A SUMOylation-Dependent Pathway Regulates SIRT1 Transcription and Lung Cancer Metastasis

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Manuscript received August 8, 2012; revised April 12, 2013; accepted April 18, 2013.

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Background
Epithelial-to-mesenchymal transition (EMT) plays a pivotal role in lung cancer metastasis. The class III deacetylase sirtuin 1 (SIRT1) possesses both pro- and anticarcinogenic properties. The role of SIRT1 in lung cancer EMT is largely undefined.

Methods
The effect of SIRT1 on migration of lung cancer cells was evaluated by wound healing assay in vitro and metastasis assay in nude mice in vivo. Protein expression in human lung cancers and cultured lung cancer cells was assessed by western blotting and immunohistochemistry. Interaction between protein and DNA was measured by chromatin immunoprecipitation assay. SIRT1 promoter activity was determined by reporter assay.

Results
SIRT1 activation antagonized migration of lung cancer cells by suppressing EMT in vitro. Activation of SIRT1 by resveratrol also statistically significantly hampered (by 68.33%; P<.001, two-sided test) lung cancer cell metastasis in vivo. Hypoxia repressed SIRT1 transcription through promoting the competition between Sp1 and HIC1 on the SIRT1 proximal promoter in a SUMOylation-dependent manner. Disruption of SUMOylation by targeting either Ubc9 or PIASy restored SIRT1 expression in and favored an epithelial-like phenotype of cancer cells, thereby preventing metastasis. Decreased SIRT1 combined with elevated PIASy expression was implicated in more-invasive types of lung cancers in humans.

Conclusions
We have identified a novel pathway that links SIRT1 down-regulation to hypoxia-induced EMT in lung cancer cells and may shed light on the development of novel antitumor therapeutics.


Lung cancer, lacking an effective and sensitive tool for early detection, is one of the deadliest forms of cancers worldwide (1). Most patients already have metastatic lesions at the time of diagnosis, leaving few if any viable therapeutic options available for effective intervention. Therefore, metastasis constitutes a critical step in the pathogenesis of lung cancer, the understanding of which may provide mechanistic insight and ultimately yield novel interventional strategies.

Epithelial-to-mesenchymal transition (EMT) is an evolutionarily conserved biological process wherein epithelial cells undergo a series of morphological alterations to gain the ability to migrate to distal sites, playing key roles in a range of human pathologies that include pulmonary fibrosis and lung cancer metastasis (2). With respect to transcription, EMT is characterized by the down-regulation of epithelial-specific genes and simultaneously the up-regulation of mesenchymal signature genes. The past decade has seen a dramatic expansion in our knowledge regarding the intricate regulatory circuit underlying EMT; many details of these pathways, however, are still poorly defined (3).

SIRT1 is a nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylase sharing significant homology with the yeast Sir2 protein that is considered a critical link to longevity as Sir2 transgene prolongs replication cycles in Saccharomyces cerevisiae and Caenorhabditis elegans (4). SIRT1 exerts its biological impacts by deacetylating histone and nonhistone proteins, and its substrates include many proto-oncogenes (eg, Myc) and tumor suppressors (eg, p53). Although SIRT1 overexpression has yet to be correlated with increased life span in mammals, it does seem to confer cancer-free aging in mice (5). SIRT1 is known to maintain the physiological integrity of the respiratory system, affording protection against emphysema (6), chronic obstructive pulmonary disease (7), and pulmonary hypertension (8). However, how SIRT1 regulates EMT in lung cancer metastasis remains enigmatic. Here we report that SIRT1 activation ameliorates the migration of lung cancer cells in vitro and lung cancer metastasis in vivo. Hypoxia, acting as a promoter of cancer metastasis and EMT, represses SIRT1 expression in a SUMOylation-dependent manner, whereas disruption of SUMOylation reverses cancer cell EMT and...
blocks metastasis. Finally, patients with late-stage metastatic lung cancer exhibit low SIRT1 expression and high PIASy expression. Therefore, our data provide a novel link between SUMOylation-dependent SIRT1 repression and lung cancer metastasis.

