Aberrant Expression and Modification of Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptors Involved in the Pathogenesis of Tumoral Cortisol Resistance

Jingjing Jiang,* Na Li,* Xiaolin Wang, Yan Lu, Yufang Bi, Weiqing Wang, Xiaoying Li, and Guang Ning

Shanghai Clinical Center for Endocrine and Metabolic Diseases (J.J., N.L., X.W., Y.L., Y.B., W.W., X.L., G.N.), Shanghai Institute of Endocrinology and Metabolism, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, and The Key Laboratory of Endocrine Tumors and the Division of Endocrine and Metabolic Diseases (X.L., G.N.), E-Institute of Shanghai Universities, Shanghai 200025, China

Ectopic ACTH syndrome (EAS) accounts for 10–15% of cases of Cushing’s syndrome and is mostly caused by small cell lung cancers or thymic carcinoids. EAS is characterized by tumoral cortisol resistance, whose underlying mechanism remains unknown. In this study, we reported that silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), a major nuclear corepressor, was aberrantly expressed in ACTH-secreting thymic carcinoids. Overexpression and knockdown of SMRT in the ACTH-secreting AtT-20 cell line demonstrated that SMRT participated in the negative feedback of dexamethasone-mediated suppression of proopiomelanocortin. Posttranslational modification by the small ubiquitin-like modifiers (SUMO), i.e., SUMOylation plays an important role in fine-tuning transcriptional activities. SUMOylation of SMRT was observed in dexamethasone-resistant cell lines. Moreover, overexpression of the deSUMOylation enzyme enhanced the suppression of proopiomelanocortin by dexamethasone in AtT-20 cells. An evolutionarily conserved consensus SUMOylation site was identified close to the histone deacetylase 3 recruiting domain of SMRT, which might interfere with the recruiting process. These results suggested that aberrant expression and modification of SMRT might be involved in the pathogenesis of tumoral cortisol resistance. A therapeutic approach targeting SMRT SUMOylation might be developed for EAS patients. (Endocrinology 151: 3697–3705, 2010)

Cushing’s syndrome is a clinical state resulting from excessive cortisol secretion. Ectopic ACTH syndrome (EAS) accounts for 10–15% of cases of Cushing’s syndrome and is caused by, among others, small cell lung cancers and thymic carcinoids (1). EAS is characterized by the ectopic expression of proopiomelanocortin (POMC) and the loss of the negative feedback regulation of the POMC gene by excessive serum cortisol. We have previously shown that hypomethylation in the promoter region of POMC gene contributed to the ectopic overexpression in thymic carcinoids (2). However, the mechanism underlying the loss of normal feedback in these tumor cells, also known as hormone resistance, remains to be elucidated. Normal feedback of hormones requires nuclear receptors as well as various corepressors. Of note, nuclear receptor corepressor (NCoR) 1 and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT; also known as NCoR2) are structurally related and extensively studied corepressors (3). A highly conserved and closely spaced pair of switching defective protein 3, adaptor 2, NCoR, transcription factor IIIB (SANT) domains identified in NCoR1 and SMRT participates in the regulation of gene expression. A therapeutic approach targeting SMRT SUMOylation might be developed for EAS patients.
transcription through recruiting histone deacetylase (HDAC)-3 (4, 5).

Posttranslational modification of proteins by the small ubiquitin-like modifiers (SUMO) influences protein stability, subcellular localization, DNA binding, gene transcription, etc. (6). So far, four SUMO paralogs (SUMO-1, SUMO-2, SUMO-3, and SUMO-4) have been reported (7, 8). SUMO is first activated by the enzymes Aos1 (activator of SUMO)/Uba2 (ubiquitin-like modifier activating enzyme 2) and then transferred to the E2 conjugation enzyme ubiquitinconjugating enzyme 9 (Ubc9) and finally conjugated to substrate proteins by an E3 ligase (9). It was reported that NCoR1 could be SUMOylated by SUMO-1 (10). However, it is still unclear whether SMRT could be SUMOylated.

