



Shock-and-kill versus block-and-lock: Targeting the fluctuating and heterogeneous HIV-1 gene expression

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Despite effective antiretroviral therapy, HIV-1 persistence in the latent reservoir remains the major barrier to cure. Current strategies for HIV-1 eradication require either inducing HIV-1 expression to expose latently infected cells for immune clearance [known as the “shock-and-kill strategy” (1)] or silencing HIV-1 expression for a prolonged drug-free remission [known as the “block-and-lock strategy” (2)]. Extensive small-molecule compound library screens have identified drugs that can reactivate HIV-1 from latency [known as latency-reversing agents (LRAs) (1)] as well as drugs that can reduce HIV-1 expression [known as HIV-1-suppressing agents (3) or latency-promoting agents (LPAs) (4)]. However, none of these agents have reached a durable HIV-1 remission in clinical trials, suggesting that more drug candidates should be identified and tested. Lu et al. (5) performed a drug screen to identify compounds that can modulate the fluctuations of HIV-1 gene expression.

Gene expression does not always follow deterministic kinetics like an on/off switch: Instead, gene expression levels frequently fluctuate (“noise”), creating stochastic variations in cell fate determination (6). For example, if HIV-1 gene expression is deterministic, maximum T cell activation should be able to reactivate all HIV-1 proviruses from latency. However, *ex vivo* studies showed that each round of maximum T cell activation can only reactivate a subset of HIV-1 expression (7). This is because HIV-1 gene expression level is determined by the stochastic fluctuation of Tat expression (8). The fluctuation and stochastic nature of HIV-1 gene expression creates a barrier for effective HIV-1 eradication strategies. Therefore, identification of drugs that can modulate the fluctuations of HIV-1 gene expression (or “noise”) may presumably increase the efficiency of HIV-1 latency reversal or silencing.

Capturing the Dynamic and Fluctuating HIV-1 Gene Expression at the Single-Cell Level

Typical drug screens for LRAs or LPAs measure the steady-state expression level of HIV-1-driven fluorescent reporter (such as green fluorescent protein

[GFP] or luciferase) at a single time point in bulk. Such methods cannot capture the dynamic changes of gene expression fluctuations and the heterogeneity in individual cells. Weinberger et al. (8) first developed a fluorescent imaging platform to track the fluctuation and heterogeneity of HIV-1–GFP reporter expression in individual cells over time. Later, Dar et al. (9) further measured the amplitude of HIV-1 gene expression fluctuations (coefficient of variation [CV]) and the duration of HIV-1 gene expression (time) to quantify the level of HIV-1 gene expression fluctuations in individual cells. Using this platform for a drug screen, Dar et al. (9) identified drugs that can change the fluctuations of HIV-1 gene expression as noise-modulating agents, including noise enhancers and noise suppressors.

In PNAS, the Dar group refine this noise-measuring method to identify drugs that can suppress HIV-1 expression upon T cell activation by tumor necrosis factor (TNF) (5) (Fig. 1A). The major advance of this study is to use automated time-lapse fluorescent microscopy to capture not only HIV-1 expression level (fluorescent intensity) but also noise magnitude ($HF-CV^2$) and the duration of gene expression fluctuation ($\tau_{1/2}$). While the previous drug screen identified only one compound (manidipine) as a noise suppressor (9), Lu et al. (5) identify three noise modulators that can suppress HIV-1 expression when challenged with different LRAs, such as TNF, protein kinase C (PKC) activator prostratin, and phorbol myristate acetate (another potent PKC activator) and ionomycin (a calcium ionophore). The authors find that two of the three compounds structurally resemble pleurotine, a thioredoxin reductase (TrxR) inhibitor. Using these two lead compounds (NSC401005 and NSC400938), the authors identify two additional Trx/TrxR pathway inhibitors, PX12 and tiopronin that can suppress HIV-1 expression. Although the mechanism of TrxR on HIV-1 is not yet fully understood, two previous studies suggest that thioredoxin may regulate the disulfide bond