Methods

Cell Culture and Treatment

HEK293, H1299, A549, and U2OS cells were maintained in Dulbecco’s modified Eagle medium (Invitrogen) and were authenticated by the Chinese Academy of Sciences Type Culture Collection Cell Bank. The SIRT1 agonist (resveratrol [RSV] and SRT1720) and antagonists (nicotinamide and sirtinol) were purchased from Sigma. Where indicated, hypoxia (1% O₂) was achieved by a mixture of ultra–high purity gases (5% CO₂, 10% H₂, 85% N₂) in a 37°C incubator (Thermo Fisher).

Plasmids and Transient Transfection

The SIRT1-reporter construct and expression constructs have been described previously (9–17). Small interfering RNA (siRNA) sequences are listed in Supplementary Table 1 (available online). Transient transfections were performed with Lipofectamine 2000 (Invitrogen). Luciferase activities were assayed 24–48 hours after transfection using a luciferase reporter assay system (Promega). Experiments were routinely performed in triplicate wells and repeated three times.

Protein Extraction, Immunoprecipitation, and Western Blotting

Whole cell protein extraction and immunoprecipitation were performed as previously described (18,19). Western blot analyses were performed with commercially available antibodies (for detailed information, see Supplementary Methods, available online). All experiments were repeated at least three times.

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (qPCR)

RNA was extracted with the RNeasy RNA isolation kit (Qiagen). Reverse-transcriptase reactions were performed using a SuperScript First-Strand Synthesis System (Invitrogen). Real-time PCR reactions were performed on an ABI Prism 7500 system. Primers used for real-time reactions are listed in Supplementary Table 2 (available online). All experiments were repeated at least three times.

Chromatin Immunoprecipitation (ChIP)

ChIP assays were performed essentially as described previously (20). Aliquots of lysates containing 200 μg of protein were used for each immunoprecipitation reaction. Precipitated genomic DNA was amplified by real-time PCR with primers listed in Supplementary Table 3 (available online). All experiments were repeated at least three times.

Scratch-Wound Healing/Migration Assay

Cells were resuspended in serum-free media. Upon confluence, scratch wounds were created by using a sterile micropipette tip. Cell migration was calculated by Image J. Data were expressed as the percentage of migration compared to control, arbitrarily set as 100%. All experiments were repeated at least three times.

In Vivo Metastasis

All animal study protocols were reviewed and approved by the intramural Ethics Committee on Humane Treatment of Experimental Animals. Male 4- to 6-week-old nude mice were randomly divided into different groups (4–15 mice per group) and inoculated with A549 cells (1 × 10⁶ per mouse, via tail vein). The next day, mice were injected peritoneally with RSV (20 mg/kg) or solvent every other day. Twenty-five days following inoculation, mice were killed and metastasized nodules were dissected and counted. All animal experiments were performed double-blind.

Human Tumor Samples and Histology

Lung cancer tissues were collected, under informed consent, from surgical resection specimens of patients who had not undergone radiotherapy or chemotherapy in the Affiliated Hospital of Nantong University following the guidelines of the intramural Committee on Human Studies. Diagnoses of all cases were confirmed by histological examination. Tumor differentiation was graded by the Edmondson grading system. Samples were processed essentially as previously described (21,22). Detailed procedures can be found in the Supplementary Materials (available online).

Statistical Analysis

One-way analysis of variance with post hoc Scheffe analyses were performed using the SPSS package (version 18.0). The differences between control and experimental groups were determined by the two-sided, unpaired Student t test.