Here we demonstrate that SMRT was markedly upregulated in thymic carcinoids obtained from EAS patients. SMRT was involved in the normal feedback of dexamethasone (Dex)-mediated suppression of POMC. SMRT overexpression enhanced the responsiveness to Dex, whereas knockdown of SMRT lead to Dex resistance. Moreover, SUMOylation of SMRT was detected in cortisol-resistant cell lines expressing POMC and secreting ACTH. Overexpression of deSUMOylation enzyme (SENPI), a deSUMOylation enzyme, enhanced the responsiveness of POMC to Dex suppression. A conserved SUMOylation site was identified close to the SANT domain of SMRT, which might potentially influence the recruitment of HDAC3 by SMRT and contribute to tumoral cortisol resistance.

Materials and Methods

Patients

Four ACTH-producing human thymic carcinoids (TCs) were obtained at surgery from patients with EAS. The patients presented with a typical Cushing habitus accompanied by hyperpigmentation and hypokalemia. For all four of these patients, the high-dose (8 mg) Dex suppression test showed lack of suppression, computed tomography scanning documented anterior mediastinal masses, and the removed mediastinal tumors were confirmed as TCs by positive ACTH and neuron-specific enolase staining (1). The four noncancerous thymuses (NTs) were from independent individuals without EAS. The study was approved by the Ruijin Hospital Ethics Committee for Human Research. Informed consents were obtained from each subject participating in the study after full explanation of the purpose and nature of all procedures used.

Plasmids and reagents

pCMX-mSMRT and pCMX-mNCoR1 plasmids were kindly provided by Jiemin Wong (East China Normal University, Shanghai, China). pcDNA3-HA-Ubc9 and its mutant C93S were gifts from F. Melchior (University Gottingen, Gottingen, Germany). FLAG-SENPI, FLAG-SENP1mutant and enhanced green fluorescent protein (EGFP)-SUMO1 were kindly provided by Jinke Cheng (M. D. Anderson Cancer Center, Houston, TX). The cDNA encoding full-length mouse Ubc9 and Ubc9 (C93S) mutant was amplified from pcDNA3-3HA-Ubc9 and pcDNA3-HA-Ubc9 (C93S) by PCR and inserted into the XbaI and XhoI sites of pCMVFa to obtain the pCMVFa-MCS-Ubc9 expression vector. The open reading frames including SANT domain of SMRT and NCoR1 were amplified from full-length pCMX-mSMRT and pCMX-mNCoR1 plasmids by PCR and inserted into the EcoRI and XbaI sites of pCMVFa-MCS-Ubc9 to generate SMRT Flag-SANT-Ubc9 (containing amino acids 394-700 of SMRT) and NCoR1 Flag-SANT-Ubc9 (containing amino acids 403-719 of NCoR1) expression vectors, respectively. SMRT SANT-Ubc9 was subsequently amplified by PCR and inserted into EcoRI and HindIII sites of pcDNA3.1/myc-His(-)C to generate SMRT SANT-Ubc9-myc-His. Detailed primer sequences are available on request.

Mutagenesis was performed to create K516R, K531R, and K668R in SANT domain using Quick Change II site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The primer sequences of sense strands for mutagenesis are: K516R, 5'-GGACGCGTGGGAGAGGAGGGAGGAGGAGGAGGAGG-3'; K531R, 5'-GACAAGGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGG-3'; K531R, 5'-GACAAGGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGG-3'. Murine POMC promoter (~819/+28 bp) was amplified using primers as follows: forward, 5'-CCGTCGAGGTAGTGCACGTGAGGT-3'; reverse, 5'-CAGGGTCTTGTCTACTTGAGCT-3'.

Dex, RU-486, and trichostatin A (TSA) were purchased from Sigma Chemicals (St. Louis, MO).

