P993 SETD2 IS A BONA FIDE TUMOR SUPPRESSOR IN SYSTEMIC MASTOCYTOSIS

**Topic:** 15. Myeloproliferative neoplasms - Biology & Translational Research

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**Background:**

The trimethylation of histone H3 at lysine 36 (H3K36me3), catalyzed by the SETD2 methyltransferase, is a post-translational modification involved in the fidelity of transcription and splicing and in the recruitment of DNA repair machinery. Recent studies have emphasized the tumor-suppressive role of SETD2, especially in renal cancer where SETD2 is often deleted or mutated. A novel mechanism of SETD2 non genomic loss of function due to proteasome-mediated degradation has recently been reported in advanced systemic mastocytosis (advSM). Proteasome inhibition has been found to inhibit clonogen growth and induce apoptosis, but whether this is due to SETD2 re-expression remains to be demonstrated.

**Aims:**

The aim of our study is to demonstrate unequivocally that forced expression of SETD2 in a cellular context in which it was lost, may restore proliferation control, clonogenic capacity and DNA damage repair mechanisms.

**Methods:**

The SETD2-deficient HMC-1.2 mast cell leukemia cell line was used as in vitro model. Forced SETD2 re-expression was obtained by nucleofection: 10⁶ HMC-1.2 cells were resuspended in 85μl of cell line Nucleofector Solution V and 2μg of a SETD2 (GFP-tagged) construct were added. An empty vector coding for GFP was used as negative control. Cells were transfected by using the Lonza Nucleofector 2b and the Lonza Amaxa Cell Line Nucleofector Kit V according to manufacturer’s instructions. GFP expression was assessed using a Cytoflex flow cytometer 24 and 48 hours post transfection; 78% of fluorescence positivity was observed at 48 hours. Neomycin selection (1mg/ml) was performed to obtain stable SETD2 expression. One month after selection, SETD2 and H3K36me3 were assessed by Western Blotting (WB). Clonogenic capacity was tested by clonogenic assays. Immunofluorescence experiments were performed to assess if SETD2 forced expression was able to restore DNA damage repair by using p-H2AX (S139), RAD51, MSH6 and THEX1 antibodies.

**Results:**

We previously characterized the HMC-1.2 cell line as deficient for SETD2/H3K36me3, as virtually all patients with advSM. To investigate whether SETD2 may indeed play a tumor suppressor role in SM, we transfected HMC-1.2 cells with an ectopic SETD2 plasmid. After transfection, the morphology of cells dramatically changed; moreover, HMC-1.2tsSETD2 showed a 70% increase in doubling time. Co-immunoprecipitation demonstrated that tsSETD2
was able to interact with p53 and to restore its expression and activity. We thus observed an increase in p21 and p27 associated with an accumulation of cells at the G1/S checkpoint. Moreover, SETD2 stable transfection restored DNA damage responses, as demonstrated by an increase in H2AX phosphorylation and RAD51 (HR), THEX1 (DNA replication) and MSH6 (MMR) expression after UV exposure. Finally, clonogenic assays in control and HMC-1.2tsSETD2 cells showed that: 1) SETD2 re-expression restores cell proliferation control; 2) reduction of clonogenic growth observed after proteasome inhibition is indeed SETD2-dependent.

Summary/Conclusion:

In advSM, SETD2 is a bona fide tumor suppressor and its loss impairs proliferation control and DNA damage responses. Its overexpression restores cell proliferation control by stabilizing p53 activity, and DNA damage repair by rescuing the H3K36me3 mark. This validates the therapeutic potential of interfering with the mechanisms responsible for SETD2 loss. Supported by AIRC IG 2019 grant (23001).