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# Putative tumor suppression function of SIRT6 in endometrial cancer

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ABSTRACT

**SIRT6, a member of the sirtuin family, has been identified as a candidate tumor suppressor. To pursue the role of SIRT6 in endometrial cancer, we investigated the anti-tumorigenic function of SIRT6. The expression of SIRT6 negatively affected the proliferation of AN3CA and KLE endometrial cancer cells. Increased expression of SIRT6 resulted in the induction of apoptosis by repressing the expression of the anti-apoptotic protein survivin. Consistent with this result, a survivin inhibitor YM155 efficiently inhibited cellular proliferation and induced apoptosis. These results revealed that SIRT6 might function as a tumor suppressor of endometrial cancer cells.**


## 1. Introduction

SIRT6 is a member of the sirtuin family, which is a family of mammalian homologs of yeast silent information regulator 2 (Sir2) that encode a class III histone deacetylase [1]. SIRT6 localizes in the nucleus and interacts with many tumor-promoting genes, including c-MYC, HIF1α, NF-κB and TNFα [2–5]. It is considered to work as a tumor suppressor by reversing the Warburg effect, which is synonymous with aerobic glycolysis [2]. Knockout of SIRT6 induced intestinal tumors and significantly shortened the lifespan in mice [6], whereas SIRT6 transgenic male mice had a longer lifespan, partially due to the inhibition of tumorigenesis [7]. SIRT6 also participates in telomere maintenance [8,9], double strand break repair [10–12] and suppression of L1 retrotransposons [13]. These findings suggest that SIRT6 is an attractive therapeutic target of cancer.

Endometrial cancer is the most common gynecologic malignancy, the incidence of which is increasing worldwide [14]. A strong relationship exists between endometrial cancer and metabolism. Diabetes mellitus (DM) and obesity have a 1.8- and 1.5-fold relative risk of endometrial cancer, respectively [15,16]. These factors trigger insulin resistance, which contributes to the development of endometrial cancer [17]. In this regard, metformin, a synthetically derived biguanide that is widely employed in the treatment of type 2 DM, is a promising anti-cancer agent for endometrial cancer [18]. Metformin inhibits the PI3K (phosphatidylinositol-3 kinase)–mTOR (mammalian target of rapamycin) pathway through AMP-activated protein kinase (AMPK) activation. Endometrial cancer has frequent mutations at PI3K or PTEN, and the PI3K–mTOR pathway is thus often activated [19,20]. Therefore, the direct inhibition of this pathway by an mTOR inhibitor might be a rationale treatment option. Among

*Abbreviations:* AMPK, AMP-activated protein kinase; DM, diabetes mellitus; EIC, endometrial epithelial immortalized cells; ERK, extracellular signal-regulated kinase; IGF1R, insulin-like growth factor 1 receptor

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many clinical trials of endometrial cancer that employed molecular-targeted agents, mTOR inhibitors such as temsirolimus and ridaforolimus are the most promising [21,22]. However, the response rates were no more than 30%. Some reports indicated the feedback activation of insulin-like growth factor 1 receptor (IGF1R) and/or Akt by mTOR inhibition [23,24]; hence, the modulation of upstream factors might be effective for the treatment of endometrial cancer.

To date, no obvious correlation between SIRT6 and endometrial cancer has been demonstrated. According to the microarray analysis of the Cancer Genome Atlas of endometrial cancer specimens, the decreased expression of SIRT1 mRNA was observed in one case (0.4%), whereas the decreased expression of SIRT6 mRNA without PTEN mRNA attenuation was observed in five cases (2.1%) [25]. SIRT6 had a protective role against heart failure through inhibition of the IGF1R–AKT–mTOR pathway [26]. Considering the frequent activation of the PI3K–mTOR pathway in endometrial cancer, SIRT6 has the potential to inhibit endometrial cancer progression.

SIRT6 has been shown to induce apoptosis in several types of human cancer. However, the precise mechanism of how SIRT6 induces apoptosis remains unclear. SIRT6 overexpression induced apoptosis through p53 and p73 activation in some cancer cells [27], whereas it did so via extracellular signal-regulated kinase (ERK) inhibition in hepatocellular carcinoma cells [28].

The purpose of this study was to determine the roles of SIRT6 in endometrial cancer cells, including the relationship between SIRT6 and survivin. Such knowledge might prove useful as a new treatment strategy for endometrial cancer.

## 2. Materials and methods

### 2.1. Chemicals and antibodies

YM155 was purchased from Cayman Chemical (Ann Arbor, MI, USA). Mouse monoclonal antibodies were anti-p53 (DO-1, Santa Cruz Biotechnology, Dallas, TX, USA), anti-Bcl-2 (100, Santa Cruz Biotechnology) and anti-β-actin (Sigma–Aldrich, St. Louis, MO, USA). Rabbit monoclonal antibodies were anti-SIRT1 (ab32441, Abcam, Cambridge, UK), anti-SIRT6 (2590), anti-cleaved PARP (9544), anti-phospho-Akt (p-Akt, Ser473) (9271), anti-phospho-p44/42 MAPK (Erk1/2) (pERK) (9101, Cell Signalling technology, Danvers, MA, USA) and anti-Survivin (NB500-201, Novus Biologicals, Littleton, CO, USA). A rabbit polyclonal antibody was anti-Bax (P-19, Novus Biologicals).

