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Regulation of neuronal ion channel activity by Polycomb and Trithorax group proteins, in unexpected manners

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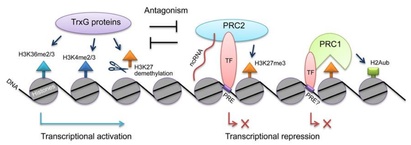
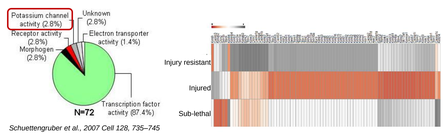
INTRODUCTION

❖ **Polycomb (PcG) and Trithorax (TrxG) group proteins are epigenetic regulatory proteins.** They regulate the expression of >1000 genes.
- Their known target genes involved in cell cycle control, cell fate decision, and development.
- They install repressive methylation and activating acetylation marks on histones, respectively. Hence, they may play opposing roles on the expression of the same genes.



PcG and TrxG proteins are assemble into multiple complexes, each with distinct role in histone modification and transcription activation/repression.
First, **PRC2** is recruited to the target gene and tri-methylates Histone H3 at K27 – producing a transcription repression mark. **PRC1** is then recruited and mediates mono-ubiquitinylation of H2A (producing mUbH2A). Chromatin condensation and DNA methylation follow, rendering the target gene **transcriptionally repressed**. **TrxG** Proteins tri-methylate H3K4 and inhibit H3K27 methylation via PRC2. This results in **transcriptional activation**, and **antagonism of PRC effects.**

❖ **Emerging neuroprotective roles of PcG proteins.**
- Roles for PcG proteins have been suggested or implicated in a number of neuronal disorders, e.g. epilepsy, AD, Parkinson's disease, TBI, pain sensing, and others.
- Using a mouse model of ischemic stroke, we identified a previously unknown, neuroprotective role of several PcG proteins against ischemic brain injury.
- Our previously published work suggest that PcG proteins regulate ion channel genes, which may explain expression changes of multiple ion channel genes under different ischemic conditions.



Schuettengruber et al., 2007 Cell 128, 735–745

(a) K⁺ channels are potential PcG protein targets

(b) ion channel expression is decreased (grey) in injury resistant conditions, and increased (orange) in injured conditions mimicking ischemia.

❖ **We ask the questions**:
- What are the molecular mechanisms underlying PcG-mediated neuroprotection?
- What are the potential roles of PcG proteins in regulating ion channel activity?
- Do TrxG proteins also regulate ion channel genes?

HYPOTHESIS & OBJECTIVE

We hypothesize that both PcG and TrxG proteins may target ion channels genes, by which they regulate neuronal excitability and subsequently either exert neuro-protective roles or exacerbate injury.

The objective of this work was to determine the roles of selected PcG and TrxG proteins on regulating electrophysiological properties of neuronal cells.

Out ultimate goal is to relate PcG and TrxG induced changes in channel function to neuronal excitability and their roles in neuronal response to ischemia.

METHODS

❖Model: Mouse brain-derived NS20Y neuroblastoma cells; differentiated with 0.5 mM 8-cpt-cAMP.
❖Inhibition of PcG protein BMI-1: 50 μM PRT 4165 (6 hrs).
❖Inhibition of TrxG protein MLL-1: 100 μM MM-102 (o/n).
❖Physiological recording: Channels that were not of interest were blocked.
- K^+ blockade: 2 mM TEA and Cs-based, intracellular.
- Na^+ blockade: 1 μM TTX.
- Ca^{2+} blockade: 100 μM $CdCl_2$.

❖All analyses were conducted on at least 3 independent cultures, with matching passage numbers and dates of recording across conditions that were compared.