Results

SIRT1 Activation Prevents Lung Cancer Metastasis by Blocking EMT

We first examined how SIRT1 activation would impact lung cancer metastasis in vitro and in vivo. Hypoxia is a major promoter of cancer metastasis by stimulating EMT (23–25). Overexpression of wild-type (WT) but not enzyme-deficient (HY) SIRT1 blocked hypoxia-induced migration of lung cancer cells (Figure 1A; Supplementary Figure 1A, available online). Meanwhile, there was an up-regulation of epithelial cell marker genes (CDH1 encoding E-cadherin; OCLN encoding occludin) and a simultaneous down-regulation of mesenchymal signature genes (VIM encoding vimentin; FN encoding fibronectin) (Figure 1, B and C; Supplementary Figure 1B, available online), indicative of a reversal of EMT in lung cancer cells. Of note, other members of the mammalian sirtuin proteins (SIRT2–SIRT7) failed to impact cancer cell migration (Supplementary Figure 1C, available online). In addition, treatment of cells with the universal sirtuin agonist RSV (Figure 1, D–F) or a more selective SIRT1 agonist SRT1720 (Supplementary Figure 1, D–G, available online) impeded hypoxia-induced cell migration and EMT, as did overexpression of the NAD⁺ salvage enzyme NAMPT (Supplementary Figure 1H, available online).

Next, we evaluated the hypothesis that SIRT1 activation might antagonize lung cancer metastasis in vivo. Indeed, intraperitoneal injection of RSV for 4 consecutive weeks in nude mice significantly attenuated metastasis of A549 cells injected via tail vein (Figure 1G; by 68.33%; P < .001, two-sided t test). Similarly, A549 cells stably expressing WT, but not HY, SIRT1 showed diminished metastasis.
Figure 1. SIRT1 activation prevents cancer metastasis by blocking epithelial-mesenchymal transition (EMT). A–C) H1299 cells were transfected with SIRT1 expression constructs followed by exposure to 1% $O_2$. A) Wound healing assays: upper panel, 0 h; bottom panel, 48 h. Expression of epithelial or mesenchymal genes was measured by quantitative polymerase chain reaction (qPCR) (B) and western blotting (C). D–F) H1299 cells were treated with resveratrol (RSV) followed by exposure to 1% $O_2$. Wound healing (D) assays were performed as described in the Methods. Upper panel, 0 h; bottom panel, 48 h. Expression of epithelial or mesenchymal genes was measured by qPCR (E) and western blotting (F). Bars represent means from three independent experiments; error bars refer to 95% confidence intervals. G) In vivo metastasis assay was performed in nude mice ($n = 14$ for the dimethyl sulfoxide [DMSO] group and $n = 15$ for the RSV group) using A549 cells. $P$ values were calculated using two-sided $t$ test. EV = empty vector; HY = enzyme deficient; WT = wild type.
in vivo (Supplementary Figure 1, I and J, available online). More importantly, SIRT1 expression, both at the mRNA level (Figure 2A) and the protein level (Figure 2, B and C; \(P < .001\), \(\chi^2\) test), was down-regulated in more-advanced human lung cancers. Together, these data support the conclusion that SIRT1 activation ameliorates cancer metastasis in vitro and in vivo by blocking cancer cell EMT.

**Hypoxia Suppresses SIRT1 Transcription Through Competition Between Sp1 and HIC1**

Next, we asked whether hypoxia, as an EMT promoter, might regulate SIRT1 expression. Lung cancer cells, when cultured under 1% O2, exhibited reduced SIRT1 expression (Figure 3A and B) and activity (Supplementary Figure 2A, available online). We then examined whether suppression of SIRT1 expression by hypoxia stemmed from decelerated transcription. To this end, cells were transfected with a series of SIRT1 promoter reporter plasmids harboring various deletions. Hypoxia repressed the activities of all the SIRT1 promoter constructs to a similar extent (Figure 3C; Supplementary Figure 2B, available online), indicating that there exists within the minimal promoter region (–115/+58) a hypoxia response element. Bioinformatic analysis of this region revealed a conserved binding site for the transcriptional activator Sp1 adjacent to a recently identified motif for the transcriptional repressor HIC1 (Figure 3D), alluding to a scenario wherein Sp1 and HIC1 compete for the binding of SIRT1 promoter. ChIP assays confirmed this hypothesis by demonstrating that increased doses of HIC1 disrupted Sp1 binding on the SIRT1 promoter and vice versa (Supplementary Figure 2C, available online). In U2OS cells lacking endogenous HIC1, reexpression of HIC1 antagonized the binding and activation of the SIRT1 promoter by Sp1 (Figure 3, E and F). Adding additional support to this model was the observation that hypoxia repressed SIRT1 in lung cancer cells, likely through favoring HIC1 over Sp1 on the SIRT1 promoter.

