Hydrogen Sulfide Inhibits Human T-cell Leukemia Virus Type-1 (HTLV-1) Protein Expression via Regulation of ATG4B

Liu Huandi (✉ huandi1984@163.com)

Xinxiang Medical University <https://orcid.org/0000-0003-4242-2335>

Jiaxiang Sun

Xinxiang Medical College: Xinxiang Medical University

Shuaifeng Guo

Xinxiang Medical University

Xuhong Cheng

Shanghai Seventh People's Hospital

Zhongxin Zhang

Xinxiang Medical University

Jia Wan

Xinxiang Medical University

Chunduo Wang

Xinxiang Medical University

Xiaoying Zhi

Xinxiang Medical University

Linghui Yuan

Xinxiang Medical University

Hui Wang

Xinxiang Medical University

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Hydrogen sulfide inhibits human T-cell leukemia virus type-1 (HTLV-1) protein expression via regulation of ATG4B

Huandi Liu^{1,2}, Jiayang Sun^{1,2}, Shuaifeng Guo², Xuhong Cheng³, Zhongxin Zhang², Jia Wan², Chunduo Wang², Xiaoying Zhi², Linghui Yuan², Hui Wang^{1,2}

¹Henan Key Laboratory of Immunology and Targeted Drugs, School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, China.

²Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical University, Xinxiang, China.

³Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Correspondence and requests for materials should be addressed to H.W. (email: wanghui@xxmu.edu.cn)

Abstract:

Background: Hydrogen sulfide (H₂S) is a redox gasotransmitter. It has been shown that H₂S has a key role in host antiviral defense by inhibiting interleukin (IL)-6 production and S-sulphydrating Keap1 lead to Nrf2/ARE pathway activation. However, it is yet unclear whether H₂S can play an antiviral role by regulating autophagy.

Results: In this research, we found that exogenous H₂S decreased the expression of HTLV-1 protein and HTLV-1 induced autophagosomes accumulation. Transmission electron microscope assays indicated that autophagosomes accumulation decreased after H₂S administration. HTLV-1-transformed T-cell lines had a high level of CSE (H₂S endogenous enzyme) which could be induced in Hela by HTLV-1 infection. Immunoblot demonstrated that overexpression of CSE inhibited HTLV-1 protein expression and autophagy. And we got the opposite after CSE knockdown. Meanwhile, H₂S could not restrain the autophagy when ATG4B had a mutant at its site of 89.

Conclusion: In a word, these results suggested that H₂S modulated HTLV-1 protein expression via ATG4B. Meanwhile, our findings suggested a new mechanism by which H₂S defended against virus infection.

Key words: human T-cell leukemia virus type 1   hydrogen sulfide   ATG4B autophagy

Background:

H₂S, hydrogen sulfide, is a colorless gas with lipid soluble. It has a smell of rotten eggs and has been supposed to be a kind of poisonous gas previously. Lately, it has been reported that H₂S could be produced in mammalian cells¹⁻⁴. There are different concentration of H₂S in different part. For example, it is about 46 μmol·L⁻¹ in rat serum and 100 μmol·L⁻¹ in rat brain⁵⁻⁶. Endogenous H₂S is produced in the cells by three enzymes, CSE (cystathionine γ-lyase), CBS (cystathionine β-synthase) and MST (3-mercaptopyruvate sulfur transferase)⁷⁻⁹. CBS is the main enzyme in the central nervous system^{6, 10, 11}, CSE is the key enzyme in the cardiovascular system^{12, 13}, whereas MST is responsible for the production of H₂S in the kidney^{14, 15}. In addition, H₂S has a pivotal role in digestive system and respiratory system¹⁶⁻¹⁹. Consequently, H₂S is considered as the third gasotransmitter, such as carbon monoxide (CO) and nitric oxide (NO)²⁰⁻²².

Sulfhydration is the main mechanism of H₂S in regulating protein activity^{23, 24}. In this modification, H₂S changes a Cys residue (–SH) of a target protein into –SSH^{23, 24}. For example, H₂S can restrain Keap1 activity by sulfhydration of Cys 151, and then Nrf2 translocates into the nucleus and combine with Antioxidant Response Element (ARE)^{25, 26}. This results in the expression of antioxidant genes.