RESULTS

1. Electrophysiological properties of NS20Y cells under normal conditions

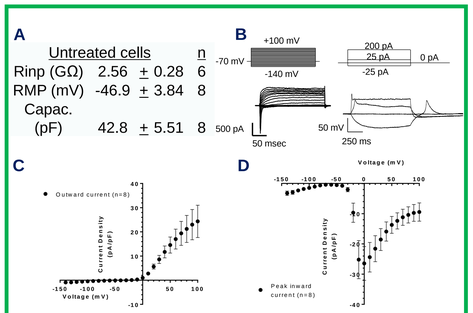


Figure 1. Differentiated NS20Y display inward and outward currents, resembling sodium and potassium channels.
A. Without current injection. B. Sample response (bottom) to voltage steps (left, top) and current injection (right, top). C. Average steady state outward current. D. Average peak inward current.

2. Pharmacological inhibition of BMI-1 or MLL-1 effectively attenuated histone modifications.

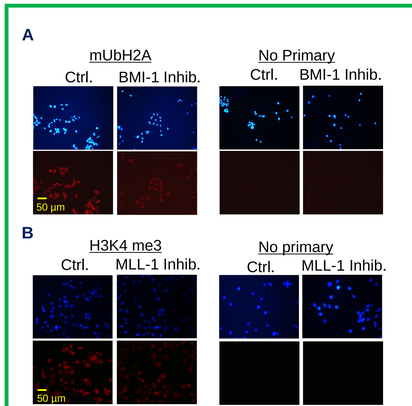


Figure 2. Decreased cellular levels of modified histone proteins with PcG or TrxG protein inhibition
A. Decreased levels of mUbH2A with BMI-1 inhibition.
B. Decreased levels of H3K4me3 with MLL-1 inhibition.

RESULTS

3. BMI-1 inhibition increased voltage-gated K^+ channel activity

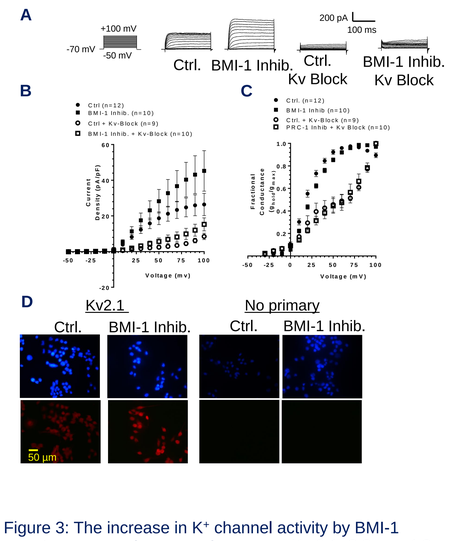


Figure 3: The increase in K^+ channel activity by BMI-1 inhibition was occluded by blockade of Kv 2.1, 2.2 and 4.3.
A and B. Increased channel activity. C. The voltage dependence of conductance was unchanged, suggesting a change only in channel number. D. increased levels of Kv2.1 proteins.

4. BMI-1 inhibition increased Na^+ channel activity

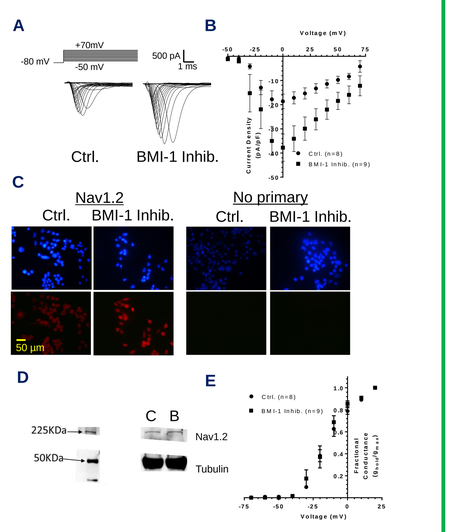


Figure 4. BMI-1 inhibition increased Na^+ channel activity.
A and B. Increased channel activity. C and D. Increased levels of Nav1.2. E. Voltage dependence of conductance was unchanged, suggesting a change only in channel number.