**SUMOylation-Dependent Mechanism Regulates SIRT1 Expression and Lung Cancer EMT**

We have previously shown that HIC1 can be SUMOylated in vivo (14). Of interest, hypoxia promoted the binding of WT, but not the SUMOylation defective (KR), HIC1 on the SIRT1 promoter.
Figure 3. Hypoxia suppresses SIRT1 transcription through competition between Sp1 and HIC1. A, B) H1299 cells were exposed to 1% O₂. Messenger RNA (A) and protein (B) levels of SIRT1 were evaluated by quantitative polymerase chain reaction and western blotting. C) SIRT1 promoter-luciferase constructs harboring serial deletions were transfected into H1299 cells followed by exposure to 1% O₂. Luciferase activities were expressed as percentage of control. D) Schematic representation of the SIRT1 proximal promoter sequences in human, mouse, and rat highlighting binding sites for Sp1 and HIC1. E) A SIRT1 promoter-luciferase construct (−115/+58) was cotransfected with indicated expression constructs into U2OS cells. Luciferase activities were expressed as percentage of control. F) U2OS cells were transfected with indicated HIC1 expression constructs. Chromatin immunoprecipitation (ChIP) assays were performed with anti-Sp1, and data were expressed as relative enrichment of control. G) H1299 cells were exposed to 1% O₂ and harvested at indicated time points. ChIP assays were performed with indicated antibodies. Bars represent means from three independent experiments; error bars refer to 95% confidence intervals. IP = immunoprecipitation; KR = lysine-to-arginine mutant; TSS = transcription start site; WT = wild type.
(Figure 4A). Similarly, HIC1 KR was refractory to hypoxia in terms of SIRT1 promoter repression (Figure 4B) and unable to compete with Sp1 (Figure 3, E and F). In addition, HIC1 SUMOylation was up-regulated by hypoxia (Supplementary Figure 3A, available online). In accordance, WT, but not KR, HIC1 promoted cell migration (Supplementary Figure 3B, available online). On the contrary, HIC1 knockdown ameliorated hypoxia-induced cell migration, which was reversed by SIRT1 knockdown or inhibition (Supplementary Figure 3, C and D, available online).

To verify the possibility that SUMOylation plays a role in SIRT1 transcriptional repression by hypoxia, we exploited a dominant negative (DN) Ubc9, the universal E2 conjugating enzyme. Overexpression of Ubc9 DN normalized the binding of Sp1 and HIC1 in response to hypoxia (Figure 4C). Importantly, Ubc9 DN restored the expression of epithelial-specific genes in hypoxia-challenged cancer cells and rendered the cells less migratory (Figure 4, D–F). Silencing SIRT1 or NAMPT (Supplementary Figure 3, E–G, available online) or treatment with two different SIRT1 antagonists (Figure 4, F and G) blunted the effect of Ubc9 DN, indicating that SUMOylation acts through SIRT1 to impact lung cancer cell EMT. Furthermore, the de-SUMOylation enzyme SENP1, but not SENP2, also blocked the migration of lung cancer cells through modulating the competition between Sp1 and HIC1 on the SIRT1 promoter (Supplementary Figure 3, H and I, available online). Together, these observations suggest the existence of a SUMOylation-dependent pathway that regulates SIRT1 expression and lung cancer EMT in response to hypoxia.

PIASy Regulates SIRT1 Expression and Lung Cancer EMT

The last step of a SUMOylation reaction is catalyzed by a family of E3 ligases known as PIAS (26). Hypoxia stimulated the expression of several PIAS proteins (Figure 3, A and B), but only the recruitment of PIASy to the SIRT1 promoter (Figure 5A). In parallel, PIASy, and to a lesser extent PIAS3 and PIASx, enhanced the repression of SIRT1 promoter activity by and SIRT1 promoter binding of WT instead of KR HIC1 (Figure 5, B–D). Moreover, PIASy overexpression, among all PIAS proteins, most significantly suppressed SIRT1 expression (Figure 5, E and F), promoted cancer cell EMT (Figure 5, H and I), and augmented migration of lung cancer cells (Figure 5G; Supplementary Figure 4, available online). On the other hand, treatment with RSV abrogated the effect of PIASy (Figure 5, G–I).

