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Killing HIV-infected resting central memory CD4$^+$ T cells by targeting inhibitor of apoptosis proteins-inhibited autophagy

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Abstract

Dysfunction of CD4$^+$ T cells by HIV infection can cause serious immune defects. Recently, Campbell and colleagues described an intriguing and simple therapeutic method for HIV-infected resting central memory CD4$^+$ T cells (HIV-T_{CM}), dependently on inhibitor of apoptosis (IAP) family proteins-targeted and second mitochondria-derived activator of caspases (SMAC) mimetics-mediated apoptosis, which is only triggered in HIV-T_{CM} and not uninfected ones. Autophagy induction and subsequent formation of a ripoptosome-like death signaling complex were observed after such treatment, which may partially explain the potential mechanism. However, the direct intracellular inhibitory effects of IAPs on autophagy, as well as the critical roles of autophagy in activating extracellular anti-infection immune responses, warrant further investigation. Thus, this pointer aims to provide potential alternative mechanisms and to suggest important avenues for follow-up study.

Keywords

Autophagy, CD4$^+$ T cell, HIV, immune signature, inhibitor of apoptosis, SMAC mimic

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Human immunodeficiency virus (HIV) attacks and destroys the body's immune system, causing acquired immunodeficiency syndrome (AIDS). CD4$^+$ T cells, as one of the most important lymphocyte types, are the main target of HIV. Dysfunction of CD4$^+$ T cells leads to impaired immune surveillance, which makes the host susceptible to a variety of infectious diseases and malignant tumors, resulting in the high fatality rate. Recently, Campbell and colleagues described an intriguing and simple therapeutic method for HIV-1-infected resting central memory CD4$^+$ T cells (HIV-T_{CM}) using inhibitor of apoptosis (IAP) family proteins-targeted second mitochondria-derived activator of caspases (SMAC) mimetics.[1,2] In brief, Campbell *et al*. found that SMAC mimetics induced apoptosis in HIV-T_{CM}, but had little influence on uninfected T_{CM}. These effects were based on autophagy induction as well as the formation of a ripoptosome-like death signaling complex. However, after carefully reading their study, we do not fully agree with the authors' interpretations. We believe that the following major concerns need to be addressed in the near future before drawing any definitive conclusions of the efficacy and the mechanism of this treatment.

IAPs are deemed the specific targets of SMAC mimetics, and the apoptosis-inducing action of IAPs inhibition is well-documented.[3,4] Surprisingly, accumulating evidence suggests that IAPs also play critical roles in regulating autophagy and its cross-talk with apoptosis. The first identified autophagic function-

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related IAP protein is Bruce, which regulates autophagy and nutrient deprivation-caused cell death during *Drosophila melanogaster* oogenesis.[5,6] X-linked inhibitor of apoptosis protein (XIAP), a representative member of the IAP family, has been demonstrated to contribute to autophagy inhibition through two individual pathways. In short, XIAP inhibits autophagy via the MDM2-p53 axis and controls starvation-induced autophagy downstream of PI3K/AKT signaling[7–10]; moreover, XIAP functions as an endogenous repressor of p62, suppressing p62 expression through ubiquitin-proteasomal degradation.[11]