### 2.2. Cell culture

We utilized 16 endometrial cancer cell lines as previously described [29]. Ishikawa cells were generous gifts from Dr. Masato Nishida (National Hospital Organization Kasumigaura Medical Center, Ibaraki, Japan). The other 10 cell lines were established by Hiroyuki Kuramoto [30]. Endometrial epithelial immortalized cells (EIC) were also gifts from Satoshi Kyo (Kanazawa University, Ishikawa, Japan) [31]. AN3CA, KLE cells and EIC were maintained in DMEM with 10% FBS at 37 °C in a humidified 5% $CO_2$ incubator.

### 2.3. Western blot analysis

Cells were harvested, and soluble protein was extracted as previously described [32], followed by immunoblotting utilizing the indicated antibodies. Signals were detected using BioRad western blotting systems (BioRad, Hercules, CA, USA) with the ECL select detection regent (GE Healthcare, Wauwatosa, WI, USA).

### 2.4. Gene silencing and transient transfection

Cells were incubated for 24 h before gene silencing with Stealth RNAi small interfering RNA (siRNA) for SIRT6 and BIRC5 (survivin) (Invitrogen, Carlsbad, CA, USA), using Lipofectamine RNAiMAX (Invitrogen). A negative control kit was employed as a control (Invitrogen). Flag-tagged wild-type SIRT6 expression plasmid was purchased from Addgene (Cambridge, MA, UK) and transfected into AN3CA and KLE cells using Effectene transfection reagent (Qiagen, Valencia, CA, USA). pcDNA 3.0 (Invitrogen) was employed as a control.

### 2.5. Trypan blue dye exclusion test with reverse transfection

Utilizing 6-well plates, AN3CA and KLE cells were plated at $3 \times 10^5$ cells per well with the indicated plasmids and siRNAs using the Lipofectamine2000 (Invitrogen) reverse transfection protocol. Cells were treated for 24 and 48 h after plating. Then, cells were harvested and counted under microscopy after trypan blue staining. These experiments were repeated three times.

### 2.6. Cell cycle analysis

Cells were seeded in 60-mm dishes and incubated for 48 h after plasmid transfection. Both floating and adherent cells were harvested using trypsin and washed twice with phosphate buffer saline (PBS). After resuspension and incubation in ice-cold 70% ethanol, cells were washed twice with PBS again and incubated in RNaseA (0.25 mg/ml) for 1 h at 37 °C, followed by staining with propidium iodide (PI) (50 μg/ml) (Sigma–Aldrich) for 30 min at 4 °C in the dark. Thereafter, cells were analyzed by flow cytometry (BD FACS Calibur HG, Franklin Lakes, NJ, USA). Utilizing the CELL Quest pro software, ver.3.1. (Beckman Coulter, Epics XL, Brea, CA, USA), cell cycle analysis was performed. These experiments were repeated three times.

### 2.7. Detection of apoptosis by annexin-V and PI double staining

Cells were seeded in 60-mm dishes for 24 h before treatment with the indicated plasmids, siRNAs and YM155, for an additional 48 h. After trypsinization, cells were harvested, washed twice with PBS, and counted. These cells were stained with annexin-V fluorescein isothiocyanate (FITC) and PI, as recommended by the manufacturers (FITC AnnexinV Apoptosis Detection Kit I, BD), and the apoptotic cell population was determined by the percentage of a double positive fraction utilizing the previously described flow cytometer. These experiments were performed three times.

### 2.8. Luciferase reporter assay

Survivin promoter activity was analyzed using a luciferase reporter assay. The luciferase expression plasmid under the control of the survivin promoter (pSRVN-Luc) was the kind gift of Chu-Xia Deng (National Institutes of Health, Bethesda, MD, USA) [33,34]. Transfection was performed with Effectene reagent (Qiagen) according to the manufacturer's recommendation. As an internal control to equalize transfection efficiency, phRL CMV-Luc vector (Promega Co., Fitchburg, WI, USA) was also transfected in all of the experiments. Individual transfections, each consisting of duplicate wells, were repeated at least three times.

### 2.9. RNA extraction and real-time PCR

Total RNAs were extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instruction. cDNAs were synthesized from total RNAs by using the ReverTra Ace α (TOYOBO,