Cell culture, transfection, and luciferase assays

AR-T20 cells were maintained in Ham’s F12K medium supplemented with 15% horse serum, 2.5% fetal bovine serum, and 2 mM t-glutamine. DMS-79 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM t-glutamine. 293T cells were cultured in DMEM supplemented with 10% fetal bovine serum and 2 mM t-glutamine. All transfections were performed using Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. For luciferase assays, cells were seeded in 24-well plates and transfection efficiency was normalized by cotransfecting Simian virus 40 (SV40) plasmid.

RT-PCR and quantitative real-time PCR

The samples were homogenized and total RNA was extracted using TRIzol reagent (Invitrogen). cDNA was transcribed from 2 µg of total RNA following the manufacturer’s instructions (Promega, Madison, WI). Primers used for RT-PCR are as follows: POMC forward, 5'-GAGCTCGGCAAGTATTATTGAC-3'; POMC reverse, 5'-GGAGTTGGTCCCATGACGT-3'; NCoR1 forward, 5'-CCCATACCCAGCAAGCAGA-3'; NCoR1 reverse, 5'-CATGGAAGACAGTTGTGAC-3'; SMRT forward, 5'-CTCCAAAGTCTTCCAGGCAACAC-3'; SMRT reverse, 5'-GGTCACTCCGTCCTGCAAG-3'; actin forward, 5'-GAGGAGGCCCAAGAAGAGGAGGAGGAGG-3'; actin reverse, 5'-GATCCCATCTTGGTCAAGTGAC-3'.
the \( \Delta \Delta C_t \) method with actin as the endogenous reference gene amplified from the samples: POMC forward, 5'-'ATGCCGAGAT-TCTGCTACAGT-3'; POMC reverse, 5'-'TCCAGCGAGAG-GTCGGATT-3'; actin forward, 5'-'GGCTGATTCCCTC-CATCG-3'; actin reverse, 5'-'CCAGTTGGAACATGC-CATGT-3.

**Antibodies and Western blots**

Antibodies were purchased from the following manufacturers: anti-Flag, anti-green fluorescent protein (GFP), and antitubulin antibodies from Cell Signaling Technology (Beverly, MA); anti-\( \text{myc} \) (9E10) and anti-POMC antibodies from Santa Cruz Biotechnology (Santa Cruz, CA); anti-FLAG M2 beads from Sigma-Aldrich (St. Louis, MO); and anti-GMP-1 antibody from Zymed (San Francisco, CA). SMRT antibody was a gift from Jiemin Wong. Novex 3–8% Tris-acetate gel (Invitrogen) was used for immunoblotting of endogenous SMRT. Otherwise, 10% SDS-PAGE was used. For comparison of SMRT expression in clinical samples, NT and TCs were homogenized and proteins were extracted using modified radioimmunoprecipitation assay buffer (50 mm Tris-HCl, pH 7.6; 150 mm NaCl; 1% Nonidet P-40; 0.25% deoxycholate; 0.1% sodium dodecyl sulfate) supplemented with protease inhibitor cocktail (Sigma). Samples were normalized for protein concentration using a BCA protein assay kit (Pierce, Rockford, IL). Proteins were transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA) and horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) was used. The proteins were visualized by an ECL chemiluminescence detection kit (Amersham Biosciences, Buckinghamshire, UK).

**Knockdown experiments**

Small interference RNA (siRNA) against mouse SMRT and RNA interference-negative control were from Dharmacon (Lafayette, CO). AtT-20 cells were used for the knockdown experiment with siRNA. Cells were transfected with the siRNA against SMRT or nonspecific RNA (100 pmol/ml) using Lipofectamine 2000 (Invitrogen). Knockdown of SMRT was confirmed by Western blot. Forty-eight hours after transfection, cells were treated with Dex for another 12 h. Cells were lysed in TRizol (Invitrogen) and prepared for real-time PCR.

