IDENTIFICATION OF SYNERGISTIC COMBINATIONS OF HIV SILENCING PROMOTING FACTORS

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BACKGROUND

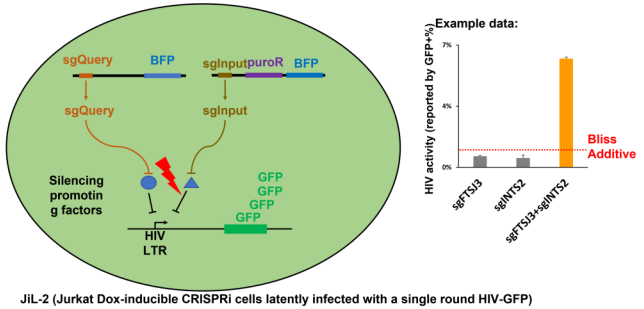
The activity of latent HIV is counter-regulated by both silencing and activating factors. To durably silence latent HIV ("block-and-lock"), we will specifically define how to enhance the activity of synergistic repressors while neutralizing HIV activators in relevant host cells. In this research, we are systematically evaluating the combinations of recently identified silencing promoting agents (SPAs) or inhibitors of activators for synergistic proviral silencing. We will also evaluate persistence of silencing when factors/inhibitors are removed—a critical hurdle we must clear for success of the block and lock strategy.

Hypothesis: Combinations of host silencing promoting agents (SPAs) and/or inhibitors of activators that act through different mechanisms will synergistically silence latent HIV even after factor/inhibitors are removed.

METHODS

To evaluate synergistic SPAs, we developed an inducible CRISPRi platform in HIV-GFP-latently-infected Jurkat cells (Fig.1). This system contains two lentiviral sgRNA vectors with different selection markers for introducing a pair of sgRNAs that target two SPAs. Using this platform, we evaluated the combinations among a selected set of 8 SPAs to assess whether synergistic networks of factors existed. Bliss-independence tests were used to validate synergy. We then conducted a proof of concept to use the network to discover synergies between the existing factors and drugs and repurpose them to block HIV.

A Platform to Identify Synergistic Interactions Between Silencing Factors



JiL-2 (Jurkat Dox-inducible CRISPRi cells latently infected with a single round HIV-GFP)

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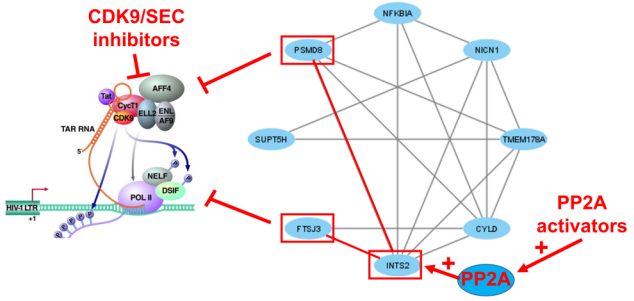
Synergies Among SPFs and Prior Knowledge Leading to Novel Drug Combinations



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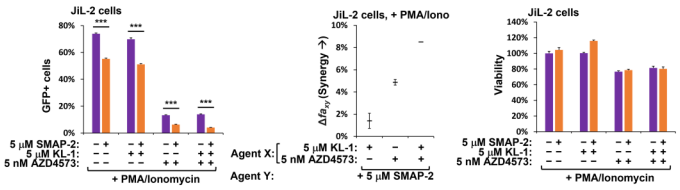
CDK9/SEC Inhibitors and PP2A Activator Synergistically Block HIV Activation by PMA/Ionomycin



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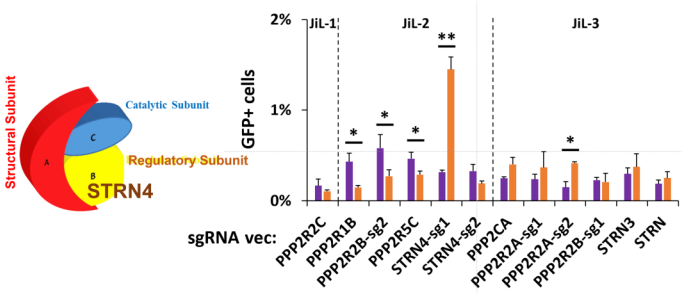
KD STRN4 (regulatory subunits of PP2A) significantly reactivated latent HIV



RESULTS

Seventeen synergies involving 8 SPFs have been identified (Fig. 2). Among them, sgINTS2 (Integrator subunit) strongly synergizes with sgPSMD8 and sgFTSJ3 (previously reported to inhibit CDK9/SEC). This led us to focus on Protein Phosphatase IIa, which interacts with and mediate the silencing promoting activity of Integrator. Small molecule activator of PP2A (SMAP-2) and CDK9/SEC inhibitors synergistically silenced HIV without inducing severe cell death (Fig.3). Knockdown of several PP2A regulatory subunits revealed that loss of STRN4 markedly activated latent HIV, indicating STRN4's key role in PP2A-induced HIV silencing (Fig. 4).

CONCLUSIONS

We established a flexible platform for assessing synergistic activities between SPFs. We find PP2A activators synergizing with CDK9/SEC inhibitors promoting silencing of latent HIV. Further, we identified the STRN4 regulatory subunit of PP2A as a key mediator of this silencing response. We will use this platform to identify additional synergies between SPFs and evaluate their ability to silence HIV in primary CD4 T cell latency models and in cells from HIV-infected patients on ART. We will also explore sequence -specific delivery of these synergistic repressors to sites of HIV provirus integration.