To gain further insight into the role of PIASy in lung cancer EMT in response to hypoxia, endogenous PIASy was depleted by siRNA. Ablation of PIASy, but not PIAS1, PIAS3, or PIASx, restored the expression of SIRT1 in lung cancer cells cultured under 1% O2 (Figure 6, A and B) and stalled cell migration (Supplementary Figure 5A, available online). On the SIRT1 promoter, PIASy elimination up-regulated Sp1 while down-regulating HIC1 binding (Figure 6C). To evaluate whether PIASy affects lung cancer EMT via SIRT1, we again treated the cells with SIRT1 inhibitors (Figure 5, D and E; Supplementary Figure 5E, available online) or knocked down the expression of endogenous SIRT1 (Supplementary Figure 5, B and C, available online) or NAMPT (Supplementary Figure 5D, available online). Indeed, suppression of SIRT1 activity and/or expression completely reversed the phenotype of lung cancer cells in the presence of PIASy siRNA.

Next, we examined the ability of lung cancer cells that lack PIASy to metastasize in vivo. As shown in Figure 6F, lung cancer cells stably expressing PIASy short hairpin RNA were less capable of spreading than the control cells. Injection of either nicotinamide or sirtinol, meanwhile, attenuated the protection conferred by PIASy silencing. Collectively, these data portray PIASy as a determinant of lung cancer cell EMT via SIRT1 repression.

Low SIRT1 Expression and High PIAS Expression Predict an Advanced Stage of Lung Cancer in Human

Finally, we made an attempt to extrapolate our finding that PIASy may be a critical regulator of lung cancer metastasis to human pathology. Examination of human lung cancer specimens, by PCR (Figure 2A) and immunohistochemistry (Figure 2, B and C; P < .001, χ2 test), revealed that an up-regulation of PIASy accompanied the down-regulation of SIRT1 expression as tumor became more malignant. In contrast, PIAS1 expression was unaltered regardless of the stages of cancer development. In conclusion, decreased SIRT1 levels combined with increased PIASy levels may herald a less favorable outcome of lung cancer in human.

Discussion

Advanced stages of lung cancer are accompanied by a gradual loss of epithelial structure and acquisition of mesenchymal characteristics in a process known as EMT (27). In these types of lung tumors, unchecked proliferation and migration instigate a high demand for oxygen, thereby creating a hypoxic niche. Hypoxia promotes lung cancer progression in part by nurturing a program that favors cancer cell EMT (28,29). Our findings, as summarized in Figure 6G, illustrate a SUMOylation-dependent pathway that connects SIRT1 repression to lung cancer metastasis, presenting a novel target for therapeutic intervention against malignant lung cancer.

Hypoxia promotes EMT via a host of transcriptional regulators including HIF1α, HIF2α, and NF-xB with HIF1α and NF-xB evoking and HIF2α neutralizing EMT (30–32). SIRT1 likely influences EMT by differentially modulating the activity of these factors. It has been demonstrated that SIRT1 deacetylases both HIF1α (33) and HIF2α (34); deacetylation by SIRT1 suppresses HIF1α but activates HIF2α. Meanwhile, SIRT1-mediated deacetylation of NF-xB weakens its affinity for target genes (35). Therefore, down-regulation of SIRT1 by hypoxia would lead to enhanced activity of HIF1α and NF-xB and concomitantly diminished activity of HIF2α resulting in accelerated EMT. Alternatively, hypoxia is known to forge conversations with several signaling pathways upstream of the EMT transcriptional program. Notch signaling and Wnt signaling are two key cascades triggered by hypoxic stimulus (25,36). On one hand, SIRT1 represses Notch dependent transcription by deacetylating histone H4K16 (37). On the other hand, SIRT1 mitigates Wnt-initiated EMT by virtue of directly deacetylating β-catenin (38). Thus, SIRT1 can be placed in a network of EMT-related signaling pathways primarily serving as a negative check. Future investigations employing proteomic tools will clarify the detailed maneuverings SIRT1 exerts during lung cancer EMT.