Autophagy acts as a kind of defensive mechanism against infecting pathogens,[4,12–16] and thus the application of autophagy-related cell death-inducing agents against infectious diseases is of great significance.[17,18] Campbell *et al.* used SMAC mimetics to treat resting memory T cells, which were obtained from the blood of AIDS patients, in their ex vivo experiments. The essential difference between T_{CM} and HIV-T_{CM} is not just HIV infection, but also the resulting upregulated expression of IAPs, which suggests that the quantity of IAPs could potentially determine the therapeutic efficacy of SMAC mimetics on HIV-T_{CM}. Although the synergistic effectiveness of SMAC mimetics in the presence of pharmacological inhibitors of apoptosis—such as pan-caspase inhibitor z-VAD-FMK, wortmannin (PI3K inhibitor), and necroptosis inhibitor—was evaluated, the direct effects of IAPs-mediated autophagic inhibition on the survival of HIV-T_{CM} and the therapeutic efficacy of SMAC mimetics should be further investigated. According to their report, although autophagy initiation could be promoted in HIV-uninfected T_{CM} at a much higher concentration (10x), IAPs-targeted SMAC mimetics were not able to induce autophagy-dependent apoptosis in these cells. Considering that HIV-uninfected T_{CM} also contains IAPs and autophagic machinery like HIV-T_{CM}, and that no genetically-depleted models of IAPs were used in the experiments, SMAC mimetics-induced confounding effects cannot be completely ruled out in this study. Hence, we propose a more reasonable model as follows: HIV infection leads to up-regulation of IAPs in T_{CM}, which enhances IAPs-mediated inhibition of autophagy and apoptosis, resulting in the survival of host T_{CM} and preventing HIV from auto-clearance. Meanwhile, amplified IAPs dominate HIV-T_{CM}, thereby playing vital roles in its biological activity. Thus, SMAC mimetics show stronger killing efficacy on IAPs-amplified HIV-T_{CM} compared to T_{CM}, the so-called "strengthening the strong" (Figure 1(a)).

In addition to being required in intracellular control of invading pathogens, autophagy is critical for activation of the extracellular immune system.[4,14,15,19] For instance, autophagy can promote host cells to release immunostimulatory molecules, such as adenosine triphosphate (ATP), lysophosphatidylcholine (LPC), calreticulin (CRT), and phosphatidylserine (PS), so as to constitute a "find me and eat me" signal in the tumor immune microenvironment (TIME). In contrast, autophagy in antigen-presenting cells (APCs) can affect the activation of Toll-like receptors, presentation of MHC II molecules, and formation of immune synapses. Not to mention, autophagy is also important for the differentiation, survival, and activity of T and B lymphocytes. Increasing evidence supports the immunomodulatory functions of IAPs, such as controlling pattern-recognition receptor (PRR) signaling and anti-tumor immune response, as well as their roles in immunodeficiency syndromes, like X-linked lymphoproliferative disease type 2 (XLP2).[20–24] Therefore, in this case, targeting IAPs by SMAC mimetics may have an immune system-dependent influence through autophagy. Moreover, considering the close association between IAPs and autophagy, the therapeutic effects of SMAC mimetics on AIDS may be dependent on autophagy-mediated immune regulation. Frustratingly, the authors did not use any animal models (like mice or monkeys) to evaluate the therapeutic efficacy of SMAC mimetics on HIV-T_{CM}; merely several experiments were done on primary resting memory T-cells from HIV infected patients, and the samples size was rather small (only four subjects). This largely diminishes the physiological significance and persuasiveness of the authors' conclusions. Is the autophagy-associated intrinsic cell death pathway sufficient to kill HIV-infected cells? What is the status of the immune system in SMAC mimetics-treated HIV-T_{CM} under pathological conditions? At least in our opinion, we cannot imagine eliminating HIV without immune system participation.

Big genomic data-based bioinformatics analyses further reveal the close relationship between IAPs and various immune signatures (molecule patterns plus signaling markers) in whole blood and spleen. Briefly, IAPs (including NAIP, CIAP1, CIAP2, XIAP, Survivin, BRUCE, Livin, and ILP2) not only directly correlate with CD4 T cells (Figure 1(b)) and central memory T cells (CCR7/SELL/IL7R) (Figure 1(c)), but are also positively related to resident memory T cells (CD69/ITGAE/CXCR6/MYADM) (Figure 1(d)), effector T cells (CX3CR1/FGFBP2/FCGR3A) (Figure 1(e)), effector memory T cells (PDCD1/DUSP4/GZMK/GZMA/IFNG) (Figure 1(f)), exhausted T cells (HAVCR2/TIGIT/LAG3/PDCD1/CXCL13/LAYN) (Figure 1(g)), effector Treg cells (FOXP3/CTLA4/CCR8/TNFRSF9) (Figure 1(h)), resting Treg cells (FOXP3/IL2RA) (Figure 1(i)), and Th1-like cells (CXCL13/HAVCR2/IFNG/CXCR3/BHLHE40/CD4) (Figure 1(j)). The detailed