Autophagy is a degradation process in cellular that transmits cytoplasmic components to lysosomes via autophagosomes^{27, 28}. Lipidation of LC3 is important for early stage of autophagy membrane formation^{29, 30}. ATG4, a kind of cysteine protease, perform as executor to process LC3 lipidation and delipidation of lipidated LC3^{31, 32}. There are four ATG4 isoforms in mammal cells, such as ATG4A, ATG4B, ATG4C and ATG4D³³. ATG4B is the key isoform for cleaving LC3/GABARAP³⁴. ATG4A has the ability of processing GABARAP. Compared with ATG4B, its activity is weak³⁵. However, ATG4D and ATG4C have almost no activity in vitro^{34, 36}.

Adult T-cell leukemia (ATL), a hematologic malignant disease of adult, is directly related to the infection of human T-cell leukemia virus type 1 (HTLV-1)³⁷⁻³⁹. It has a variety of symptoms like acute type, chronic type or insidious type^{40, 41}. Chronic and insidious patients are often supported by symptomatic support treatment; Chemical and biological therapy are often used to cure acute lymphoma. However, the effect is unsatisfactory, and the median survival of the acute patients is about 2 ~ 6 months^{40, 42-44}.

Tax, HTLV-1 protein, can continuously activate autophagy and generate a lot of autophagosomes. And then, HTLV-1 is wrapped in the autophagosomes. HTLV-1 in autophagosomes not only can get away from intracellular immunity but also complete self-replication in autophagosomes^{45, 46}. The massive virus promoted the expression of Tax. Lots of Tax continue to activate autophagy and advance virus replication via positive feedback, resulting in the generation and development of ATL finally⁴⁷.

In this research, we demonstrated that CSE was induced by HTLV-1 infection and suppressed HTLV-1 protein expression. We showed that exogenous H₂S inhibited the accumulation of autophagosomes during HTLV-1 infection. Furthermore, we found that CSE and exogenous H₂S were associated with ATG4B which is essential for autophagy early period. Collectively, our findings may shed some new lights on HTLV-1 therapy and contribute to our understanding of H₂S defenses against virus infection.

Results

H₂S treatment suppressed HTLV1 replication, reduces autophagy level, and has no effect on cells' viability.

The increase of autophagy is a common manifestation of viral infection. HTLV-1 induces autophagy and complete their replication in autophagosomes. H₂S, an endogenous gas signal molecule, can inhibit autophagy. That's the reason we explore the relationship between HTLV-1 infection and H₂S. HTLV-1 positive cells, MT2, MT4 and C8166, have high level of autophagy. LC3 has two isoforms, LC3A (upper line) and LC3B (below line). LC3B is a marker of autophagy⁴⁸. The expression of LC3B in MT2 reduced after H₂S administration for 48 hours (Fig. 1A.), and virus protein Tax and p19 also decreased. We saw the same results in MT4 (Fig. 1B.) and C8166 (Fig. 1C.). Meanwhile, the level of Tax gene expression was inhibited by H₂S (Fig. 1D.).

significantly. In order to further determine whether the decrease of viral protein expression is related to the decrease of cell activity, MTT assay was performed. As shown in Fig. 1E, H₂S had no effect on MT2 viability for 48 hours. And the cells viability increased after H₂S administration for 72 hours. Consistently, the accumulation of autophagosomes was decreased in H₂S-treated MT2 cells compared to MT2 without H₂S administration (Fig. 1F). These results suggests that the effect of H₂S on HTLV-1 protein expression was associated with autophagy.

Figure 1 The inhibition of virus replication by H₂S was related to autophagy. MT2 cells (A), MT4 (B) and C8166 cells (C) were stimulated by NaHS. After 48 h, the cell lysates were immunized with anti-Tax, anti-p19, anti-LC3 as indicated. (D) MT2 cells were stimulated by NaHS. After 2 d or 3 d, the cells were lysated for real-time PCR assay. (E) MT2 cells were stimulated by NaHS. After 2 days (d) or 3 days (d), MTT Cell Proliferation and Cytotoxicity Assay Kit was used as manufacturer's instructions. (F) MT2 cells were stimulated by NaHS. After 2 d, the cells were fixed with the TEM fixative. Autophagosomes were indicated by the black arrows.

