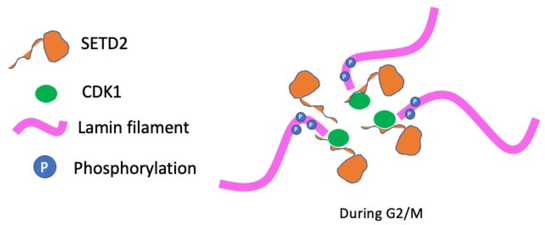
Cells were synchronized to the G1/S boundary using a double thymidine block. Lamin phosphorylation at Serine 22 on Lamin A/C was monitored as cells progressed through S, G2, and M. The results show a clear decrease in phosphorylation levels between control and SETD2-KD as well as between Wild Type and tSETD2, suggesting SETD2's N-terminus is necessary for Lamin phosphorylation during mitosis.

SETD2's N-terminus is required for SETD2-CDK1 Interaction

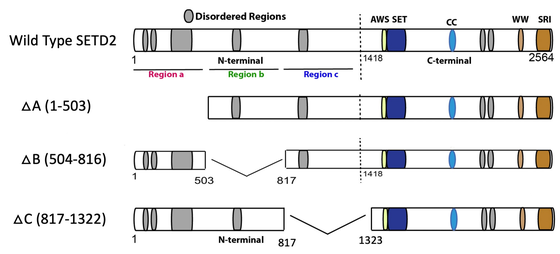


(Left. Immunoprecipitation of endogenous SETD2 in asynchronous and mitotic populations. CDK1 is enriched in the IP of the mitotic population, suggesting the SETD2-CDK1 interaction also occurs primarily during mitosis. **(Right**. Immunoprecipitation of CDK1 showing that full length SETD2 interacts with CDK1, but not tSETD2. This suggests that SETD2's N-terminus is necessary for the SETD2-CDK1 interaction

Model/Hypothesis



Future Directions



- Western for Ser22p Lamin A/C in SETD2 mutant cells
- Live cell imaging with Lamin A-GFP during G2/M
- Co-IP of CDK1 and SETD2 mutants
- Co-IF of CDK1 and SETD2 mutants

References

1. Chen, R., Zhao, W.-Q., Fang, C., Yang, X., & Ji, M. (2020, March 5). *Histone methyltransferase SETD2: A potential tumor suppressor in solid cancers*. Journal of Cancer.
2. Dittmer, T. A., and Misteli, T. (2011, May 31). *The lamin protein family - genome biology*. BioMed Central.
3. Paschal, B. M., & Kelley, J. B. (2013). *Nuclear lamina*. Nuclear Lamina - an overview. Encyclopedia of Biological Chemistry.