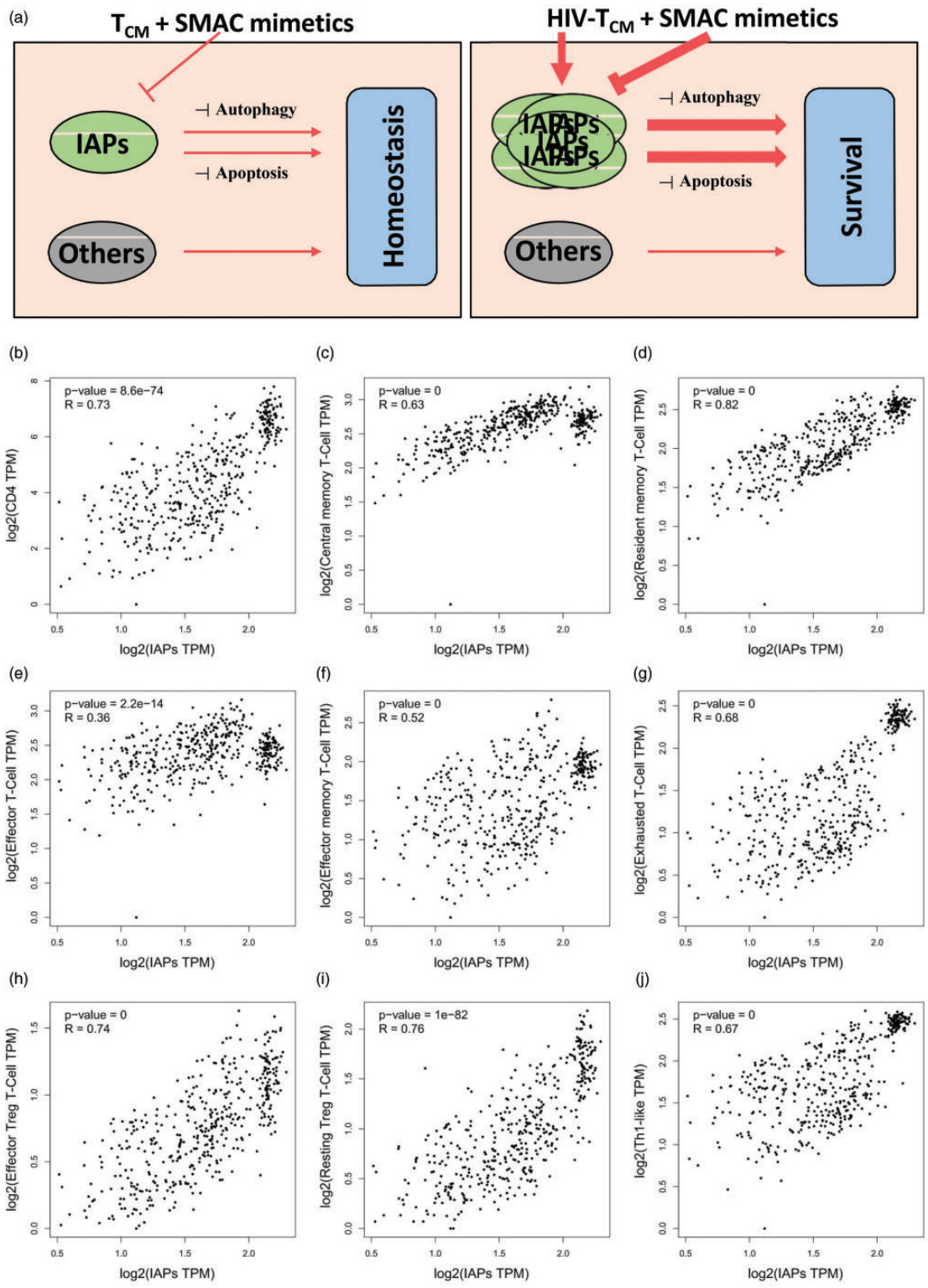

Figure 1. Potential roles of autophagy and the immune system in IAPs-targeted antiviral chemotherapy. (a) Hypothetical diagram. (b) Correlation analysis between IAPs and CD4. (c) Correlation analysis between IAPs and central memory T cells (CCR7/SELL/IL7R). (d) Correlation analysis between IAPs and resident memory T cells (CD69/ITGAE/CXCR6/MYADM). (e) Correlation analysis between IAPs and effector T cells (CX3CR1/FGFBP2/FCGR3A). (f) Correlation analysis between IAPs and effector memory T cells (PDCD1/DUSP4/GZMK/GZMA/IFNG). (g) Correlation analysis between IAPs and exhausted T cells (HAVCR2/TIGIT/LAG3/PDCD1/CXCL13/LAYN). (h) Correlation analysis between IAPs and effector Treg cells (FOXP3/CTLA4/CCR8/TNFRSF9). (i) Correlation analysis between IAPs and resting Treg cells (FOXP3/IL2RA). (j) Correlation analysis between IAPs and Th1-like cells (CXCL13/HAVCR2/IFNG/CXCR3/BHLHE40/CD4). The p and r values are individually shown for each group. $p < 0.01$ was considered statistically significant.

p and r values are individually shown, as indicated in each panel. Taken together, this information strongly suggests that the potential impacts on the immune system in the application of SMAC mimetics need to be taken into consideration.

Of note, although we propose a more reasonable model pointing to future direction, the current *in silico* analyses do not provide sufficient evidence to support the idea that the immune system is a key player in autophagy-dependent apoptosis of HIV. Moreover, due to the lack of HIV-infected patient samples, general correlations between IAPs and multiple T cell populations do not clearly elucidate that the extent of IAP-T cell association is different in HIV-infected patients compared to uninfected ones. Therefore, further investigation is required to demonstrate the precise interactions among SMAC, IAPs, autophagy and immune response in SMAC mimetics-based anti-HIV chemotherapy, at least before such a strategy can be translated into clinical applications.