Autophagy plays a key role in the inhibition of virus replication by H₂S.

We determined the role of H₂S in MT2 cells in the presence of RA (rapamycin) or 3-

MA (3-Methyladenine), which are believed to activate or block the autophagy. We found that RA could reduce the effect of H₂S, and 3-MA could enhance the effect of H₂S (Fig.2A). Similar results were observed in MT4 and C8166 (Fig. 2B and C). Meanwhile, NaHS could also restrain the autophagy of Hela (Fig. 2D). It means that H₂S depressed autophagy not only for HTLV-1 positive cells. And its effect on Hela laid a foundation for our further study of the mechanism.

Figure 2 The effect of ATG4B in H₂S-induced suppression of HTLV1 replication. MT2 cells (A), MT4 (B) and C8166 cells (C) were stimulated by NaHS, RA, 3-MA, or both two. After 48 h, the cell lysates were immunized with anti-Tax, anti-p19, anti-LC3 as indicated. (D) Hela cells were stimulated by NaHS for several different time. The cell lysates were immunized with anti-LC3.

H₂S interacts with ATG4B.

To characterize the mechanism by which H₂S regulated autophagosome accumulation, we tested whether H₂S was able to interact with ATG4B involved in canonical autophagy. ATG4B is a cysteine proteinase, which activity depends on the cysteine located in the active center⁴⁹. ATG4B has twelve cysteines according to NCBI database (Fig. 3). It has been reported that ATG4B has no activity if the cysteine at site 74 is mutated^{50, 51}. Meanwhile, H₂S signals is by modification of cysteine residues on target proteins in a process designated as sulfhydration or persulfidation. Therefore, we constructed 11 mutants which had one cysteine mutated separately except the site 74. The plasmid of ATG4B and its cysteine mutants were transfected in Hela cells. NaHS was administrated. To confirm the effect of these mutants on ATG4B activity, Hela cells were treated by RA or 3-MA and then subjected to immunoblot analysis. The results

showed that RA and 3-MA could active or depress autophagy when ATG4B had these cysteine mutated (Fig. 4A and 4B). It suggested that these cysteine were not essential for ATG4B activity. However, H₂S had no effect on autophagy when the cysteine at site 89 was mutated (Fig. 4C). These data indicated that cysteine 89 of ATG4B was the target of H₂S in the regulation of autophagy.

Figure 3 Alignment of the amino acid sequence of ATG4B and its homologues in human and other species. Sequence of human (NCBI Reference Sequence: NP_037457.3), mouse (NCBI Reference Sequence: NP_777363.1), bovine (NCBI Reference Sequence: NP_001001170.1), dog (NCBI Reference Sequence: XP_038291966.1), pig (NCBI Reference Sequence: NP_001177212.1), and trachemys scripta elegans (NCBI Reference Sequence: XP_034636799.1) were compared by ESPRIPT 3.x⁵². Twelve cysteine sites, marked by box, are highly conservative.

Figure 4 The effect of ATG4B in H₂S-induced suppression of autophagy. (A) Hela cells were transfected with ATG4B or its cysteine mutants. After 48 h, the cells were stimulated by RA. The cell lysates were immunized with anti-LC3 as indicated. (B) Hela cells were transfected with ATG4B or its cysteine mutants. After 48 h, the cells were stimulated by 3-MA. The cell lysates were immunized with anti-LC3 as indicated. (C) Hela cells were transfected with ATG4B or its cysteine mutants. After 48 h, the cells were stimulated by NaHS. The cell lysates were immunized with anti-LC3 as indicated.

CSE expression increases in early period of HTLV-1 infection.

