SIRT1 Suppresses Human T-Cell Leukemia Virus Type 1 Transcription

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ABSTRACT

Human T-cell leukemia virus type 1 (HTLV-1)-associated diseases are poorly treatable, and HTLV-1 vaccines are not available. High proviral load is one major risk factor for disease development. HTLV-1 encodes Tax oncoprotein, which activates transcription from viral long terminal repeats (LTR) and various types of cellular promoters. Counteracting Tax function might have prophylactic and therapeutic benefits. In this work, we report on the suppression of Tax activation of HTLV-1 LTR by SIRT1 deacetylase. The transcriptional activity of Tax on the LTR was largely ablated when SIRT1 was overexpressed, but Tax activation of NF-κB was unaffected. On the contrary, the activation of the LTR by Tax was boosted when SIRT1 was depleted. Treatment of cells with resveratrol shunted Tax activity in a SIRT1-dependent manner. The activation of SIRT1 in HTLV-1-transformed T cells by resveratrol potently inhibited HTLV-1 proviral transcription and Tax expression, whereas compromising SIRT1 by specific inhibitors augmented HTLV-1 mRNA expression. The administration of resveratrol also decreased the production of cell-free HTLV-1 virions from MT2 cells and the transmission of HTLV-1 from MT2 cells to uninfected Jurkat cells in coculture. SIRT1 associated with Tax in HTLV-1-transformed T cells. Treatment with resveratrol prevented the interaction of Tax with CREB and the recruitment of CREB, CRTC1, and p300 to Tax-responsive elements in the LTR. Our work demonstrates the negative regulatory function of SIRT1 in Tax activation of HTLV-1 transcription. Small-molecule activators of SIRT1 such as resveratrol might be considered new prophylactic and therapeutic agents in HTLV-1-associated diseases.

IMPORTANCE

Human T-cell leukemia virus type 1 (HTLV-1) causes a highly lethal blood cancer or a chronic debilitating disease of the spinal cord. Treatments are unsatisfactory, and vaccines are not available. Disease progression is associated with robust expression of HTLV-1 genes. Suppressing HTLV-1 gene expression might have preventive and therapeutic benefits. It is therefore critical that host factors controlling HTLV-1 gene expression be identified and characterized. This work reveals a new host factor that suppresses HTLV-1 gene expression and a natural compound that activates this suppression. Our findings not only provide new knowledge of the host control of HTLV-1 gene expression but also suggest a new strategy of using natural compounds for prevention and treatment of HTLV-1-associated diseases.
fluctuational in this process (16, 17). In view of the essential regulatory roles of LKB1 and SIKs in Tax activation of CREB, we have further proposed that metformin and other pharmaceutical activators of LKB1 and SIKs might be repurposed for the prevention and treatment of ATL and TSP (17).

SIRT1 is the best-studied mammalian homolog of yeast Sir2p that extends life span by preventing genome instability (18, 19). SIRT1 is a sirtuin with NAD⁺−dependent deacetylase activity on histones, transcription factors, and other transcriptional regulatory proteins. This reaction is the reverse of acetylation catalyzed by histone and transcription factor acetyltransferases, such as CBP and p300. In response to metabolic signals and stress, SIRT1 can positively and negatively regulate transcription by modifying its substrate proteins (20). In addition to having a function in healthy aging, SIRT1 has been implicated in metabolism, stress response, cancer, diabetes, and human immunodeficiency virus (HIV) infection (18). SIRT1 can deacetylate transcription factors, such as NF-κB, p53, E2F1, and CREB, as well as transcriptional effectors, such as p300 and CRTCs (18). Particularly, the activity of CREB and CRTCs is compromised when they are deacetylated by SIRT1 (21–23). On the other hand, HIV-1 transactivator Tat interacts with SIRT1 to suppress its deacetylation of p65 (24).