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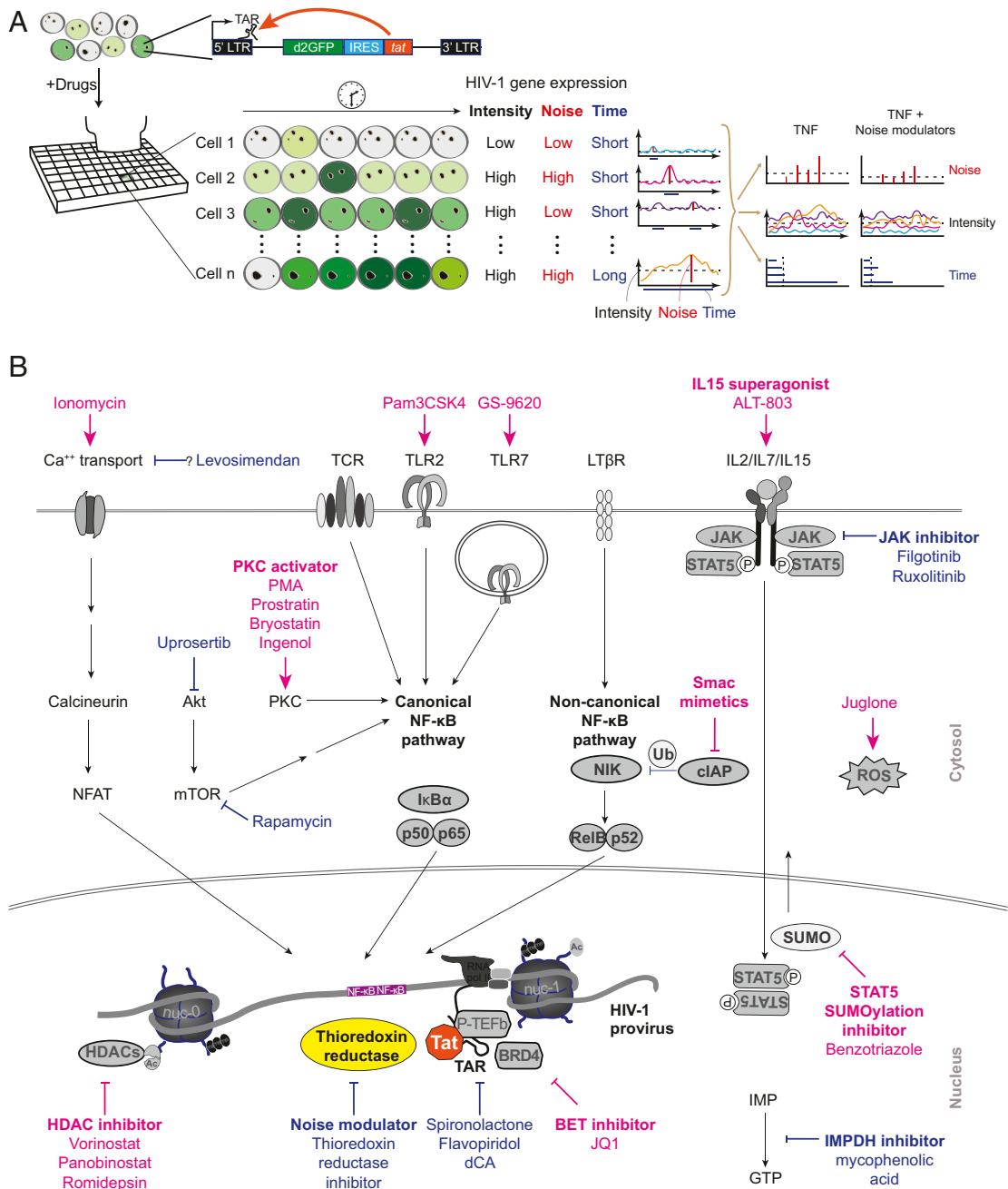


Fig. 1. Identification of cellular targets for LRAs versus LPAs. (A) Measuring the fluctuating and heterogeneous HIV-1 gene expression by automated time-lapse fluorescent microscopy. In individual culture wells, HIV-1–driven, Tat-dependent GFP expression is captured by tracking the GFP signal intensity over time, allowing the measurement of HIV-1 expression level (intensity), fluctuations (noise) in HIV-1–GFP expression, and the duration of HIV-1 expression (time). As opposed to measuring the static level of HIV-1 expression, this approach quantifies the trajectory of the fluctuating HIV-1 gene expression over time at the single-cell level. **(B)** Shock-and-kill versus block-and-lock strategies: Drug screens for LRAs (magenta) versus LPAs (blue) identified molecular mechanisms and therapeutic targets that are important for HIV-1 expression. ROS, reactive oxygen species.

binding of Tat (10) or NF-κB (11). It remains unclear whether these drugs can indeed promote HIV-1 latency at the epigenetic level or serve merely as a Tat inhibitor at the RNA transcription elongation level.

Understanding the Control of HIV-1 Gene Expression Is a Key to a Cure

Drug screens not only identify candidate therapeutic targets but also serve as probes to identify pathways that are critical for HIV-1 gene expression, latency, and reactivation. While T cell activation

remains the most effective way to reactivate HIV-1, the systemic side effect and potential of increasing the proliferation of HIV-1–infected cells make global T cell activation not feasible for clinical use. Drug screens on HIV-1 latency reversal identified multiple viral and cellular pathways: HIV-1 can be reactivated by 1) increasing Tat-dependent transcriptional elongation by inhibiting BRD4 interaction with the positive transcription elongation factor b (P-TEFb) [such as JQ1 (12)], 2) removing epigenetic silencing by histone deacetylation inhibitors [such as vorinostat (13),

panobinostat (14), and romidepsin (15)), 3) direct or indirect activation of NF- κ B through Toll-like receptor (TLR) activation [such as TLR1/2 agonist Pam3CSK4 (16) and TLR7 agonist GS-9620 (17)], PKC activation [such as bryostatin (18) and ingenol (19)], or non-canonical NF- κ B activation [such as SMAC mimetics (20, 21)], 4) activation by common γ -chain receptor cytokines [such as IL-15 agonist (22)] and JAK/STAT pathways such as STAT5 SUMOylation inhibitor [such as benzotriazoles (23)], and 5) other cellular pathways such as reactive oxidative stress inducer [such as juglone (24)] (Fig. 1B). Similarly, drug screens on HIV-1-suppressing agents also identified viral and cellular pathways as therapeutic targets: HIV-1 reactivation can be suppressed by 1) inhibition of Tat-dependent transcription by Tat inhibitor [such as didehydrocortistatin A (dCA) (2)], CDK9 inhibitor [such as flavopiridol (3)], and DNA helicase transcription factor IIH inhibitor [such as spironolactone (3, 25)], 2) inhibition of NF- κ B activation by mTOR inhibitor [such as rapamycin (26)] and Akt inhibitor [such as uprosertib (3)], 3) inhibition of JAK/STAT pathways by JAK

inhibitors [such as filgotinib (3) and ruxolitinib (3, 27)], or 4) other cellular pathways such as calcium sensitizer levosimendan (3, 4), nucleotide synthesis inhibitor [such as mycophenolic acid and MMF (3)], and RNA splicing inhibitor [such as filgotinib (3)]. Ideally, the platform that Lu et al. (5) developed can be applied to different reporter systems and may potentially identify gene expression noise modulators involving these cellular pathways to fine-tune both the shock-and-kill and the block-and-lock strategies.

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