Then we tried to explore the functions of H₂S endogenous enzyme during HTLV-1 infection. Jurkat and MT2 cells were lysated, and H₂S endogenous enzymes were investigated. Immunoblot assays demonstrated that the expression of H₂S endogenous enzymes were associated with HTLV-1 infection (Fig. 5A). Hela cells were co-cultured with MT2 cells and the expression levels of Tax was investigated. PCR assays demonstrated that Tax could be detected in Hela cells after co-cultured for 24 h (Fig. 5B). The expression of CSE reached a maximum on the 48 h and the level of autophagy decreased after co-culture (Fig. 5C). However, the expression of CBS and MST

decreased after co-culture for 48 h (Fig. 5C). These results suggested that CSE played a key role in early period of HTLV-1 infection. In order to confirm that the decreased of autophagy was associated with CSE and HTLV-1. Hela cells were cultured without FBS. Immunoblot assays found that the increase of CSE could not reduce the level of autophagy without HTLV-1 infection (Fig. 5D). And then, we detected the effect of exogenous H₂S on Hela cells. The result showed that exogenous H₂S had no effect on the expression of endogenous enzymes (Fig. 5E). In summary, the expression of CSE decreases the level of autophagy of HTLV-1 positive cells.

Figure 5 The effect of HTLV-1 infection on the expression of H₂S endogenous enzyme. (A) MT2 cells and Jurkat cells were lysated, and the lysates were immunized with anti-CBS, anti-CSE, anti-MST, anti-LC3 as indicated. (B) Hela cells were seeded for 24 h, and then co-cultured with MT2 cells for another 24 h. The cells were lysated for PCR assay and agarose electrophoresis. (C) Hela cells were seeded for 24 h, and then co-cultured with MT2 cells for another 24 h. The cells lysates were immunized with anti-CBS, anti-CSE, anti-MST, anti-LC3 as indicated. (D) Hela cells were cultured without FBS for 48 h. The cell lysates were immunized with anti-CBS, anti-CSE, anti-MST, anti-LC3 as indicated. (E) Hela cells were stimulated with NaHS. After 48 h, the cells lysates were immunized with anti-CBS, anti-CSE, anti-MST, anti-LC3 as indicated.

CSE can inhibit HTLV-1 replication via ATG4B.

Next, we wanted to make sure whether CSE had the same effect like exogenous H₂S. Hela cells with overexpressing or low expressing of CSE were co-cultured with MT2 cells and the expression levels of HTLV-1 proteins and autophagy were investigated. Immunoblot assays demonstrated that the expression of CSE was associated with lower levels of HTLV-1 proteins and autophagy (Fig. 6A and 6B). Meanwhile, we investigated

whether RA and 3-MA could impact the effect of CSE. We found that RA could reduce the effect of CSE, and 3-MA could enhanced the effect of CSE (Fig. 6C and 6D). In order to confirm that ATG4B was associated with the effect of CSE. Hela cells, overexpressing of CSE and ATG4B mutant, were co-cultured with MT2 cells. Immunoblot assays showed that the level of HTLV-1 protein was restored partly (Fig. 6E). Taken together, these data suggested that the expression of CSE decreased HTLV-1 protein via the cysteine 89 of ATG4B.

Figure 6 CSE can effectively regulate HTLV1 replication in early infection stage via ATG4B. (A) Hela cells were transfected with empty vector (–) or pCMV3-CSE (+). At 24 h after transfection, the cells were co-cultured with MT2 cells for another 24 h. Then the cells were washed with PBS three times to remove MT2 cells and lysed for immunoblot assays. (B) Hela cells were transfected with negative control or CSE-siRNA. At 24 h after transfection, the cells were co-cultured with MT2 cells for another 24 h. Then the cells were washed with PBS three times to remove MT2 cells and lysed for immunoblot assays. (C) Hela cells were transfected with empty vector (–) or pCMV3-CSE (+). At 24 h after transfection, the cells were co-cultured with MT2 cells for another 24 h. Then the cells were stimulated by RA or 3-MA for 24 h. Finally, the cells were washed with PBS three times to remove MT2 cells and lysed for immunoblot assays. (D) Hela cells were transfected with negative control or CSE-siRNA. At 24 h after transfection, the cells were co-cultured with MT2 cells for another 24 h. Then the cells were stimulated by RA or 3-MA for 24 h. Finally, the cells were washed with PBS three times to remove MT2 cells and lysed for immunoblot assays. (E) Hela