Methods

Gene Expression Profiling Interactive Analysis 2 (GEPIA2, http://gepia2.cancer-pku.cn) is a web server for interactive exploration of RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects.[25] GEPIA2 was applied to analyze gene expression correlations between IAPs and characteristic signatures of multiple immune cells using Spearman's correlation coefficient. Raw RNA-Seq data downloaded from GTEx were recomputed by the UCSC Xena project using a uniform pipeline to avoid data imbalance. The parameter settings were consistent for all the inquired genes in each individual analysis. A p-value < 0.05 was considered to indicate statistically significant differences, and no adjustments to the p-values were made.

Abbreviations

HIV-T_{CM}, HIV-infected resting central memory $CD4^+$ T cells; IAP, inhibitor of apoptosis; SMAC, second mitochondria-derived activator of caspase; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; XIAP, X-linked inhibitor of apoptosis protein; ATP, adenosine triphosphate; LPC, lysophosphatidylcholine; CRT, calreticulin; PS, phosphatidylserine; TIME, tumor immune microenvironment; APC, antigen-presenting cell; PRR, pattern-recognition receptor; XLP2, X-linked lymphoproliferative disease type 2; GEPIA2, Gene Expression Profiling Interactive Analysis 2.

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Authors' contribution

X.H. conceived the study, performed the literature search and bioinformatics analysis, and prepared the figures; G.Z. helped with data collection, analysis, and interpretation. X.H. wrote and revised the manuscript; G.Z. proof-read the final version. X.H. and G.Z. contributed equally to the drafting process.

Data accessibility statement

All data generated and described in this article are available from the corresponding web servers, and are freely available to any scientist wishing to use them for noncommercial purposes, without breaching participant confidentiality. Further information is available from the corresponding author on reasonable request.