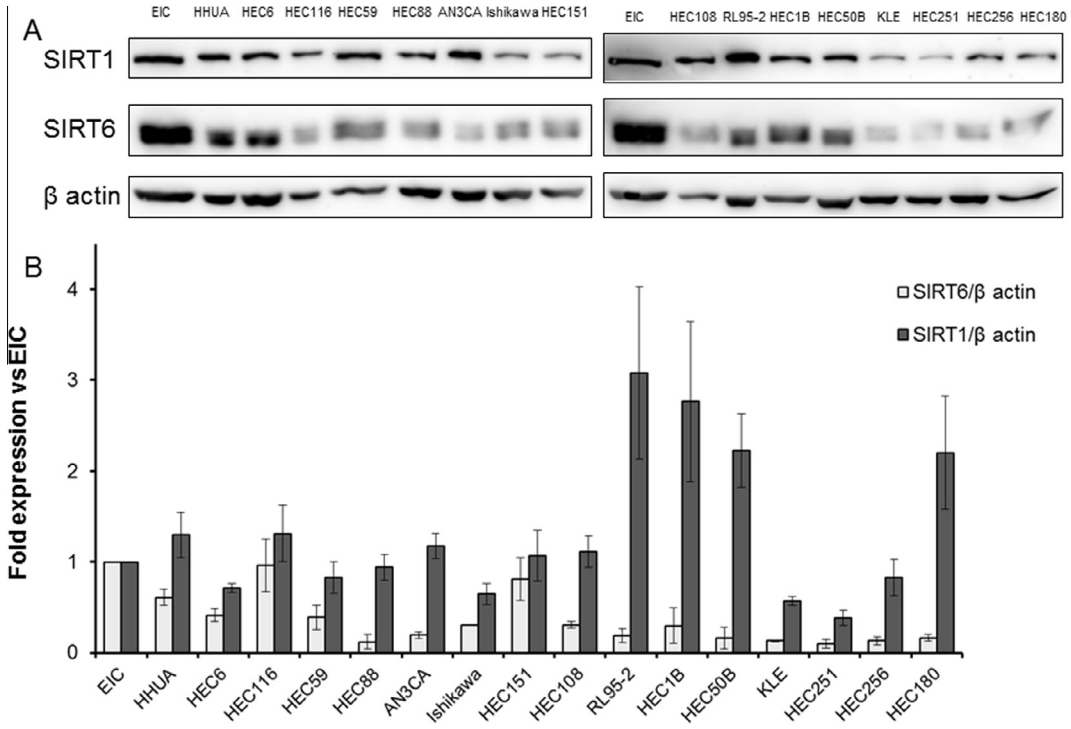


**Fig. 1.** SIRT6 protein expression is more attenuated in endometrial cancer cells than in endometrial epithelial immortalized cells. Immunoblot analysis of SIRT1 and SIRT6 (A) in EIC (endometrial epithelial immortalized cells) and 16 endometrial cancer cell lines. Relative protein expression of SIRT1 and SIRT6 compared with EIC was quantified in 16 endometrial cancer cells by Image J software (B). β Actin was used as an internal control. The results are shown as the mean ± SE of three independent experiments.