5. MLL-1 inhibition increased voltage-gated K^+ channel activity

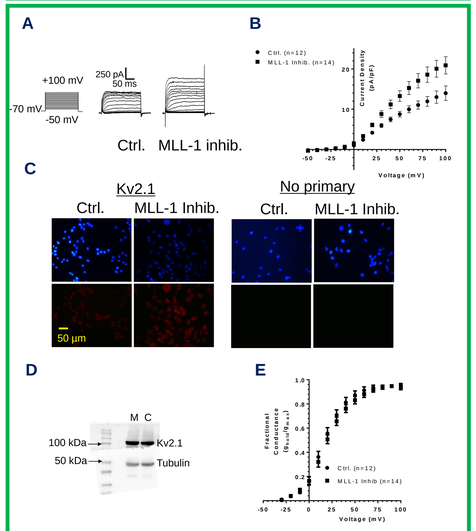


Figure 5: Unexpected increase in K^+ currents with MLL-1 inhibition.
A and B. Increased K^+ channel activity. C and D. Increased levels of Kv2.1. E. Voltage dependence of conductance was unchanged, suggesting a change only in channel number.

6. MLL-1 inhibition decreased Na^+ channel activity

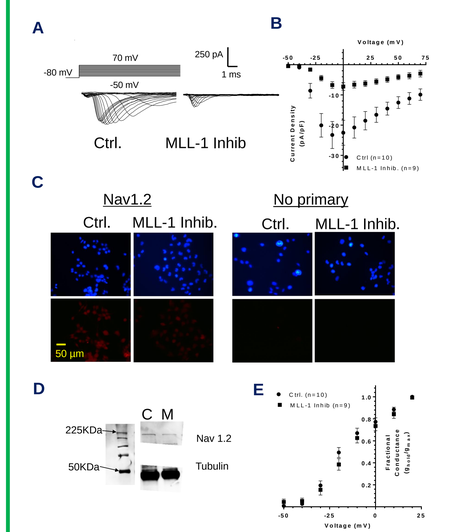


Figure 6: MLL-1 inhibition decreased Na^+ channel activity. A and B. Decreased channel activity. C and D. Decreased levels of Nav1.2. E. Voltage dependence of conductance was unchanged, suggesting a change only in channel number.

7. BMI-1 or MLL-1 inhibition do not affect KIR channel activity or Resting Membrane Potential

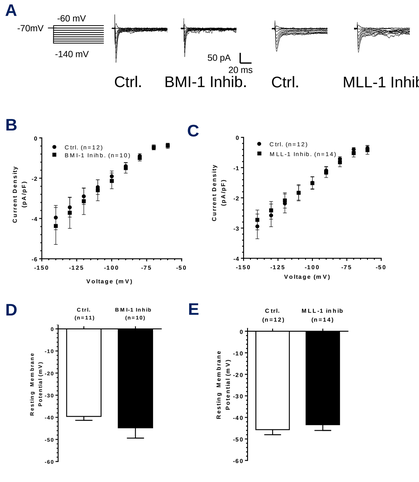


Figure 7: Neither KIR nor K^+ channels open at rest were affected by BMI-1 inhibition or MLL-1 inhibition.
A. Sample traces of KIR. B and C. No change in KIR channel activity by BMI-1 inhibition or MLL-1 inhibition, respectively. D and E. Resting membrane potential was unchanged by BMI-1 inhibition or MLL-1 inhibition, respectively.

SUMMARY AND FUTURE DIRECTION

❖ BMI-1 and MLL-1 inhibition resulted in decreased levels of mUbH2A or H3K4me3 proteins, respectively, as expected.
❖ K^+ channel activity was increased with BMI-1 inhibition, as expected, but also with MLL-1 inhibition, unexpectedly.
❖ BMI-1 inhibition and MLL-1 inhibition resulted in opposing changes in Na^+ channels, as expected.
❖ The observed changes are suggestive of changes in neuronal excitability.

❖ Future directions
- Roles of other TrxG proteins in regulation of neuronal ion channels, to determine whether the effects of MLL-1 seen in this study are MLL-1-specific or general to TrxG proteins as gene activators.
- Assess roles of PcG and TrxG proteins in regulating neuronal excitability in primary neurons.
- Impacts of PcG and TrxG-induced changes in excitability on the brain's response to ischemic stress.

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