Another major finding of the present study is that a Ubc9-PIASy axis promotes lung cancer EMT in response to hypoxia.
Figure 4. SUMOylation-dependent mechanism regulates SIRT1 expression and epithelial-mesenchymal transition. A) HEK293 cells were transfected with indicated expression constructs followed by exposure to 1% O₂. Chromatin immunoprecipitation (ChIP) assays were performed with indicated antibodies. B) A SIRT1 promoter-luciferase construct (~115/+58) was cotransfected with indicated expression constructs into H1299 cells followed by exposure to 1% O₂. Luciferase activities were expressed as percentage of control. C) HEK293 cells were transfected with indicated expression constructs followed by exposure to 1% O₂. ChIP assays were performed with indicated antibodies. D, E) H1299 cells were transfected with dominant negative (DN) Ubc9 followed by exposure to 1% O₂. Messenger RNA (D) and protein (E) levels of SIRT1 and epithelial and mesenchymal markers were assessed by quantitative polymerase chain reaction (qPCR) and western blotting. F, G) H1299 cells were transfected with DN Ubc9 and treated with nicotinamide (NAM) or sirtinol followed by exposure to 1% O₂. F) Wound healing assay: upper panel, 0h; bottom panel, 48h. G) Expression levels of epithelial and mesenchymal markers were measured by qPCR. Bars represent means from three independent experiments; error bars refer to 95% confidence intervals. DMSO = dimethyl sulfoxide; DN-H = dominant negative-high dose; DN-L = dominant negative-low dose; DN-M = dominant negative-medium dose; EV = empty vector; IP = immunoprecipitation; KR = lysine-to-arginine mutant; WT = wild type.
It is noteworthy that SUMOylation has been indicated in distinct aspects of carcinogenesis. For example, Ubc9-mediated SUMOylation of pontin, a coactivator for β-catenin, promotes proliferation in prostate cancer cells (39). Ubc9 has also been demonstrated to antagonize a handful of tumor suppressors, either directly or indirectly, including p53 (40), WT1 (41), and VHL (42). Accordingly, high expression levels of Ubc9 have been noted in advanced-stage melanoma (43), malignant head and neck...
carcinoma (44), chemoresistant breast cancer (45), acute myeloid leukemia (46), and hepatocellular carcinoma (47). Paradoxically, evidence has been presented that Ubc9 is capable of engaging a BRCA1-containing complex in double-stranded DNA break repair in response to genotoxic stress (48). Furthermore, Zhang et al have reported that Ubc9 mediates arsenic oxide induced degradation of the PML-RARα oncoprotein, essentially arguing for a tumor-suppressing role of Ubc9 (49). Thus, although SUMOylation is involved in biologically conserved processes intimately related to carcinogenesis, these conflicting observations likely reflect the delicate nature of this process and suggest that its precise role has to be dissected and analyzed in a tissue- and event-specific manner.