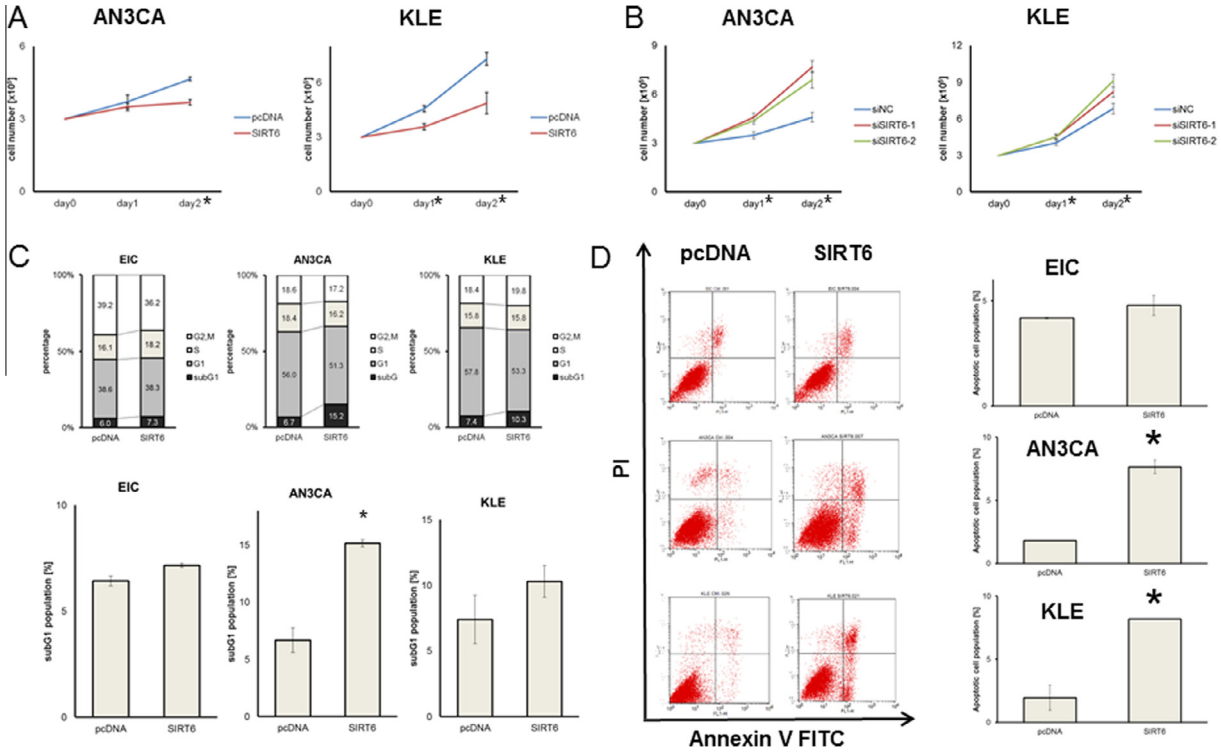


**Fig. 2.** SIRT6 suppresses endometrial cancer cell proliferation by apoptosis induction. (A) Cell number count with SIRT6 expression plasmid transfection in AN3CA (left panel) and KLE (right panel) endometrial cancer cells. Empty pcDNA vector (pcDNA) was utilized as a control. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. pcDNA. (B) Cell number count with SIRT6 knockdown in AN3CA (left panel) and KLE (right panel) cells. Two siRNAs (siSIRT6-1, siSIRT6-2) and a negative control (siNC) were employed for this assay. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. siNC. (C) Cell cycle analysis with SIRT6 overexpression in EIC (upper left), AN3CA (upper middle) and KLE (upper right) cells. pcDNA was utilized as a control. Lower panels show the percentage of subG1 population in EIC (left), AN3CA (middle) and KLE (right), respectively. These results are shown as the mean (±SE) of three independent experiments. $^{*}P < 0.05$ vs. pcDNA. (D) AnnexinV-PI double staining with SIRT6 overexpression. Three independent experiments were performed, employing pcDNA as a control. The left panel represents one of the three experiments of EIC (upper), AN3CA (middle) and KLE (lower) cells. The right panel presents the percentage of the double positive population in the EIC (upper), AN3CA (middle) and KLE (lower) cells. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. pcDNA.

Osaka, Japan). Employing the ABI7300 (Applied Biosystems, San Mateo, CA, USA), real-time RT-PCR fluorescence detection was performed in 96-well plates with the SYBR Green PCR Master Mix (Applied Biosystems). Primers for BIRC5 (survivin) were TGCTT CAAGGAGCTGGAAGG (forward) and AGAAGCACCTCTGGTGCCAC (reverse). Primers for GAPDH were previously described [35]. The threshold cycle number (Ct) for each sample was determined in triplicate. The Ct values for BIRC5 were normalized against GAPDH.

### 2.10. MTT assay

Employing 96-well plates, 3000 cells were seeded per well on DMEM and treated with increasing doses (6.25–10,000 nM) of YM155 for 72 h, beginning 24 h after seeding. After applying 10 μl of the Cell Counting Kit-8 using the tetrazolium salt WST-8 (Dojindo, Kumamoto, Japan), the absorbance at 450 nm was monitored by a microplate reader (BioTek, Winooski, VT, USA). Proliferation was normalized relative to the absorbance of cell cultures treated with dimethyl sulfoxide (DMSO) alone.

### 2.11. Statistical analysis

Data represent the mean ± SE from at least three independent determinations. The significance of differences between more than three samples was analyzed by one-way ANOVA and post hoc test, whereas the significance between two samples was analyzed by the Mann–Whitney *U* test using the GraphPad Prism software ver. 6.0 (GraphPad Software, San Diego, CA, USA), and a *P* value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. SIRT6 protein expression is lower in endometrial cancer cells than in EIC

To assess the expression level of SIRT6 protein, we conducted immunoblotting with EIC and 16 endometrial cancer cell lines. There was an absence of a pattern in SIRT1 protein expression, whereas SIRT6 protein expression was uniformly lower in endometrial cancer cells than in EIC (Fig. 1). In addition, we performed an immunohistochemistry analysis of tissue microarrays from 104 endometrial endometrioid adenocarcinoma patients. The immunohistochemical findings revealed that low SIRT6 nuclear staining had relevance with worse overall survival (Fig. S1). However, no statistical significance was found. These data suggest that SIRT6 attenuation is relevant to endometrial carcinogenesis.

### 3.2. SIRT6 inhibits endometrial cancer cell growth by apoptosis induction

To investigate the function of SIRT6 in endometrial cancer cells, we selected AN3CA and KLE cells, which had lower levels of SIRT6 protein expression (Fig. 1). First, we performed a cell number count with both SIRT6 overexpression and SIRT6 knockdown. Exogenous