Small-molecule activators of SIRT1, such as resveratrol, have been extensively tested for prevention and treatment of diseases in clinical trials (25). Resveratrol is a natural compound found in the skin of red grapes and widely sold as a nutritional supplement. Oral administration of resveratrol to mice has been shown to extend life span and improve health and survival (26). However, whether and how SIRT1 and resveratrol might affect HTLV-1 infection and transcription remain to be understood.

In this study, we set out to determine the influence of SIRT1 on Tax activation of HTLV-1 LTR and to provide proof of concept for the utility of resveratrol in anti-HTLV-1 therapy. We found that SIRT1 and resveratrol potently suppress HTLV-1 transcription. Our findings support the model in which SIRT1 suppresses Tax activation of HTLV-1 LTR by impeding the recruitment and function of CREB and CRTCs. Our work also has implications in the prevention and treatment of ATL and TSP.

**MATERIALS AND METHODS**

**Cell culture and transfection.** HEK293 and HEK293T cells were grown in Dulbecco modified Eagle medium. Jurkat, Molt-4, and HTLV-1–transformed T cells (MT2, MT4, and C8166) were propagated in RPMI 1640 medium.

HEK293T cells were transfected using GeneJuice (Novagen, Madison, WI). Jurkat, MT2, MT4, and C8166 cells were transfected using Lipofectamine 2000 (Invitrogen, Grand Island, NY).

**Plasmids, antibodies, and reagents.** Luciferase reporter plasmids pLTR-Luc and pkb-Luc as well as expression plasmids for Tax, CREB, and SIRT1 have been detailed elsewhere (17, 27–29). Tax expression is driven by a cytomegalovirus promoter. Site-directed mutagenesis was carried out as described previously (30–32).

Rabbit anti-V5, mouse anti-α-tubulin, and mouse anti-Flag were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-V5 was from Invitrogen. Rabbit anti-SIRT1 (H-300), rabbit anti-p300 (C-20), and mouse antibody against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were from Santa Cruz Biotechnology (Dallas, TX). Rabbit antibodies against CRTC1, CREB, and acetyl-lysine (Ac-K) were from Cell Signaling (Danvers, MA). Mouse anti-Tax has been described previously (29). Resveratrol, sirtinol, and Ex527 were bought from Merck (Whitehouse Station, NJ), Millipore-Calbiochem (Danvers, MA), and Sigma-Aldrich, respectively.

**Reporter assays and protein analysis.** Cells were harvested 36 or 48 h after transfection. Dual luciferase assay and protein analysis were performed as described previously (33, 34). pSV-RLuc reporter (Promega, Madison, WI) was used as an internal control to normalize for transfection efficiency. Three independent experiments were carried out, and standard deviations (SD) were calculated. Two-tailed Student’s t test was performed to statistically assess the differences between the desired groups.

**Coimmunoprecipitation.** Cells were harvested to immunoprecipitation buffer (20 mM Tris-HCl [pH 7.5], 100 mM NaCl, 0.5 mM EDTA, 0.5% NP-40, 1 mM dithiothreitol, 20 mM β-glycerophosphate, 1 mM sodium vanadate, and 1 mM phenylmethylsulfonyl fluoride). To precipitate Flag-SIRT1 and V5-Tax proteins, cleared cell lysates were incubated with mouse anti-Flag (M2; Sigma-Aldrich) and anti-V5 (Invitrogen), respectively, for 2 h at 4°C. Antibody-antibody complex was collected with protein G agarose (Invitrogen), washed three times with immunoprecipitation buffer, and resuspended in sample buffer (50 mM Tris-Cl, pH 8.0, 0.5% SDS, 5% glycerol, 5% β-mercaptoethanol, and 0.002% bromophenol blue) for subsequent SDS-PAGE and Western blot analysis.

For immunoprecipitation of endogenous Tax, HTLV-1–transformed cells (MT2, MT4, and C8166) and Jurkat cells were grown to confluence and harvested to 1 ml of immunoprecipitation buffer. Endogenous Tax protein was precipitated from the cleared lysate by a 2-h incubation at 4°C with mouse anti-Tax.