cells were transfected with empty vector (–) and negative control or pCMV3-CSE (+) and ATG4B C89A mutant. At 24 h after transfection, the cells were co-cultured with MT2 cells for another 24 h. Then the cells were washed with PBS three times to remove MT2 cells and lysed for immunoblot assays. (F) The mechanism diagram of the effect among HTLV-1, autophagy, ATG4B, CSE and H₂S. ①HTLV-1 induced the formation of autophagosomes and enhanced HTLV-1 protein expression. ② HTLV-1 increased the expression of CSE. ③CSE expression and exogenous H₂S decreased autophagy accumulation and lower HTLV-1 protein expression levels via ATG4B.

Discussion:

As a gasotransmitter, H₂S plays an important role in the physiological and pathological process of the organism¹⁶⁻¹⁹. Nowadays, the mechanism of H₂S in the process of virus infection is unclear. It has been reported that H₂S could play an antiviral role through its antioxidant and anti-inflammatory effects, such as COVID-9, HSV, HIV and paramyxovirus⁵³⁻⁵⁸. Our study found that both endogenous and exogenous H₂S could inhibit autophagy caused by HTLV-1 and reduced the expression of viral proteins by autophagy related protein ATG4B.

Our results showed that HTLV-1-positive T-cell lines had a strong expression of CSE and CSE expression could be induced in Hela after HTLV-1 infection. Further studies indicated that the overexpression of CSE inhibited HTLV-1 protein expression and CSE knockdown was associated with higher levels of HTLV-1 protein expression. There was significant relevance between autophagy level and the expression of CSE. These results drove us to find other possible mechanism to explain the role of CSE in autophagy.

There is a violent war between autophagy and pathogenic microorganisms⁵⁹. Paradoxically, autophagosomes is benefit for HTLV-1 to complete its replication⁴⁵⁻⁴⁷. We used LC3B as the marker of autophagy and examined the effect of CSE on HTLV-1 induced autophagy. Our findings confirmed the hypothesis by three steps. Firstly, H₂S inhibited the autophagosomes accumulation in MT2 cells. Secondly, CSE inhibited the autophagosomes accumulation induced by HTLV-1 infection in Hela. Thirdly, when RA was used to activate the early stage of autophagy, we found that CSE overexpression lost the abilities to inhibit autophagosomes accumulation and HTLV-1 viral protein expression increased. These results suggested that the regulatory role of CSE in autophagosomes accumulation was important to its effect on HTLV-1 protein expression.

How could H₂S regulate autophagosomes accumulation? It has been reported that ATG4B has an important role in LC3 II and autophagosomes formation³⁴. As a cysteine enzyme, ATG4B is a good target for H₂S regulation. Our findings indicated that H₂S interacted with ATG4B. Using a series of ATG4B cysteine mutants, we found ATG4B cysteine 89 was essential for the effect of H₂S. However, ATG4B will almost lost the ability when it has a mutant at the site of cysteine 74⁶⁰. Therefore, we could not confirm whether the cysteine 74 was associated with H₂S function.

It is reported that the function of ATG4B can be changed via post-translational modification. For example, its activity is enhanced when the serine at 383 site was phosphorylated⁶¹; After phosphorylation of serine at 34 and 316 sites or S-nitrosation

of cysteine at 189 and 292 sites, the opposite results will appear^{62, 63,64}; In addition, when the level of reactive oxygen species increases, a disulfide bond will be formed between cysteines at site 292 and 361 resulting the level of autophagy increases⁶⁵. Our study suggested that H₂S could sulfurize cysteine at site 89 and inhibit autophagy. However, we had no direct evidence to confirm the sulfhydration of ATG4B because of technical limitations.