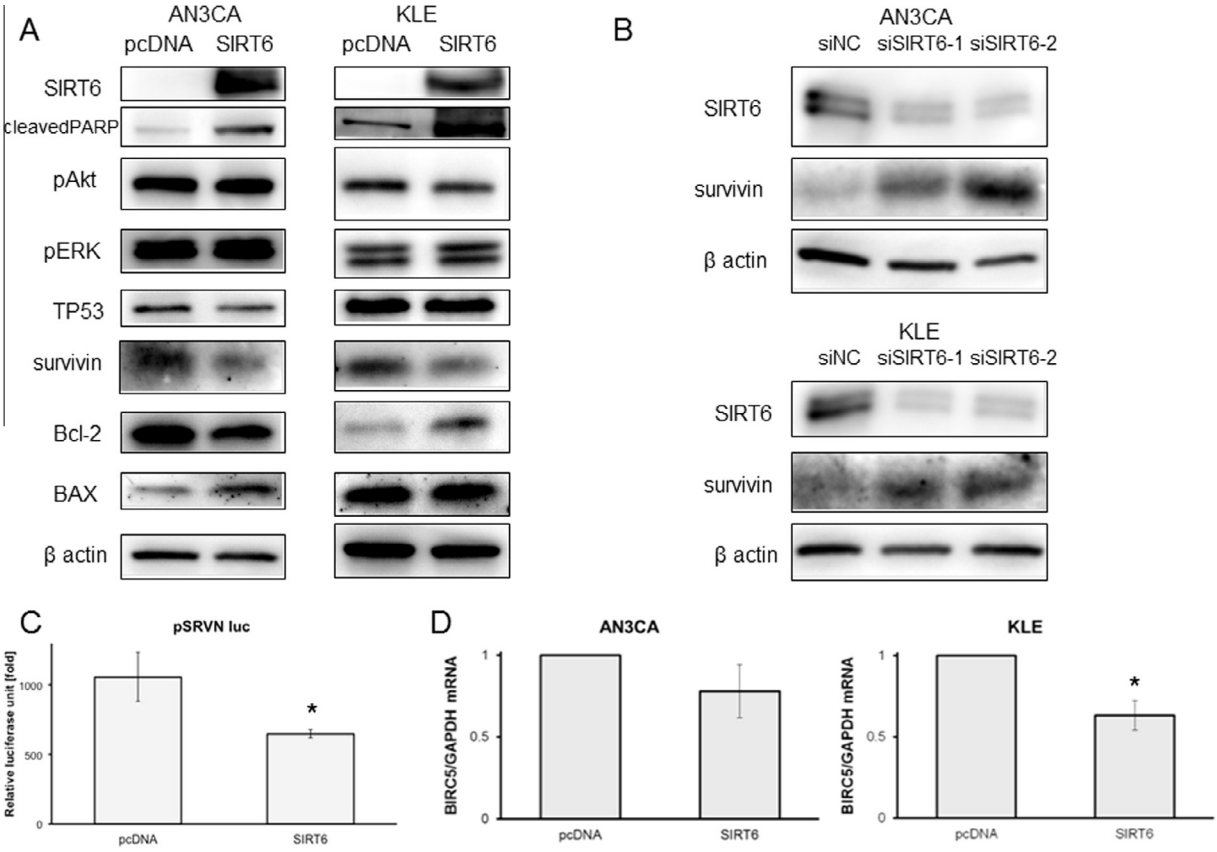


**Fig. 3.** SIRT6 induces apoptosis to endometrial cancer cells by repressing survivin. (A) Immunoblot analysis of apoptosis-related proteins with SIRT6 overexpression compared with empty vector (pcDNA) in the AN3CA (left panel) and KLE (right panel) cells. β Actin was employed as a loading control. (B) Immunoblot analysis of SIRT6 and survivin with SIRT6 knockdown in the AN3CA (upper panel) and KLE (lower panel) cells. Two siRNAs (siSIRT6-1, siSIRT6-2) and a negative control (siNC) were identical with Fig. 2B. β Actin was utilized as a loading control. (C) Luciferase reporter assay of survivin promoter with pcDNA and SIRT6 expression plasmid in AN3CA cells. Relative luciferase activities were assessed by pSRVN-Luc/phRL CMV-Luc activity. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. pcDNA. (D) Real-time PCR analysis of BIRC5 (survivin) mRNA levels after SIRT6 overexpression in the AN3CA (left panel) and KLE (right panel) cells. BIRC5 mRNA levels were normalized by GAPDH mRNA levels. These results are shown as the mean ± SE of triplicate samples. $^{*}P < 0.05$ vs. pcDNA.

SIRT6 expression inhibited cell growth in both cell lines (Fig. 2A). In contrast, SIRT6 knockdown accelerated cell growth in both cells (Fig. 2B). We also performed cell cycle analysis to elucidate whether growth inhibition by SIRT6 was attributed to cell cycle arrest or cell death. Cell cycle analysis demonstrated that SIRT6 caused a significant and slight increase in the sub-G1 population in the AN3CA and KLE cells, respectively (Fig. 2C). In addition, AnnexinV-PI double staining demonstrated a significant accumulation of double positive populations in both cells (Fig. 2D). In contrast, SIRT6 induced neither the sub-G1 increase nor the accumulation of AnnexinV-PI double positive populations in EIC (Fig. 2C and D). These results indicate that SIRT6 specifically inhibits the growth of endometrial cancer cells with apoptosis induction.

### 3.3. SIRT6 induces apoptosis to endometrial cancer cells by repressing survivin

To examine the detailed mechanism of apoptosis induced by SIRT6, we performed immunoblotting of apoptosis-related proteins in the AN3CA and KLE cells (Fig. 3A). PARP cleavage, which is another marker for apoptosis, was confirmed in both cells based on SIRT6 overexpression. Although there existed no constant alteration of pAkt, pERK, p53, Bax and Bcl-2 in these cells, SIRT6 clearly inhibited survivin protein expression in both cells. Inversely, SIRT6 knockdown clearly enhanced the survivin protein expression in both cells (Fig. 3B). We also conducted luciferase reporter assay to confirm whether survivin inhibition by SIRT6 was attributed to transcriptional suppression or post-transcriptional modification. SIRT6 significantly inhibited the pSRVN luciferase activity in the AN3CA cells (Fig. 3C), which meant that SIRT6 repressed the transcription of survivin. As expected, SIRT6 also inhibited BIRC5 (survivin) mRNA expression in both cells (Fig. 3D). These results suggest that SIRT6 induced apoptosis to endometrial cancer cells through survivin repression.