**Chromatin immunoprecipitation.** Chromatin immunoprecipitation was carried out as previously described (16, 35). In brief, MT2 cells were cross-linked with 1% formaldehyde for 10 min at room temperature. Cells were washed and cell lysis was performed in the presence of protease inhibitor cocktail. After sonication, the cell debris was removed by centrifugation. The DNA-protein complex was immunoprecipitated and the DNA was extracted by phenol-chloroform. The DNA sequence spanning the three 21-bp Tax-responsive elements in HTLV-1 LTR was analyzed by quantitative PCR (qPCR) with primers 5′-GGCTTAGAGCCTCCAGT G-3′ and 5′-CCCTGAAACTGTCTCCACGC-3′.

**RNA interference.** HEK293 cells were transfected with 100 nM small interfering RNA (siRNA) using Lipofectamine 2000 (Invitrogen). RNA interference experiments were performed as described previously (17, 36). siRNA sequences were as follows: siGFP, 5′-GCAAGCGUGACCCUG AAGUU-3′; siSIRT1-1, 5′-GGUAGGCUGAUAACUUCU-3′; and siSIRT2-2, 5′-CCACCUGAUUGGAGUA-3′.

**Real-time RT-PCR.** Real-time reverse transcription-PCR (RT-PCR) was performed essentially as described previously (17, 37). Primer sequences for Tax, Env, and GAPDH transcripts were also described previously (17). Total RNA extraction was performed using RNAiso Plus reagents (TaKaRa, Otsu, Shiga, Japan). CDNA synthesis was achieved with a Transcriptor First Strand cDNA synthesis kit (Roche, Indianapolis, IN) using random hexamer primers. Real-time PCR was performed with SYBR Premix Ex Taq reagents (TaKaRa) in the StepOne real-time PCR system (Applied Biosystems, Foster City, CA). The normalized value in each sample was derived from the relative quantity of target mRNA divided by the relative quantity of GAPDH mRNA. Relative mRNA expression level was derived from the threshold cycle (2−ΔΔCt) by use of the comparative Cq method.

**Quantitation of HTLV-1 virions.** MT2 cells were seeded at 10⁶/ml overnight in culture medium in the presence of escalating amounts of resveratrol (10, 20, or 40 µM). Culture supernatants were collected and ultracentrifuged for 2 h at 200,000 × g. The supernatant from Jurkat cells was used as a negative control. HTLV-1 viruses were resuspended in 100 µl of lysis buffer and quantified by use of a colorimetric reverse transcription–ase kit (Roche) as described previously (17).

**Measurement of HTLV-1 transmission.** MT2 cells were seeded at 10⁶/ml and treated with resveratrol for 24 h. Jurkat cells were seeded at 5 × 10⁵/ml and transfected with pLTR-Luc and pRL-TK for 24 h. Treated MT2 cells and transfected Jurkat cells were then mixed and cocultured for an extra 48 h before being harvested for dual luciferase reporter assay.
RESULTS

SIRT1 inhibits Tax activation of HTLV-1 LTR. Acetylation and deacetylation of histones, transcription factors, and transcriptional coregulators are critically involved in the regulation of HTLV-1 transcription (6). Small-molecule activators of SIRT1 deacetylase have been extensively tested as targeted therapeutics for cancer (25). With these thoughts in mind, we sought to investigate how SIRT1 might affect HTLV-1 transcription.

We first determined the effect of SIRT1 expression on Tax activation of HTLV-1 LTR in HEK293 cells. Increasing expression of SIRT1 effectively blunted Tax activation of the LTR to the basal level (Fig. 1A, compare groups 5 and 6 to group 4). In contrast, when we expressed a catalytically inactive H363Y (HY) mutant of SIRT1, which has previously been shown to be deacytalytic defective (27), no suppression of Tax-mediated LTR activation was observed (Fig. 1A, compare group 7 to group 4), indicating the requirement of the deacetylation activity of SIRT1 for its suppression of Tax activation of the LTR.