**Immunoprecipitation assay**

For endogenous SMRT SUMoylation analysis, DMS-79 cells were lysed in modified radioimmunoprecipitation assay buffer as mentioned above, supplemented with 20 mm N-ethylmaleimide and protease inhibitor cocktail. Cell debris was removed by centrifugation at 13,000 \( \times g \) for 15 min. One milligram of cell lysate was incubated with SMRT antibody or mouse IgG on a rotator at 4 \(^\circ\)C overnight. The immunoprecipitated complexes were captured with protein-G agarose (Santa Cruz) for 2 h at 4 \(^\circ\)C, washed three times with the same buffer, boiled in loading buffer, and subjected to Novex 3–8% Tris-acetate gel followed by Western blotting. For Flag-SANT-Ubc9 immunoprecipitation, 293T cells were harvested and lysed in 24 h after transfection in FLAG lysis buffer [50 mm Tris-HCl (pH 7.4), with 150 mm NaCl, 1 mm EDTA, and 1% Triton X-100] supplemented with 20 mm N-ethylmaleimide and protease inhibitor cocktail. After brief sonication, cell debris was removed by centrifugation at 13,000 \( \times g \) for 15 min. The supernatants were collected and incubated with anti-Flag M2 beads on a rotator at 4 \(^\circ\)C overnight. Immunoprecipitates were washed with the same buffer for three times, boiled in loading buffer, and subjected to SDS-PAGE followed by Western blotting.

**Results**

**Aberrant expression of SMRT in TCs from EAS patients**

We previously performed microarray studies on TCs from EAS patients and revealed novel molecular mechanisms underlying the pathogenesis of EAS (11). To address the underlying mechanism causing tumoral cortisol resistance, special attention was paid to misregulated transcription factors and cofactors potentially involved in the normal POMC feedback. Interestingly, SMRT, also known as NCoR2, but not NCoR1, was found to be up-regulated in tumor samples compared with normal tissues. RT-PCR was subsequently performed using cDNAs from TCs, verifying the array data (Fig. 1A). The expression of SMRT in TCs was also examined by Western blot. As expected, tumors expressed much higher level of SMRT protein, which was consistent with the results of RT-PCR analysis, supporting a potential role of SMRT in EAS. Notably, two bands of SMRT were detected (Fig. 1B).

**SMRT involved in physiological suppression of POMC**

To explore the physiological role of SMRT in the negative feedback of POMC, SMRT was examined in two ACTH-secreting cell lines, a human small cell lung cancer cell line DMS-79 and a murine pituitary corticotroph tumor cell line AtT-20, both of which expressed POMC. DMS-79 expressed a high level of SMRT, whereas AtT-20 expressed a relatively lower level of SMRT, as compared with normal controls. The expression of SMRT in TCs was also examined by Western blot. As expected, tumors expressed much higher level of SMRT protein, which was consistent with the results of RT-PCR analysis, supporting a potential role of SMRT in EAS. Notably, two bands of SMRT were detected (Fig. 1B).

**FIG. 1.** Aberrant expression of SMRT in TCs from patients with EAS. A, POMC and corepressor expression was detected by RT-PCR in TCs and NTs. B, SMRT protein expression level was detected in tumors and normal controls. Fourty micrograms of each sample were analyzed by Novex 3–8% Tris-acetate gel and Western blotted using SMRT antibody diluted 1:1000. Tubulin served as an internal control.
with DMS-79 (data not shown). DMS-79 was totally non-responsive to Dex suppression, whereas ArT-20, although derived from pituitary corticotroph tumor, responded to Dex as reflected by a POMC reporter assay (Fig. 2A). RU-486, a glucocorticoid receptor (GR) antagonist, abolished the effect of Dex (Fig. 2B). Thus, ArT-20 cells were selected for functional studies of SMRT in normal POMC feedback.