### 3.4. Survivin knockdown selectively induces apoptosis only in endometrial cancer cells

Firstly we assessed the expression level of survivin protein. Immunoblotting revealed uniformly higher SIRT6 protein expression in most of 16 endometrial cancer cells than in EIC (Fig. S2). To confirm the involvement of survivin inhibition in apoptosis induced by SIRT6, we conducted a genetic inhibition of BIRC5 (survivin) using two siRNAs in AN3CA and KLE cells and EIC. Survivin knockdown was confirmed by immunoblotting. Two siRNAs significantly inhibited the survivin protein expression in all three cell types (Fig. 4A). Survivin knockdown induced an accumulation of cleaved PARP in the AN3CA and KLE cells, whereas cleaved PARP was not detected in EIC (Fig. 4A). Additionally, AnnexinV-PI double staining presented a significant accumulation of double positive

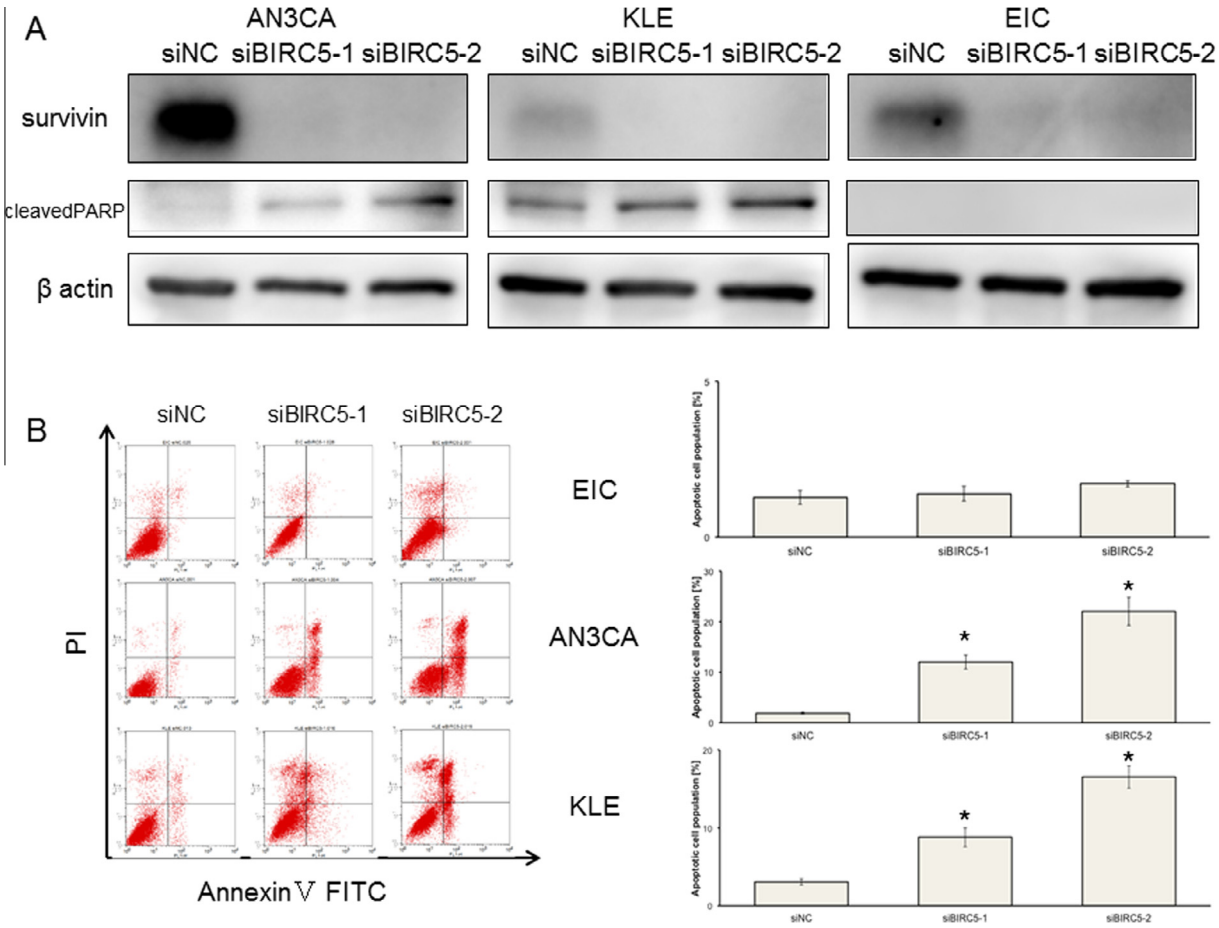


**Fig. 4.** Survivin knockdown selectively induces apoptosis only in endometrial cancer cells. (A) Immunoblot analysis of survivin and cleaved PARP with survivin knockdown in AN3CA (left) and KLE cells (middle) and EIC (right). Two siRNAs for survivin (siBIRC5-1, siBIRC5-2) and a negative control (siNC) were employed for this assay. β Actin was loaded as a internal control. (B) AnnexinV-PI double staining with BIRC5 (survivin) knockdown in AN3CA and KLE cells and EIC. Two siRNAs (siBIRC5-1, siBIRC5-2) and a negative control (siNC) were the same as in Fig. 4A. Three independent experiments were performed. The left panel represents one of the three experiments of EIC (upper), AN3CA (middle) and KLE (lower) cells. The right panel shows the percentage of the double positive population in EIC (upper), AN3CA (middle) and KLE (lower) cells. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. siNC.

populations only in endometrial cancer cells (Fig. 4B). These data demonstrate that survivin inhibition selectively induces apoptosis only in endometrial cancer cells.