Because SIRT1 is also known to target p65 and other substrates that mediate NF-κB activation (18, 38), we next investigated whether SIRT1 might also affect Tax activation of NF-κB. To our surprise, expression of SIRT1 or its HY mutant had no influence on Tax-induced activation of NF-κB (Fig. 1B, compare groups 4 and 6 to group 2). In other words, SIRT1 exerts a suppressive effect specifically on HTLV-1 LTR.

To further verify the role of SIRT1 in Tax activation of the LTR, we depleted SIRT1 expression in HEK293 cells using two independent siRNAs (siSIRT1-1 and siSIRT1-2). The specificity of the silencing effect of siSIRT1-1/2 was verified by Western blotting of SIRT1 (Fig. 1C, inset). Silencing of SIRT1 augmented Tax activation of the LTR significantly (Fig. 1C, compare groups 5 and 6 to group 4). Hence, SIRT1 functions as a negative regulator of HTLV-1 transcription.

Resveratrol inhibits Tax activation of LTR in a SIRT1-dependent manner. The implication of SIRT1 in suppressing HTLV-1 transcription provides new opportunities for rational design and development of anti-HTLV-1 agents. Toward this end, we tested the effect of resveratrol, a well-known activator of SIRT1 (25). Consistently with our observation of SIRT1-overexpressing cells, treatment of HEK293 cells with increasing amounts of resveratrol abrogated Tax-mediated LTR transactivation progressively (Fig. 2A, compare groups 5 and 6 to group 4). Notably, the expression of Tax protein driven by the cytomegalovirus promoter remained unchanged upon administration of resveratrol (Fig. 2A, inset), indicating that its suppressive effect on the LTR occurs primarily at a step subsequent to the production of Tax. In line with the lack of suppressive effect of SIRT1 on Tax activation of NF-κB (Fig. 1B), treatment of cells with 20 μM resveratrol did not affect Tax-induced activation of NF-κB activity (Fig. 2B, compare group 4 to group 2). The suppression of Tax activation of NF-κB by resveratrol was observed only at the high concentration of 40 μM (Fig. 2B, compare group 6 to group 2), which might be attributed to a nonspecific and SIRT1-independent effect.

When we depleted endogenous SIRT1 with two independent siRNAs, the suppressive effect of resveratrol on LTR activation was completely reversed (Fig. 2C, compare groups 4 and 5 to group 3). Hence, resveratrol potently suppressed Tax-mediated LTR transcription in a SIRT1-dependent manner. In other words, the effect of resveratrol on Tax activation of the LTR was mediated primarily through SIRT1.

In addition to the results in HEK293 cells, similar observations concerning the suppression of Tax activity on the LTR by resveratrol were made in Jurkat T cells (Fig. 2D, compare groups 5 and 6 to group 4). Furthermore, LTR activation was severely compromised in MT2 and C8166 cells treated with resveratrol (Fig. 2E, compare group 3 to group 5, and group 4 to group 6). All these data consistently support the notion that resveratrol represses HTLV-1 transcription.

Resveratrol suppresses proviral transcription, virion production, and cell-to-cell transmission of HTLV-1. To assess the effect of resveratrol on HTLV-1 transcription in a more physiological context, we treated three lines of HTLV-1+ T cells (i.e., MT2, MT4, and C8166) with resveratrol. Treatment with resveratrol repressed LTR-driven Tax and Env mRNA expression in a...
HHTV-1 from MT2 cells to uninfected Jurkat cells in coculture (Fig. 3H, compare group 2 to group 1). Collectively, our results demonstrated the anti-HTLV-1 effect of resveratrol in HTLV-1-transformed cells.