Overexpression and knockdown of SMRT were performed in ArT-20 cells. The expression of SMRT was confirmed by Western blots in overexpression and knockdown experiments (Fig. 2, C and D). To our surprise, overexpression of SMRT actually sensitized ArT-20 to Dex, whereas knockdown of SMRT using siRNA rendered ArT-20 more resistant to Dex suppression (Fig. 2, E and F). These findings suggested that SMRT functioned as a corepressor in the normal POMC feedback.

**HDAC activity required for POMC suppression**

Previous studies showed that SMRT exerted repressive function through binding with HDAC3 via its SANT domain (4, 5). It was also reported that on Dex stimulation, GR and SMRT associated to form a repression complex and reduce histone acetylation level in the GSTA2 promoter region (12). We explored whether repression of POMC by SMRT also required HDAC activity. We determined whether treatment of cells with TSA, a HDAC inhibitor, could reverse the inhibitory effect of Dex suppression of POMC expression. Suppression of POMC expression was almost completely reversed by treatment with TSA, as reflected by both POMC reporter assay and Western blot in ArT-20 cells (Fig. 3, A and B), suggesting that a GR-SMRT-HDAC complex was recruited to the POMC promoter on Dex treatment.

**SUMOylation of SMRT detected in cortisol-resistant cell lines**

Posttranslational modifications played an important role in the regulation of nuclear factors. Phosphorylation of SMRT by inhibitory-κB kinase-α in colorectal tumors lead to nuclear export and specific Notch target gene derepression (13). However, no cytoplasmic accumulation of SMRT was detected in our study (data not shown). SUMOylation emerged as an important posttranslational modification for a variety of transcription factors, including NCoR1 (10). We examined whether SMRT was also SUMOylated.

Immunoprecipitation using highly cortisol-resistant DMS-79 cells clearly demonstrated that SMRT was modified by SUMO1 (Fig. 4A). Interestingly, two bands rec-
ognized by the SMRT antibody were both detected by SUMO1. Notably, SUMOylation of SMRT was also detectable in AtT-20 cells, although at a lower level than that of DMS-79 (data not shown). To determine the functional consequences of SUMOylation, a deSUMOylation enzyme, SENP1 (14), was transfected into AtT-20 cells. Interestingly, SENP1 sensitized response of AtT-20 to Dex, supporting a potential role of SUMOylation in cortisol resistance (Fig. 4B). Due to the low transfection efficiency, we were unable to observe the effect of SENP1 overexpression in DMS-79 cells. These results prompted us to speculate that SUMOylation of SMRT might be involved in the pathogenesis of cortisol resistance.

Multiple potential SUMO motifs identified in SMRT

Analysis using multiple prediction software including SUMOplot (http://www.abgent.com.cn/doc/sumoplot/login.asp), SUMOsp2.0 (15), and SUMOpre (16) revealed that SMRT had four classic SUMOylation consensus motifs, namely LKME, LKVE, VKQE, and LKRE (Fig. 5A), suggesting high probability of SUMOylation for SMRT.

Of the four classic motifs found in SMRT, LK\(^{668}\)ME, VK\(^{1174}\)QE, and LK\(^{1381}\)RE were evolutionarily conserved (Fig. 5B). Of note, VK\(^{1174}\)QE and LK\(^{1381}\)RE in SMRT well corresponded to previously verified SUMOylation motifs (IK\(^{1117}\)QE and IK\(^{1330}\)RE) in NCoR1 and VK\(^{1174}\)QE actually fitted the phosphorylation-dependent SUMOylation motif, composed of a SUMO consensus site and an adjacent proline-directed phosphorylation site (\(\psi KxExxSP\)) (17). Interestingly, several bioinformatic approaches predicted high probability of SUMOylation for LK\(^{668}\)ME located at the immediate end of the SANT domain, which was found only in SMRT across species but not in NCoR1 (Fig. 5B). These observations prompted us to determine whether K668 was a real target for SUMOylation.

