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SETD2 LOSS CONTRIBUTES TO CHRONIC MYELOID LEUKEMIA PROGRESSION AFFECTING DNA INTEGRITY AND EPIGENETIC LANDSCAPE

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INTRODUCTION

SETD2 is a tumor suppressor that trimethylates histone H3 at Lys36 (H3K36Me3), a key mark for transcription and DNA damage repair (DDR). We previously reported SETD2 nongenomic loss of function in blast crisis (BC)-CML, but not in chronic phase at diagnosis.

AIM

The aim of this study was to assess in CML models whether the contribution of SETD2 loss to disease progression occurs through the impairment of DDR fidelity and the perturbation of the epigenetic landscape

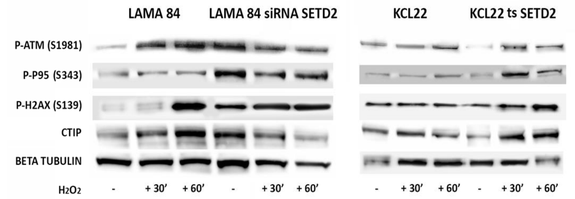
METHOD

- SETD2 forced expression in the SETD2-deficient KCL22 cell line was performed by nucleofection
- SETD2 knock-down in the SETD2-proficient LAMA 84 cell line was performed by RNA
- Western blotting (WB) and immunofluorescence assays were used to assess DNA damage and DNA repair activation in cell lines using an Homologous Recombination (HR) DNA Repair Antibody Kit (Cell Signaling Technology)
- ChipSEQ and RNASEQ were performed in SETD2 deficient and proficient cells to investigate its role in transcriptional regulation of different pathways

RESULTS

To investigate the activation and proficiency of homologous recombination (HR) of double strand breaks (DSB) and of mismatch repair (MMR), we used a single exposure to hydrogen peroxide, inducing DNA damage in SETD2 siRNA-depleted LAMA 84 cells. Compared to parental cells, cells silenced for SETD2 lost the ability to activate the ATM-dependent repair pathway. As a confirmation, SETD2 forced expression in KCL22 cells was able to restore DDR, as demonstrated by ATM and H2AX phosphorylation after hydrogen peroxide exposure.

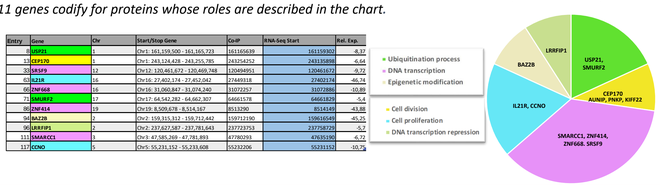
Figure 1: Western blotting assay shows phosphorylation status of different players of ATM pathway (P95, ATM, H2AX) and CTIP in the 4 cell lines at three stages of damage (controls, 30′ and 60′) (loading control, β-Tubulin)



ChIP-seq showed that forced SETD2 re-expression rescued H3K36Me3 in coding sequences but not promoters and identified a series of genes that are targeted by SETD2 trimethylation in CML and may thus be more prone to DSBs and mutations in vivo. In addition, H3K36Me3/RNAPolII colocalization identified genes whose transcription is SETD2/H3K36Me3-dependent (eg, AUNIP, CEP170, PNKP, and KIF22, which are involved in mitosis, cytokinesis, and DNA damage repair). Significant changes in expression of the same genes were detected in our SETD2 on/off models by RNA-seq.

Figure 4: In the top table we can see all loci in which co-immuno precipitation and over-transcription in SETD2 positive cells align. Those 11 genes codify for proteins whose roles are described in the chart.

Entry	Gene	Chr	Start/Stop Gene	Co-IP	RNA-Seq Start	Rel. Exp.
4	USP21	1	Chr1: 161,159,500 - 161,165,723	161165639	161159302	-8,33
19	CEP170	1	Chr1: 243,124,428 - 243,255,785	243135898	243154252	-6,64
30	SRSF9	12	Chr12: 120,461,672 - 120,469,748	120469551	120494951	-6,72
62	KZ14	16	Chr16: 27,402,174 - 27,452,042	27449318	27403174	-46,74
66	ZNF668	16	Chr16: 31,060,847 - 31,074,240	31072257	31072857	-10,85
71	SMARCF2	17	Chr17: 64,542,282 - 64,662,307	64661578	64661829	-5,4
86	ZNF414	19	Chr19: 8,509,678 - 8,514,167	8513290	8514149	-43,88
94	BAZ2B	2	Chr2: 159,315,312 - 159,712,442	159616549	159713190	-45,25
96	LRRFIP1	2	Chr2: 237,627,587 - 237,781,643	237723753	237758729	-5,7
111	SMARCC1	3	Chr3: 47,585,269 - 47,781,893	47782093	47635190	-6,72
117	CCNO	5	Chr5: 55,231,152 - 55,233,608	55233206	55231153	-10,75



We also found that ATM inactivation impaired H2AX localization to the sites of DNA damage in vitro, and prevents the formation of RAD51 (HR) and MSH6 (MMR) repair foci.

Figure 2: Immunofluorescence results of HR assessment in KCL22, KCL22 tsSETD2, LAMA84 and LAMA84 siSETD2 cell lines before and after 60′ of H2O2 exposure. DAPI is used as an intercalating probe for DNA to mark nuclei. MERGE represents the superimposition of the 3 images in blue (DAPI), green (p-H2AX) and red (RAD51)

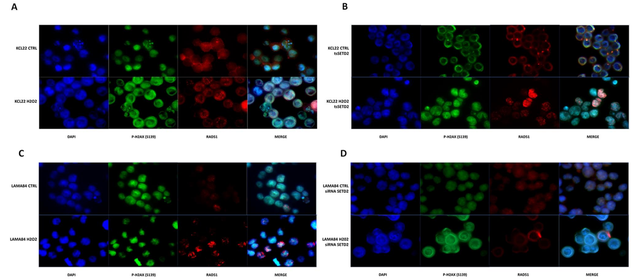
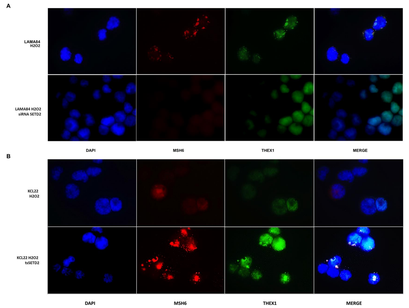


Figure 3: Immunofluorescence results of MMR assessment in LAMA84, LAMA84 siSETD2 , KCL22 and KCL22 tsSETD2 cell lines after 60′ of H2O2 exposure. DAPI is used as an intercalating probe for DNA to mark nuclei. MERGE represents the superimposition of the 3 images in blue (DAPI), green (THEX1) and red (MSH6).



CONCLUSIONS

SETD2/H3K36Me3 deficiency is a novel, BCR::ABL1-independent mechanism of genetic and genomic instability in CML

SETD2/H3K36Me3 loss perturbs the expression of key cellular genes compromising their integrity after DNA damage

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