### 3.5. Pharmacologic inhibition of survivin by YM155 also induces apoptosis in endometrial cancer cells

Next, we conducted a pharmacologic inhibition of survivin by YM155, which is a clinically used survivin inhibitor [36–38]. YM155 clearly induced cell shrinkage in AN3CA and KLE cells (Fig. 3A). To assess the growth inhibitory effect of YM155, we performed MTT assay using these two cells. YM155 attenuated the proliferation of these cells in a dose-dependent manner, according to the microscopic findings (Fig. 5B). The half-maximal inhibitory concentration ($IC_{50}$) values of AN3CA and KLE cells were 25 and 250 nM, respectively. The inhibition of survivin protein expression by YM155 was confirmed by immunoblotting (Fig. 5C). Finally, AnnexinV-PI double staining showed an induction of apoptosis by YM155 in both cells (Fig. 5D). These data demonstrate that YM155 suppresses endometrial cancer cell growth through apoptosis induction.

## 4. Discussion

It has already been reported that SIRT6 induces apoptosis in some cancer cells, such as brain, breast, uterine cervix and liver [27,28]. However, to our knowledge, this is the first report to reveal the tumor suppressive function of SIRT6 in endometrial cancer cells.

Unlike SIRT1, SIRT6 protein expression was uniformly attenuated in endometrial cancer cells. SIRT1 primarily localizes in the nucleus, similar to SIRT6 [1]. SIRT1 has bifurcated roles in tumor progression. It inhibits tumor formation by improving genomic stability [39], but it induces tumor progression by promoting genomic instability, partially by inhibiting p53 through its deacetylation [40]. SIRT6 has common interacting partners with SIRT1, such as c-MYC, HIF1α, and NF-κB [2–4]. However, in contrast to SIRT1, SIRT6 does not have the function of p53 deacetylation [1]. Considering its attenuation in endometrial cancer cells, SIRT6 is a more appropriate therapeutic target than SIRT1.

In our study, SIRT6 suppressed the proliferation of endometrial cancer cells by inducing apoptosis. AN3CA and KLE cells are p53 mutants [41]. Therefore, the apoptosis induced by SIRT6 was p53 independent. Unlike the case of cardiomyocytes [26], no significant Akt inhibition was observed in these cells. Instead, survivin protein expression was clearly inhibited in both cells. The SIRT6-dependent inhibition of survivin was reported to markedly impair liver cancer development, and immunohistochemistry demonstrated that SIRT6 expression was significantly attenuated in hepatocellular carcinoma patients [42]. Thus, there could be an overlap between hepatic and endometrial carcinogenesis. For instance, current oral contraceptive use has been associated with a modest increased risk of liver cancer [43]. In AN3CA cells, survivin inhibition by SIRT6 was more evident at the protein level than at the mRNA level, despite the significant suppression of survivin promoter activity. This result implies that SIRT6 might also inhibit survivin via post-transcriptional modification.

Survivin is a member of the family of inhibitors of apoptosis proteins [44]. It inhibits apoptosis through the direct inactivation of caspase-9 and the stabilization of X-linked IAP [45,46]. Survivin inhibition by siRNA was already reported to induce apoptosis in Ishikawa endometrial cancer cells [47]. Our data support