Declaration of conflicting interests

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References

1. Campbell GR, Bruckman RS, Chu YL, et al. SMAC mimetics induce autophagy-dependent apoptosis of HIV-1-infected resting memory CD4+ T cells. *Cell Host Microbe* 2018; 24: 689–702 e687.
2. Campbell GR and Spector SA. DIABLO/SMAC mimetics selectively kill HIV-1-infected resting memory CD4 (+) T cells: a potential role in a cure strategy for HIV-1 infection. *Autophagy* 2019; 15: 744-746.
3. Fulda S. Smac mimetics as IAP antagonists. *Semin Cell Dev Biol* 2015; 39: 132–138.
4. Lalaoui N and Vaux DL. Recent advances in understanding inhibitor of apoptosis proteins [version 1; peer review: 2 approved]. F1000Research 2018; 7(F1000 Faculty Rev): 1889. https://doi.org/10.12688/f1000research.16439.1
5. Hou YC, Hannigan AM and Gorski SM. An executioner caspase regulates autophagy. *Autophagy* 2009; 5: 530–533.

6. Hou YC, Chittaranjan S, Barbosa SG, et al. Effector caspase dcp-1 and IAP protein Bruce regulate starvation-induced autophagy during *Drosophila melanogaster* oogenesis. *J Cell Biol* 2008; 182: 1127–1139.
7. Huang X, Wu Z, Mei Y, et al. XIAP inhibits autophagy via XIAP-Mdm2-p53 signalling. *Embo J* 2013; 32: 2204–2216.
8. Merlo P and Cecconi F. XIAP: inhibitor of two worlds. *Embo J* 2013; 32: 2187–2188.
9. Tasdemir E, Chiara Maiuri M, Morselli E, et al. A dual role of p53 in the control of autophagy. *Autophagy* 2008; 4: 810–814.
10. Tasdemir E, Maiuri MC, Galluzzi L, et al. Regulation of autophagy by cytoplasmic p53. *Nat Cell Biol* 2008; 10: 676–687.
11. Huang X, Wang XN, Yuan XD, et al. XIAP facilitates breast and colon carcinoma growth via promotion of p62 depletion through ubiquitination-dependent proteasomal degradation. *Oncogene* 2019; 38: 1448–1460.
12. Levine B and Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell* 2019; 176: 11–42.
13. Levine B and Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008; 132: 27–42.
14. Deretic V and Levine B. Autophagy balances inflammation in innate immunity. *Autophagy* 2018; 14: 243–251.
15. Levine B, Mizushima N and Virgin HW. Autophagy in immunity and inflammation. *Nature* 2011; 469: 323–335.
16. Sumpter R Jr and Levine B. Selective autophagy and viruses. *Autophagy* 2011; 7: 260–265.
17. Galluzzi L, Bravo-San Pedro JM, Levine B, et al. Pharmacological modulation of autophagy: therapeutic potential and persisting obstacles. *Nat Rev Drug Discov* 2017; 16: 487–511.
18. Rubinsztein DC, Codogno P and Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov* 2012; 11: 709–730.
19. Ma Y, Galluzzi L, Zitvogel L, et al. Autophagy and cellular immune responses. *Immunity* 2013; 39: 211–227.
20. Pedersen J, LaCasse EC, Seidelin JB, et al. Inhibitors of apoptosis (IAPs) regulate intestinal immunity and inflammatory bowel disease (IBD) inflammation. *Trends Mol Med* 2014; 20: 652–665.
21. Vandenabeele P and Bertrand MJ. The role of the IAP E3 ubiquitin ligases in regulating pattern-recognition receptor signalling. *Nat Rev Immunol* 2012; 12: 833–844.
22. Beug ST, Cheung HH, LaCasse EC, et al. Modulation of immune signalling by inhibitors of apoptosis. *Trends Immunol* 2012; 33: 535–545.
23. Dougan SK and Dougan M. Regulation of innate and adaptive antitumor immunity by IAP antagonists. *Immunotherapy* 2018; 10: 787–796.
24. Mo X, Tang C, Niu Q, et al. High-Throughput immunomodulator phenotypic screening platform to reveal IAP antagonists as anti-cancer immune enhancers. *Cell Chem Biol* 2018; 26: 331–339.
25. Tang Z, Kang B, Li C, et al. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* 2019; 47: W556–W560.