A classic SUMO motif confirmed close to the SANT domain in SMRT

To explore the possibility of LK\(^{668}\)ME, a recently described Ubc9 fusion-directed SUMOylation system was used (18, 19). As shown in Fig. 6A, the SANT domain was fused to the N terminal of Ubc9 to generate a SANT-Ubc9 fusion protein. Multiple mutations were introduced to either the SANT part or the Ubc9 part.

As expected, an additional band indicating the SUMOylation of SANT domain was observed in coexpression with EGF-P-SUMO1, whereas no additional band could be detected in coexpression with Ubc9 (C93S) mutant (Fig. 6B). The immunoprecipitation study also showed that the slow migrating band could be detected by both Flag and GFP antibodies, suggesting SANT-Ubc9 was SUMOylated (Fig. 6C). SUMOylation of SANT-Ubc9 was further examined by coexpression of SENP1. Overexpression of SENP1 rather than mutant SENP1 pre-
vented the SUMOylation of SANT-Ubc9(Fig. 6D). To define the SUMOylation site, K668 and two other lysines, K516 and K531, were replaced by arginines, individually or in combination. The mutant K668R alone abolished the SUMOylation band, whereas mutants K516R or K531R, alone or in combination, did not change the SUMOylation (Fig. 6E). The corresponding SANT domain of NCoR1 was also fused to Ubc9. However, no SUMOylated form of NCoR1 SANT was observed (Fig. 6F), which was consistent with previous studies (10). Taken together, these findings indicated that K668 beside the SANT domain of SMRT was a real target for SUMOylation.

Discussion

The mechanism underlying tumoral cortisol resistance is complex and diverse. In some rare cases of cortisol resistance, inherited or sporadic mutations of the gene encoding the GR account for cortisol insensitivity (20–24). For most cases, other poorly defined mechanisms appear to be implicated (25). In our study, no mutation was detected in tumor samples (data not shown), although a mutation in GR was detected in the DMS-79 cell line as previously reported, which did not impair its normal function (26). A previous study suggested that loss of Brg1 (Brahma-related gene) or HDAC2 contributed to cortisol resistance in about 50% of glucocorticoid-resistant human and dog corticotroph adenomas (27). However, both NT and TC samples expressed a similar level of Brg1, HDAC2, and HDAC3 (data not shown). In this study, we have shown that SMRT was up-regulated in TCs derived from patients with EAS.

SMRT and its paralog NCoR1 serve as key corepressors for a large assortment of different transcription factors, including PLZF (promyelocytic leukemia zinc finger), c-Jun, phosphorylated mothers against decapentaplegics, -1, pituitary transcription factor-1, nuclear factor-kB, BCL-6 (B cell leukemia/lymophma 6), and many nuclear hormone receptors, including GR (12, 28, 29). The two proteins share a common molecular architecture, form similar complexes with other corepressor proteins, and exert overlapping biological functions. Significantly, SMRT and NCoR not only physically recruit but can also kindle the enzymatic activity of their associated HDACs, mainly via its SANT domain (5). Upon Dex activation, GR binds to SMRT to form a repression complex (12). Overexpression and knockdown of SMRT confirmed its function as a corepressor in GR-mediated POMC suppression because the repression of SMRT was largely dependent on the recruitment and activation of HDAC via its SANT domain. Repression of POMC should also rely on HDAC activity. Consistent with this concept, TSA completely abrogated the repression on POMC by Dex, suggesting a functional GR-SMRT-HDAC complex on POMC promoter on Dex stimulation.

SUMOylation has emerged as an important posttranslational modification and was frequently shown to influence the transcriptional activity of nuclear factors. SUMOylation of SMRT has not been previously reported, although NCoR1 has been proved to be a target of SUMOylation (10). In this study, we have provided direct evidence that SMRT was a real target for SUMO modification. SUMOylation of SMRT was detected in both DMS-79 and AtT-20 cell lines. Previous studies demonstrated that knockdown of SENP1 in AR-positive LNCaP cells desensitized the cells to androgen-stimulated effects (30). Intriguingly, overexpression of SENP1 enhanced the responsiveness to Dex suppression, although it should be noted that SENP1 transfection led to a decline of overall SUMOylation level, which might also lead to change of SUMOylation level of proteins other than SMRT.