Acetylation of Tax has no influence on LTR activation. Acetylation of Tax at K346 might be influential on its transforming potential and its activation of NF-κB (39, 40). If acetylation and deacetylation of Tax are important in transcription and transformation, it will be of interest to see whether and how SIRT1 deacetylates Tax. To shed light on this, we immunoprecipitated Tax from cells expressing SIRT1 or its HY mutant and probed the precipitates with antibodies recognizing acetyl-lysine. The steady-state amount of acetylated Tax decreased in the presence of SIRT1 but increased when SIRT1-HY was expressed (Fig. 3F, compare lanes 2 to 4 to lanes 1 and 3 to 5). The decrease of Tax expression became more prominent when we progressively increased the concentration of resveratrol in MT4 and C8166 cells (Fig. 3D, compare lanes 2 to 4 to lane 1, and lanes 6 to 8 to lane 5).

In addition to SIRT1 activators, highly specific small-molecule inhibitors of SIRT1 are also well developed (36). To further validate the specificity of SIRT1’s effect, we applied two different SIRT1-specific inhibitors, sirtinol and Ex527, individually to groups 5 and 6. Consistently with earlier results, pharmaceutical inhibition of SIRT1 augmented the LTR-driven production of Tax mRNA in a dose-dependent manner (Fig. 3E, compare groups 3 and 5 to group 1, and groups 4 and 6 to group 2). A similar effect of sirtinol on Tax mRNA expression was also observed in MT2 and MT4 cells (Fig. 3F, compare groups 4 to 6 to groups 1 to 3). More importantly, treatment of MT2 cells with resveratrol perturbed the production of cell-free HTLV-1 virions in a dose-dependent manner (Fig. 3G, compare groups 2 to 4 to group 1). Consistently with this, resveratrol blocked the transmission of HTLV-1 from MT2 cells to uninfected Jurkat cells in coculture (Fig. 3H, compare group 2 to group 1). Collectively, our results demonstrated the anti-HTLV-1 effect of resveratrol in HTLV-1-transformed cells.
pared the activity of Tax and its acetylation mutants in LTR activation. Whereas K346Q mimics a constitutively acetylated state, K346R is a nonacetylatable mutant (40). Tax and its K346Q and K346R mutants were equally competent in the activation of the LTR (Fig. 4B, compare groups 3 and 4 to group 2). In addition, all three forms were suppressed to similar extents by resveratrol (Fig. 4B, compare groups 7 and 8 to group 6, and groups 11 and 12 to group 10). Thus, acetylation of Tax does not affect LTR activation or the suppression of HTLV-1 transcription by resveratrol.

**SIRT1 interacts with Tax in HTLV-1-transformed cells.** The potent suppressive effect of SIRT1 on Tax activation of HTLV-1 LTR (Fig. 1) suggested that SIRT1 and Tax might interact with each other. To explore this possibility, we performed coimmunoprecipitation in HEK293T cells overexpressing Tax and Flag-tagged SIRT1. The presence of both Tax and SIRT1 in the anti-Flag precipitate obtained from cells expressing both proteins (Fig. 5A, compare lane 3 to lanes 1 and 2) indicated the association of the two entities in these cells. The catalytically inactive HY mutant of SIRT1 retained the ability to associate with Tax, albeit to a lesser extent (Fig. 5A, compare lane 4 to lane 3). To extend our analysis to endogenous proteins in HTLV-1-transformed T cells, similar experiments were also done with MT2, MT4, and C8166 cells. Endogenous SIRT1 was detected in the anti-Tax precipitate obtained from extracts of MT2, MT4, and C8166 cells (Fig. 5B, compare lanes 3 to 5 to lanes 1 and 2). The SIRT1 band in the MT2 precipitate was less prominent, probably due to the smaller amount of Tax recovered (Fig. 5B, compare lane 3 to lanes 4 and 5). Nevertheless, our results demonstrated the association of Tax and SIRT1 in HTLV-1-transformed T cells.