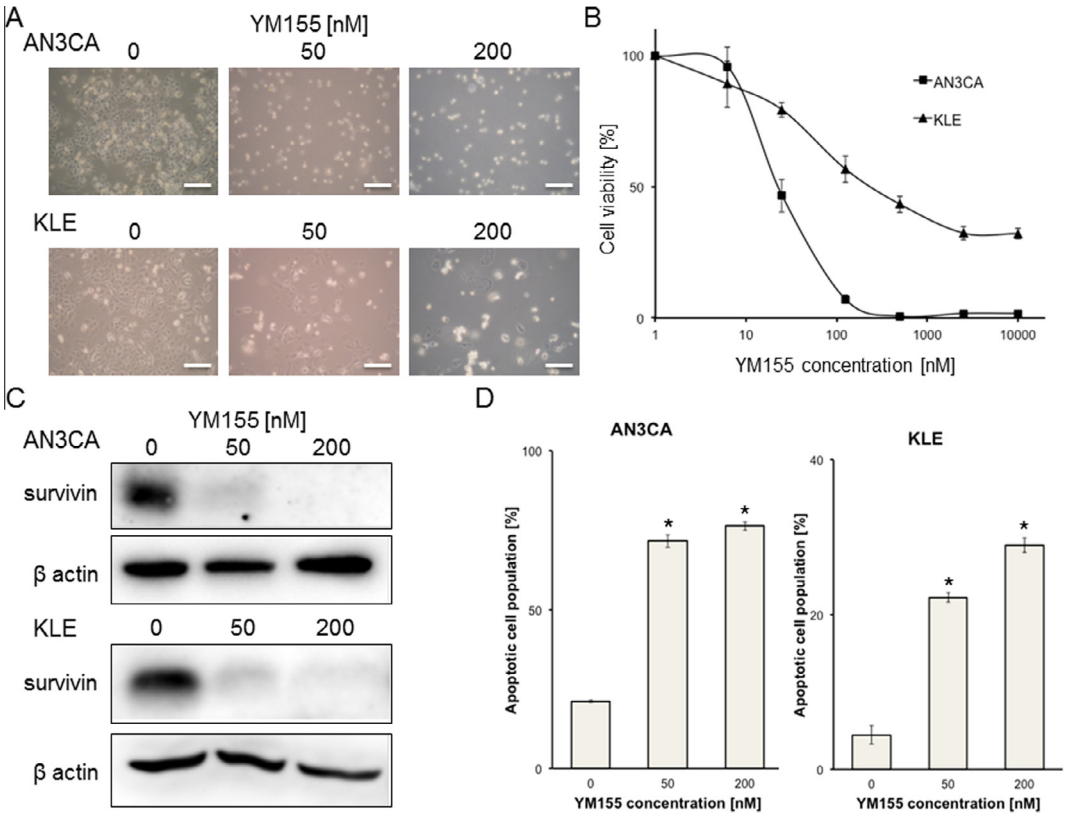


**Fig. 5.** Pharmacologic inhibition of survivin by YM155 induces apoptosis in endometrial cancer cells. (A) Microscopic findings with YM155 treatment for 72 h in AN3CA (upper) and KLE (lower) cells. The three different concentrations used were 0 (DMSO alone), 50 and 200 nM. Scale bar = 100 μM. (B) MTT assay of YM155 for 72 h in the AN3CA and KLE cells. YM155 concentration ranged from 6.25 to 10,000 nM. The results are shown as the mean ± SE of four samples. (C) Immunoblot analysis of survivin with YM155 treatment for 72 h in the AN3CA (upper) and KLE (lower) cells. The concentrations were the same as in Fig. 5A. β Actin was employed as an internal control. (D) AnnexinV-PI double staining with YM155 treatment for 48 h in the AN3CA (left panel) and KLE (right panel) cells. The concentrations were the same as in Fig. 5A. Both panels present the percentage of the double positive cell population. The results are shown as the mean ± SE of three independent experiments. $^{*}P < 0.05$ vs. 0 nM (DMSO alone).

this report and demonstrate that survivin inhibition selectively triggers apoptosis only in cancer cell lines, regardless of p53 status. In addition, survivin protein expression was uniformly upregulated in endometrial cancer cells. Although most chemotherapeutic agents have adverse effects on normal cells and tissues, survivin inhibitor could avoid these effects.

Among several survivin inhibitors, YM155 is a very successful drug. It was selected via a high-throughput screening assay with a survivin-promoter luciferase assay [48]. It inhibited the proliferation of various cancer cell lines [49]. In addition, phase II clinical trials were performed in melanoma, prostate cancer and non-Hodgkin's lymphoma patients [36,37,50]. In our research, YM155 also induced massive apoptosis in endometrial cancer cells and subsequently suppressed cellular proliferation. Although the suppression of survivin protein expression was in the same range following treatment with both YM155 and siBIRC5, the apoptotic cell population was significantly higher in YM155 than in siBIRC5. Thus, YM155 might function as more than a survivin inhibitor. Recent reports have demonstrated an upregulation of death receptor 5 (DR5) and a downregulation of Mcl-1 by YM155 [51,52]. Therefore, further investigation will be needed to clarify the mechanism of YM155-inducible apoptosis in endometrial cancer cells.

Our study has some limitations. First, SIRT6 attenuation was confirmed in the cell lines. However, the immunohistochemical findings did not show a significant difference in overall survival according to nuclear SIRT6 expression. The prognosis of endometrial cancer patients is relatively good, and we therefore need to assess more patients to determine statistical significance. Survivin upregulation has been reported in endometrial cancer specimens [53,54], whereas only one report has focused on SIRT6 expression in endometrial cancer patients [55]. The latter report presented higher SIRT6 expression compared with the normal endometrium levels based on real-time RT-PCR, in conflict with our data. Our data demonstrated the attenuation of SIRT6 protein expression. Therefore, this discrepancy might result from the post-transcriptional modification of SIRT6. Second, we focused on the SIRT6-dependent inhibition of survivin to explain the mechanism of apoptosis induction. However, Bax activation and Bcl-2 inactivation coexisted in AN3CA cells, and these effects thus also contribute to apoptosis induction by SIRT6. In contrast to the AN3CA cells, these effects were not observed in KLE cells, and the apoptosis induced by SIRT6 might thus be cell type dependent. Finally, our data suggest that SIRT6 is a potent tumor suppressor in endometrial cancer. However, there exist no specific SIRT6 activators that are available in the clinic. In addition, a detailed regulatory mechanism of SIRT6 has not shown more than ubiquitination [56]. Therefore, unveiling the mechanism would be helpful for identifying a specific SIRT6 activator.

In this study, we clarify the tumor suppressive role of SIRT6 and the efficacy of YM155 in endometrial cancer cells. Our findings may shed light on endometrial carcinogenesis. Given that YM155 is a clinically used drug, it could be a new therapeutic option for endometrial cancer.

## Conflict of interest statement

These authors have no conflict of interest to declare.

## Role of the funding source

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.06.043.

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