



SETD2 Loss of Function Induces Genomic Instability in CML and May Contribute to Disease Progression to Blast Crisis



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Introduction

Genetic/genomic instability is a hallmark of CML. SETD2, a histone methyltransferase that trimethylates histone H3 on K36 (H3K36me3), has recently demonstrated a crucial role in preserving genomic integrity by modulating DNA Mismatch Repair (MMR) and Homologous Recombination (HR) repair. By Western blotting (WB), we previously observed SETD2 and H3K36me3 loss (resulting from aberrant SETD2 turnover) in 85% of blast crisis (BC) CML patients but not in newly diagnosed chronic phase (CP) patients who will achieve optimal responses.

Objective

1. To investigate whether SETD2 is involved in the maintenance of genetic/genomic stability in CML.
2. To assess whether SETD2 loss precedes or follows progression from CP to blast crisis BC

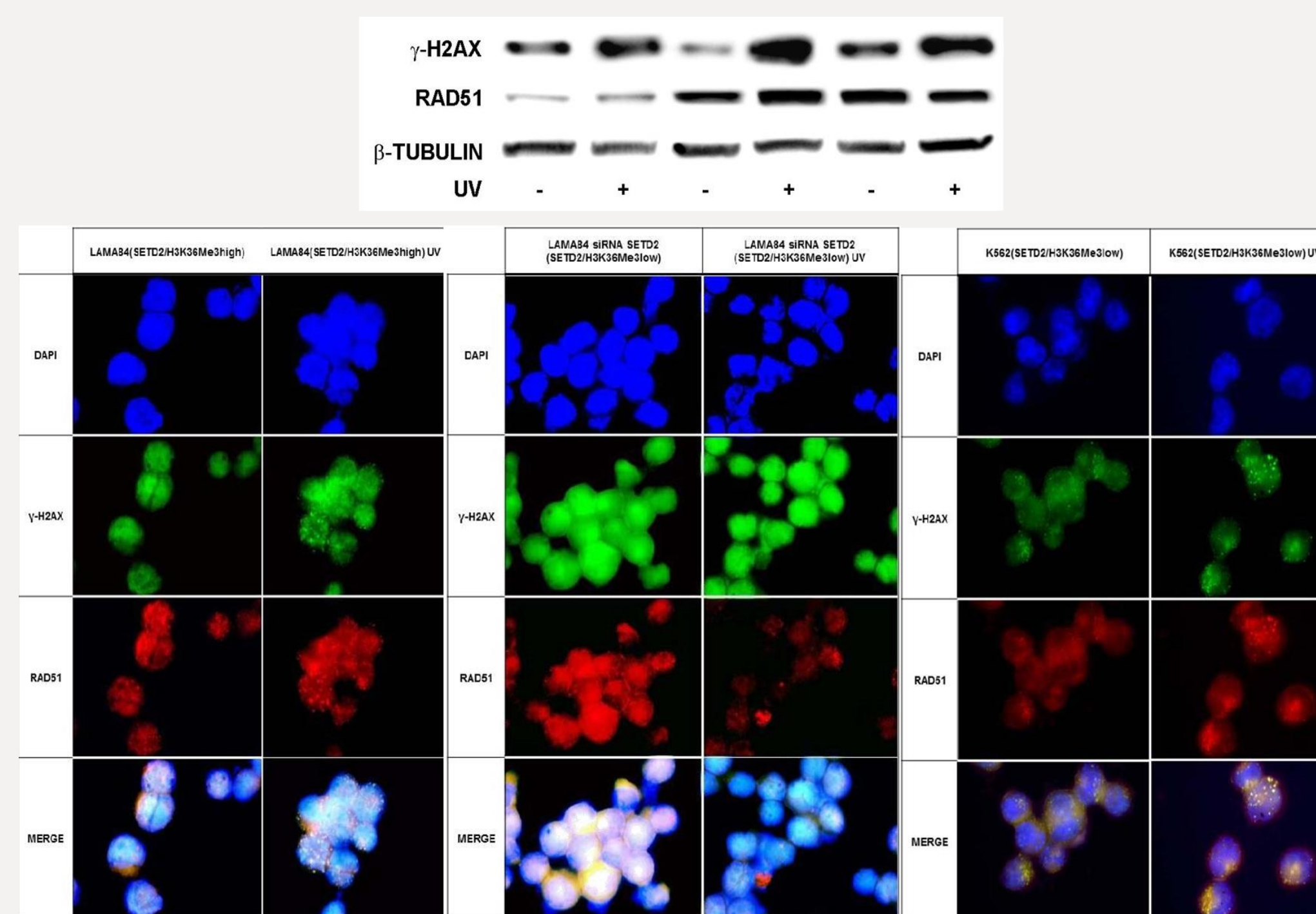
Materials

SETD2-proficient (LAMA84) and -deficient (KCL22) CML cell lines and primary patient samples (n=86) were studied.