Conclusion: Together, our study demonstrated that H₂S modulated autophagosomes accumulation by regulating ATG4B, thereby affecting the expression of HTLV-1 protein. Our research may expand our understanding of H₂S on virus infection and suggest a new role of ATGs sulfhydration in autophagy.

Methods:

Reagents

The following antibodies were used for immunoblot analysis: anti-Gapdh (60004-1-Ig, Proteintech), anti-CBS (14782, Cell Signaling Technology), anti-CSE (19689, Cell Signaling Technology), anti-HTLV-1 p19 (ab9080, Abcam), anti-MST (ab224043, Abcam), anti-Tax (sc-57872, Santa Cruz Biotechnology), anti-ATG4B (15131-1-AP, Proteintech), anti-LC3A/B (18725-1-AP, Proteintech). The 3-MA (M9281), NaHS (151627) and RA (553211) were obtained from Sigma-Aldrich. SYBR Green quantitative PCR master mix were purchased from TOYOBO Ideas and Chemistry (QPK-201). MTT Cell Proliferation and Cytotoxicity Assay Kit was obtained from Beyotime Biotechnology.

ATG4B mutants' preparation

ATG4B plasmid was bought from Sino Biological (Cat. HG20407-ANG). GeneArt® Site-Directed Mutagenesis PLUS Kit was used to construct cysteine mutants. Gene-specific primer sequences were as follow:

C78A:

Forward: CACAGGCTGGGGCTGCATGCTGCGGGCTGGACAGATGATCTTTGCCCAAGCC

Reverse: GGCTTGGGCAAAGATCATCTGTCCAGCCCGCAGCATGCAGCCCCAGCCTGTG

C89A:

Forward: GATGATCTTTGCCCAAGCCCTGGTGGCCCGGCACCTAGGCCGAGATTGGAGG

Reverse: CCTCCAATCTCGGCCTAGGTGCCGGGCCACCAGGGCTTGGGCAAAGATCATC

C183A:

Forward: GTTGTGATGGAGGAAATCAGAAGGTTGCCAGGACCAGCGTTCCTGTGCAGGC

Reverse: GCCTGCACAGGGAACGCTGGTCCTGGCAACCTTCTGATTTCCTCCATCACAAAC

C189A:

Forward: GAAGGTTGTGCAGGACCAGCGTTCCCGCTGCAGGCGCCACTGCGTTTCCTGCAG

Reverse: CTGCAGGAAACGCAGTGGCGCCTGCAGCGGGAACGCTGGTCCTGCACAACCTTC

C203A:

Forward: GTTTCCTGCAGATTCCGACCGGCACGCCAACGGATTCCCTGCCGGAGCTGAG

Reverse: CTCAGCTCCGGCAGGGAATCCGTTGGCGTGCCGGTCGGAATCTGCAGGAAAC

C246A:

Forward: GGCCTACGTGGAGACGCTGAAGCACGCCTTCATGATGCCCCAGTCCCTGGGC

Reverse: GCCCAGGGACTGGGGCATCATGAAGGCGTGCTTCAGCGTCTCCACGTAGGCC

C292A:

Forward: GCCAGCCGTGGAGCCCACTGATGGCGCCTTCATCCCGGACGAGAGCTTCCAC

Reverse: GTGGAAGCTCTCGTCCGGGATGAAGGCGCCATCAGTGGGCTCCACGGCTGGC

C301A:

Forward: CTTTCATCCCGGACGAGAGCTTCCACGCCCAGCACCCGCCGTGCCGCATGAGC

Reverse: GCTCATGCGGCACGGCGGGTGCTGGGCGTGGAAGCTCTCGTCCGGGATGAAG

C323A:

Forward: CCCGTCCATCGCTGTGGGGTTTTTCGCTAAGACTGAAGATGACTTCAATGATTG

Reverse: CAATCATTGAAGTCATCTTCAGTCTTAGCGAAAAACCCACAGCGATGGACGGG

C333A:

Forward: GACTGAAGATGACTTCAATGATTGGGCCCAGCAAGTCAAAAAGCTGTCTCTG

Reverse: CAGAGACAGCTTTTTGACTTGCTGGGCCCCAATCATTGAAGTCATCTTCAGTC

C361A:

Forward: GGAGCTGCAGCCTTCACATCTGGCCGCCCCGACGTCCTGAACCTGTCCCTAG

Reverse: CTAGGGACAGGTTTCAGGACGTCGGGGGCGGCCAGATGTGAAGGCTGCAGCTCC

Cell culture and transfection

Hela cells was cultured in DMEM with high glucose. MT2, MT4, C8166 and Jurkat cells were grown in RPMI 1640. All cells were provided with 10% FBS (Gibco), 4 mM L-glutamine under humidified conditions with 5% CO₂ at 37 °C. Transfection of Hela cells was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Western blot

Immunoblot analysis was performed. Hela cells were transfected with various combinations of plasmids or siRNA. At 24 h - 36 h after the transfection, the cell lysates were prepared in RIPA lysis buffer plus a protease inhibitor 'cocktail' (Roche). MT2, MT4, C8166 and Jurkat cells were simulated by NaHS (100 μ mol/L) for 2 days or 3 days. The cell lysates were incubated with indicated antibodies and analyzed by immunoblot.

Real-time PCR

Total RNA was extracted from the cultured cells with TRIzol reagent (Invitrogen) as described by the manufacturer. All gene transcripts were quantified by real-time PCR with the ABI 7500 Fast real-time PCR system (Applied Biosystems). The results were analyzed using the $2^{-\Delta\Delta C_t}$ method.

The primers used for real-time PCR were as follows:

Tax,

Forward, 5'-CGGATACCCAGTCTACGTGT-3',

Reverse, 5'-GAGCCGATAACGCGTCCATCG-3';

GAPDH,

Forward, 5'-GCCAAAAGGGTCATCATCTC- 3',

Reverse, 5'-GTAGAGGCAGGGATGATGTTC-3'.

Transmission electron microscope

After indicated stimulation, MT2 cells were fixed with the TEM fixative. And then let the precipitation re-suspended in the fixative and fixed at 4°C for preservation and transportation. The operation was performed according to the instructions. The copper grids are observed under TEM (HITACHI: HT7800/HT7700) and take images.

RNA interference

The siRNA, CSE-silencer, was obtained from Genepharma. The sequences used were as follows:

K1, forward, 5'-GCCGAGAGCUUGGGAGGAUTT-3', reverse, 5'-

AUCCUCCCAAGCUCUCGGCTT-3'; K2, forward, 5'-GCAGCCACUGUAACUAUUATT-3',

reverse, 5'-UAAUAGUUACAGUGGCUGCTT-3'. The negative control was purchased from

Genepharma (catalog no. A06001).

Co-culture

Hela cells were cultured for 24 hours. MT2 cells were seeded and co-cultured with Hela cells at a ratio of 1:100 (1 $\times$ 10⁴ HTLV-1 donor cells: 1 $\times$ 10⁶ uninfected recipient cells ratio) and allowed to incubate for an additional 24 hours prior to harvesting for downstream assays.

Statistical analysis

The data are presented as the mean $\pm$ standard error from at least three independent experiments. All p-values were calculated using two-tailed Student's t-tests (Graphpad) and were considered statistically no significant when $p > 0.05$.

List of abbreviations

HTLV-1: human T-cell leukemia virus type-1; H₂S: hydrogen sulfide; CSE: cystathionine γ -lyase; CBS: cystathionine β -synthase; MST: 3-mercaptopyruvate sulfur transferase; CO: carbon monoxide; NO: nitric oxide; ARE: Antioxidant Response Element; ATL: Adult T-cell leukemia; RA: rapamycin; 3-MA: 3-Methyladenine;

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Authors' Contributions

H.L. and H.W. conceived the idea for the project. J.S., S.G., X.C., Z.Z., J.W., C.W., X.Z., L.Y. did the experiments. H.L. designed experiments, analyzed the results and wrote the paper.

Ethics approval and consent to participate

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Its publication has been approved by all co-authors.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Henan Key Laboratory of Immunology and Targeted Drugs, School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, China.

²Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical University, Xinxiang, China.

³Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, China.

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