**SIRT1 and resveratrol prevent Tax-CREB interaction.** From the above-described results, we knew that resveratrol suppressed Tax activation of HTLV-1 LTR through SIRT1 (Fig. 2). In

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**FIG 3** Resveratrol and SIRT1 suppress proviral transcription of HTLV-1. (A and B) Effect of resveratrol on viral mRNA expression. HTLV-1-transformed MT2, MT4, and C8166 cells were treated with increasing doses (20 and 40 μM) of resveratrol (Res) for 24 h. Cells in control groups 1 to 3 were treated with DMSO. Cells were harvested and total RNA was extracted for cDNA synthesis. Real-time quantitative RT-PCR was performed to analyze Tax (A), Env (B), and GAPDH transcripts. Quantification of mRNA expression was achieved by the comparative CT method. Tax or Env mRNA expression relative to GAPDH transcript in DMSO-treated cells (groups 1 to 3) was taken as 1. The differences between groups 2 and 5 in panel A and between groups 2 and 5 in panel B are statistically significant by two-tailed Student’s t test, with P values equal to 0.040 (indicated by “*”) and 0.0042 (indicated by “**”), respectively. (C and D) Effect of resveratrol on Tax expression. Cells were treated as indicated with 20 μM (C) or 20, 40, and 80 μM (D) resveratrol for 24 h. Expression of Tax and α-tubulin (α-tub) was detected by Western blotting. (E) Effect of SIRT1 inhibition on Tax mRNA expression. C8166 cells were treated with increasing doses (10 and 20 μM) of SIRT1 inhibitors (SIRT1i) sirtinol and Ex527 for 24 h. RT-qPCR was performed as for panel A. The difference between groups 1 and 5 is statistically significant by two-tailed Student’s t test, with a P value equal to 0.018 (indicated by “*”). (F) Effect of sirtinol on Tax mRNA expression. MT2, MT4, and C8166 cells were treated with the indicated dose of sirtinol for 24 h. RT-qPCR was performed as for panel A. The difference between groups 3 and 6 is statistically significant by two-tailed Student’s t test, with a P value equal to 0.0072 (indicated by “**”). (G) Inhibition of HTLV-1 virion production by resveratrol. Cells were treated with resveratrol (10, 20, or 40 μM). Overnight production of cell-free HTLV-1 virions was assessed by measuring reverse transcriptase activity recovered from live HTLV-1 in the culture supernatant with a colorimetric assay kit (Roche). HTLV-1-negative Jurkat cells (group 5) served as a negative control. The difference between groups 1 and 2 is statistically significant by two-tailed Student’s t test, with a P value equal to 0.0036 (indicated by “***”). (H) Inhibition of cell-to-cell transmission of HTLV-1 by resveratrol. MT2 cells were treated with either DMSO or resveratrol (20 μM) for 24 h. Jurkat cells were transfected with pLTR-Luc and pRL-TK for 24 h. Treated MT2 cells and transfected Jurkat cells were then mixed and cocultured for an additional 48 h. Cell were harvested for dual luciferase reporter assay. Statistical analysis was performed using two-tailed Student’s t test, with a P value equal to 0.0020 (indicated by “**”).
addition, SIRT1 interacts with Tax (Fig. 5). Because Tax activates HTLV-1 LTR primarily through the interaction with CREB (6), we asked whether SIRT1 and resveratrol might perturb this interaction.

To test this idea, we coexpressed SIRT1 or its HY mutant together with Tax and CREB in HEK293T cells. In the absence of SIRT1, CREB associated with Tax (Fig. 6A, compare lane 3 to lanes 4 and 5). Interestingly, CREB was not detected in the Tax-containing immunoprecipitate when SIRT1 was overexpressed (Fig. 6A, compare lane 2 to lane 3). However, the association between Tax and CREB remained unaffected in SIRT1-HY-overexpressing cells (Fig. 6A, compare lane 1 to lanes 2 and 3). These results suggested that SIRT1 interferes with Tax-CREB interaction in an activity-dependent manner. Consistently with this, addition of resveratrol prevented Tax-CREB association, even at the lowest dose (Fig. 6B, compare lanes 4 and 5 to lane 3). Hence, SIRT1 and resveratrol block the interaction between Tax and CREB, plausibly perturbing the function of Tax and CREB in HTLV-1 transcription.