A careful bioinformatic study of SMRT protein sequence revealed four consensus motifs, of which three were evolutionarily conserved. K668 was unique in at least two aspects. First, K668 was located at the end of the SANT domain, which was responsible for repression. Sec-
ond, unlike the others, K668 was present only in SMRT. A previous study demonstrated that SUMOylation of a corepressor prospero-related homeobox protein-1 reduced its interaction with HDAC3 and as a result downregulated its corepressor activity (31). We propose that if K668 of SMRT could be SUMOylated, it might interfere with the SANT recruitment of HDAC3 in a similar manner. When the ability to recruit and activate HDAC3 was blocked by SUMOylation, SMRT could no longer function normally as a corepressor, thus leading to derepression of POMC.

It should be noted that two bands were detected in tumor samples as shown by Western blot. Previous studies identified at least two splices of SMRT, whose expression varied in different tissues (32, 33). The longer isoform, designated as SMRTα, refers to full-length SMRT, and the shorter isoform, designated as SMRTβ, harbors a natural deletion of amino acids 36-254 close to the N terminal (34). The SMRT antibody used in this study recognized the middle part of SMRT, which was intact in both SMRTα and SMRTβ. It appeared that both isoforms of SMRT could be SUMOylated. Because the SUMO consensus motifs were all far behind, the natural deletion did not seem to influence SUMOylation of SMRT anyway. Due to the heavy molecular weight of SMRT, SUMOylation resulted in only minimal shift as detected by SUMO1 antibody, which was also the case for NCoR1 (10).

In conclusion, up-regulation of SMRT was observed in cortisol-resistant TCs. SMRT functioned as a corepressor in Dex-GR-mediated repression of POMC. SUMOylation
of SMRT was detected in ACTH-secreting cell lines and SENP1 improved the responsiveness to Dex. K668 was proved to be a real target for SUMOylation, which might interfere with the recruitment of HDAC3 by SANT of SMRT. It remains unknown whether SUMOylation of SMRT could influence its interaction with other proteins and whether SUMOylation of different SMRT isoforms functioned differently. Questions remain as to why SMRT was SUMOylated during cortisol resistance and how SMRT SUMOylation was regulated. Nevertheless, a lower SUMOylation level caused by SENP1 transfection enhanced the sensitivity to Dex. It is reasonable to assume that SUMOylation inhibitors could be developed as a therapeutic alleviation of tumoral cortisol resistance in ectopic ACTH-secreting tumors. Further studies are required to address these issues, which should provide clues toward better understanding the pathogenesis of cortisol resistance and expand the knowledge of SMRT as a transcriptional corepressor.

Acknowledgments

We are grateful to Imelda Lee (Shanghai Clinical Center for Endocrine and Metabolic Diseases) for critical readings.

Address all correspondence and requests for reprints to: Guang Ning, M.D., Ph.D., Department of Endocrinology and Metabolism, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China. E-mail: gning@sibs.ac.com.

This work was supported by National Natural Science Foundation of China Grants 30725037, 30771018, and 30871203, China. E-mail: gning@sibs.ac.com.

Disclosure Summary: The authors have nothing to disclose.

References

3. Lazar MA 2003 Nuclear receptor corepressors. Nuc Recept Signal 1:e001
25. Dahia PL, Honegger J, Reincke M, Jacobs RA, Mirtella A, Fahlbusch


32. Park EJ, Schroen DJ, Yang M, Li H, Li L, Chen JD 1999 SMRTe, a silencing mediator for retinoid and thyroid hormone receptors-extended isoform that is more related to the nuclear receptor corepressor. Proc Natl Acad Sci USA 96:3519–3524


Up-To-Date links on JCEM provide a wealth of additional information!

www.endo-society.org