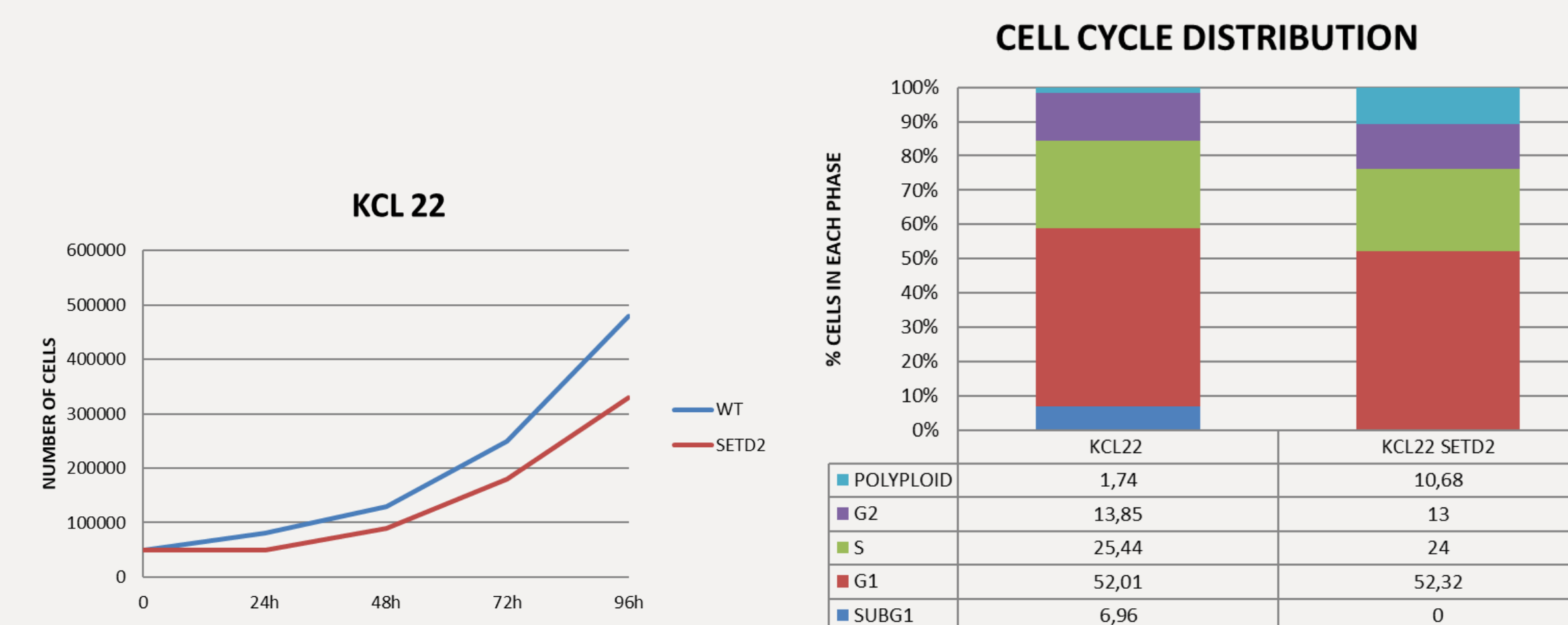
Results

To investigate whether SETD2/H3K36me3 loss impinges on the activation and proficiency of HR, we used UV rays to induce DNA damage in SETD2 siRNA-depleted LAMA 84 (SETD2-proficient) cells. Compared to control cells, cells silenced for SETD2 displayed a marked increase in γ H2AX (a marker of DNA damage) and fewer RAD51 foci (markers of ongoing HR).

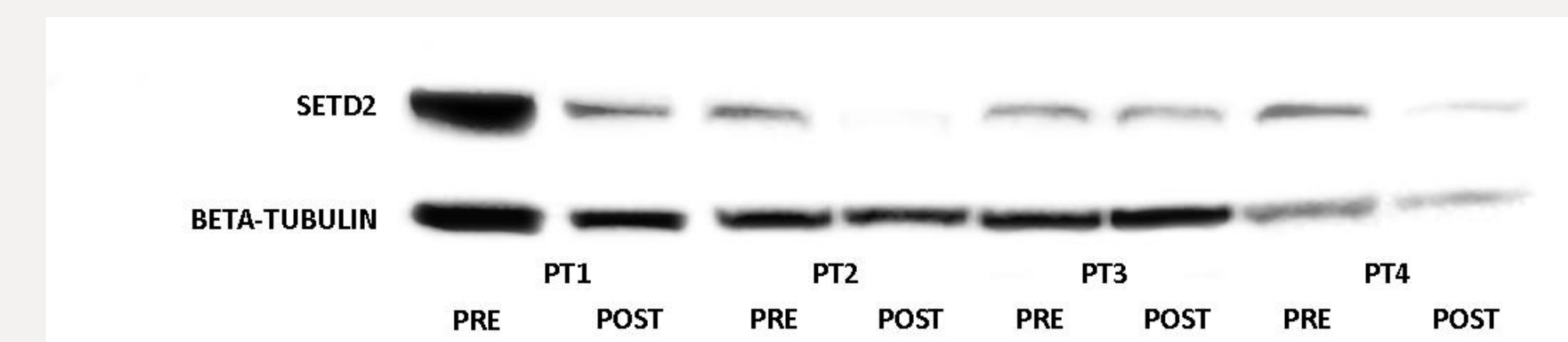
DNA damage repair's proficiency was evaluated by WB and Immunofluorescence. Cells with low or silenced SETD2 have significantly higher levels of γ H2AX and are unable to induce homologous recombination (HR) repair after DNA damage.



To further confirm the role of SETD2 as a tumor suppressor implicated in maintaining genomic stability in CML, we transfected KCL22 (SETD2-deficient) cells with an ectopic SETD2 plasmid. Preliminary results showed that SETD2 re-expression induced a reduction in cell doubling time, an accumulation of cells at G1/S checkpoint and a significant reduction in clonogenic potential.



Studies are ongoing to assess the effects on DNA damage repair pathways. We next wondered when during disease history SETD2/H3K36me3 loss occurs. WB analysis of paired (diagnosis/progression) samples from four CML patients suggested that patients who will progress still have intact and functional SETD2 at diagnosis. Additional analyses are ongoing to assess whether SETD2/H3K36me3 loss precedes, hence allows to predict, progression to BC.



Conclusions

Loss of SETD2/H3K36me3 is a novel, BCR-ABL1-independent mechanism of genetic instability in CML. Further investigations are needed to establish to what extent SETD2/H3K36me3 loss contributes to (and may be predictive of) disease progression.