Resveratrol perturbs CREB, CRTC1, and p300 recruitment to HTLV-1 LTR. Tax activation of the LTR requires CREB, CRTCcs, and p300 (14). CREB is a master regulator of cell metabolism and a bona fide oncoprotein in different contexts (41). Various CREB regulatory signals converge on CRTCcs to exert their effects (42). CRTCcs cooperate with p300 and CBP to achieve optimal activation of CREB-dependent transcription (14, 43). Perturbation of CREB, CRTC, and p300 signaling has been implicated in other malignancies, including those associated with infections by Epstein-Barr virus and hepatitis B virus (35, 44, 45). SIRT1 interacts with Tax (Fig. 5). SIRT1 and resveratrol also prevent the interaction between Tax and CREB (Fig. 6). It will therefore be of great interest to see whether resveratrol might perturb...
Tax activation of HTLV-1 transcription by affecting the recruitment of CREB, CRTC1, and p300 to the LTR.

To shed light on the mechanism by which resveratrol counteracts Tax function, we performed chromatin immunoprecipitation to examine the relative recruitment of endogenous CREB, CRTC1, and p300 to HTLV-1 LTR. CREB, CRTC1, and p300 were bound to the LTR in MT2 cells (Fig. 7A, B and C, compare group 2 to group 1). When we treated MT2 cells with resveratrol, the recruitment of CREB, CRTC1, and p300 to the LTR was compromised (Fig. 7A and B, compare groups 3 and 4 to group 2, and Fig. 7C, compare group 3 to group 2). These data suggest that resveratrol represses HTLV-1 transcription by restricting LTR occupancy of CREB, CRTC1, and p300.

**DISCUSSION**

In this study, we obtained the first evidence for SIRT1 suppression of HTLV-1 gene expression. We first demonstrated the inhibition of Tax activation of HTLV-1 LTR but not NF-kB by SIRT1. We went on to show a SIRT1-dependent repression of HTLV-1 proviral gene transcription, virion production, and cell-to-cell transmission by resveratrol, a small-molecule agonist of SIRT1. Mechanistically, SIRT1 interacts with Tax and prevents Tax from interacting with CREB, resulting in decreased recruitment of CREB, CRTC1, and p300 to the LTR. Our work reveals the interplay between SIRT1 and Tax and thereby provides a new regulatory mechanism in HTLV-1 transcription, a control step in disease development. Our findings on SIRT1 and resveratrol may also guide future development of new anti-HTLV-1 agents for prevention of and intervention against ATL and TSP.

The suppression of HTLV-1 transcription by SIRT1 was supported by results from both gain-of-function and loss-of-function experiments conducted with HTLV-1-transformed T cells. Importantly, our analysis of the catalytically dead mutant of SIRT1 indicated the requirement of SIRT1 deacetylase activity for the suppression of HTLV-1 transcription. The deacetylase-dead mutant of SIRT1 can still interact with Tax but loses its ability to impede the interaction of Tax with CREB or to suppress Tax activation of the LTR. Thus, for the suppression of LTR activation, the deacetylase activity of SIRT1 is more important than the interaction between SIRT1 and Tax, which might be required but is not sufficient. The perturbation of the Tax-CREB interaction and the inhibition of the recruitment of CREB, CRTC1, and p300 to the LTR are two functional consequences of the action of SIRT1 deacetylase. Our findings are generally consistent with the model for SIRT1-mediated transcriptional regulation (18–20), and they...
provide the foundation for further analysis of the suppressive effect of SIRT1 on HTLV-1 transcription and transformation.