Figure 6. PIASy depletion restores SIRT1 expression and ameliorates epithelial-to-mesenchymal transition. A-C) H1299 cells were transfected with indicated PIAS-targeting small interfering RNA (siRNA) or scrambled siRNA (SCR) followed by exposure to 1% O₂. Messenger RNA (A) and protein (B) levels of SIRT1 were assessed by quantitative polymerase chain reaction (qPCR) and western blotting. C) Chromatin immunoprecipitation (ChIP) assays were performed with anti-Sp1 or anti-HIC1. D-E) H1299 cells were transfected with siRNA targeting PIASy or SCR and treated with nicotinamide (NAM) or sirtinol followed by exposure to 1% O₂. D) Wound healing assay: upper panel, 0h; bottom panel, 48h. Messenger RNA (E) levels of epithelial/mesenchymal signature genes were measured by qPCR. Bars represent means from three independent experiments; error bars refer to 95% confidence intervals. F) In vivo metastasis assay was performed in nude mice (n = 10 for each group) using A549 cells stably expressing short-hairpin RNA targeting PIASy or SCR. G) A model depicting that SIRT1 down-regulation by hypoxia in a SUMOylation-dependent manner promotes epithelial-mesenchymal transition and eventually leads to cancer metastasis. DMSO, dimethyl sulfoxide; IP = immunoprecipitation.
Germline deletion of PIAS1 (50), PIASx (51), and PIASy (52) has been created in mice with the revelation that these mice all appeared normal in terms of gross histology after birth suggesting that individually these proteins play nonessential roles during embryogenesis. There remains a controversy, however, regarding the redundancy among members of the PIAS family in regulating distinct pathobiological processes in adult life. PIAS1 is suggested as the potential driving force behind transforming growth factor β-induced EMT in mammary epithelial cells (53). Increased expression of PIAS1 is also associated with worsened survival in patients with myeloma (54). In the meantime, PIAS3 has been shown to stimulate lamellipodia, cell migration, and invasion by SUMOylating the GTPase Rac1 (55). We provide solid evidence here that PIASy is the only SUMO E3 ligase that regulates lung cancer EMT by repressing SIRT1 transcription. Unlike PIAS1, PIASy expression is specifically up-regulated in patients with more-aggressive types of lung cancer (Figure 2). It is possible that a specific PIAS protein may steer distinct transcriptional programs in a spatiotemporally controlled manner. Further effort to unveil this mystery by using tissue-specific animal models is warranted.

Lung cancer metastasis is a multifaceted process to which SIRT contributes by fine-tuning EMT. There exist alternative explanations underlying weakened lung cancer cell migration in vitro and in vivo following SIRT1 activation. Accumulation of proinflammatory mediators can promote lung cancer metastasis (56). SIRT1 is widely believed to exert potent anti-inflammatory effects by suppressing the master regulators of proinflammatory transcription, NF-κB (35) and AP-1 (57). On the other hand, the Warburg effect dictates that malignant cancer cells favor glycolysis over oxidative phosphorylation for energy, alluding to a connection between metabolism and cancer metastasis (58). Mounting evidence has placed SIRT1 as a critical node within the metabolic network, preferentially promoting aerobic metabolism by stimulating mitochondrial biogenesis and fatty acid oxidation (59). Thus, SIRT1 activation in lung cancer cells may rewire the metabolic machinery and deprive these cells of energy resources, putting a hold on metastasis. A comprehensive profiling of gene expression in lung cancer will likely uncover additional SIRT1 targets and provide mechanistic insight for SIRT1-dependent regulation of lung cancer metastasis.

A major limitation of the current study is the lack of rigorous testing of the in vitro model in a large population in humans. Recently, Tseng et al have reported that elevated SIRT1 expression along with decreased HIC1 expression/p53 acetylation heralds a poorer prognosis in a cohort of patients with lung cancer (60). In addition, several independent investigations have implicated SIRT1 as a promoter, rather than an inhibitor, of EMT in different cancer cells (61–63). In contrast, a number of recent reports have implicated SIRT1 as an important antagonist of EMT in different settings (64–67). Therefore, although the current study has contributed to the mechanistic understanding of SIRT1 in lung cancer, the issue as to how SIRT1 precisely regulates EMT in the process of metastasis is unlikely to be settled any time soon. More rigorous examinations using tissue-specific animal models and a much larger cohort of patients are warranted before any of the current conclusions can be extrapolated to real-world applications.

In summary, our findings reinforce the notion that SIRT1 plays essential roles in regulating EMT. As such, targeting Ub9-PIASy may bring up SIRT1 expression may yield novel therapeutic solutions against aggressive lung cancer.

References


Funding

National Basic Science Research “973” Program of China (2012CB517503, 2011CB910604, 2012CB822104); National Natural Science Foundation of China (30730044, 30870230, 31070721, and 81172879); Program for New Century Excellent Talents in University of China (NCET-11-0991); Science and Technology Administration of Jiangsu Province (BK2012043); Priority Academic Program Development of Jiangsu Higher Education Institutions.
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