Acetylation of Tax has previously been shown to modulate NF-κB activation and oncogenic transformation (39, 40). In this study, we clarified that acetylation of Tax at K346 does not affect LTR activation and might be irrelevant to the suppressive effect of resveratrol on HTLV-1 transcription. We also demonstrated that SIRT1 has no influence on Tax activation of NF-κB. The suppressive effect of SIRT1 and resveratrol is therefore highly specific to HTLV-1 LTR. Although direct deacetylation of Tax by SIRT1 cannot be excluded, it would not be surprising if SIRT1 could act on multiple substrates relevant to Tax activity and HTLV-1 transcription. Thus, how SIRT1 affects deacetylation and the functions of histones, CREB, CRTCs, and other transcriptional regulators in the context of HTLV-1 infection merits further investigations. Particularly, it is intriguing to see exactly how SIRT1 impedes the interaction of Tax with CREB.

Whereas Tax plays a major role in the initiation of ATL and TSP, its expression subsides at the later stage of ATL development (11). In contrast, HBZ is expressed both in untransformed infected cells and when ATL is fully developed (10). Since SIRT1 and resveratrol are not known to affect HBZ expression, the use of resveratrol might be more feasible when Tax expression is high in HTLV-1 carriers, TSP patients, and a small subset of ATL patients.

Resveratrol has already been sold as a nutritional supplement and might prove useful in many other diseases, including various types of cancer (17, 24). In view of the potential of repurposing resveratrol for ATL, we characterized its inhibitory effect on Tax activity, HTLV-1 transcription, and virion production. Particularly, the negative impact of resveratrol on Tax-CREB interaction and on the LTR occupancy of CREB, CRTCs, and p300 was documented. These and further analyses along this line will elucidate the mechanism of action of resveratrol in the context of HTLV-1 transcription and transformation. A detailed comparison of resveratrol with other anti-HTLV-1 agents, such as interferon alpha and zidovudine, which have been used clinically in ATL (46), might fully establish the utility of resveratrol for ATL and other HTLV-1-associated diseases, such as TSP.

The dose of resveratrol that we used in our study is comparable to or lower than those tested in other cell-based models (47, 48). In one recent study, the in vitro beneficial effects obtained with 25 μM resveratrol in cultured cells were found to be highly relevant in mice treated with a moderate dose (230 mg/kg of body weight per day) of resveratrol. SIRT1-independent toxic effects of resveratrol in cultured cells were observed only when high doses (≥50 μM) were administered (48). Nevertheless, to establish the physiological relevance of our in vitro results, it will be mandatory to test the effects of resveratrol in primary ATL cells and in humanized mouse models of HTLV-1 infection (49) in the future.

Our analysis of the anti-HTLV-1 activity of resveratrol provides the proof of principle that SIRT1 activators might have beneficial effects in ATL and TSP. A large panel of small-molecule activators of SIRT1 has already been tested in clinical trials (18, 25). Particularly, second- and third-generation SIRT1-activating compounds such as SRT1720 and STAC-5 are available (25). Compared to resveratrol, these synthetic SIRT1 activators offer greater potency, enhanced solubility, and improved bioavailability (25, 50). These agents might also exhibit potent anti-HTLV-1 activity. In this sense, our work will pave the way for further development of synthetic SIRT1 activators as novel anti-HTLV-1 agents for the treatment of ATL and TSP.

ACKNOWLEDGMENTS

We thank members of the Jin laboratory for critical readings of the manuscript.

This work was supported by the S. K. Yee Medical Research Fund (2011), Hong Kong Research Grants Council (grants HKU7674/12M, HKU7686/13M, and HKU1/CRF/11G), and Hong Kong Health and Medical Research Fund (grant 13121052).

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SIRT1 Suppresses HTLV-1 Transcription


August 2015 Volume 89 Number 16 jvi.asm.org