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Aberrant survivin expression in endometrial hyperplasia: another mechanism of progestin resistance

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Up to 30% of failure rate in endometrial hyperplasia patients treated by progestin urges more detailed understanding of the mechanisms involved in progestin resistance. Survivin is a key regulator in the antiapoptotic network, and overexpression of survivin has been reported in endometrial hyperplasia and cancer. This study investigated the role of survivin in progestin resistance in endometrial hyperplasia. Pre- and post-treatment endometrial hyperplasia tissue samples from 23 women were examined for changes in survivin expression related to the administration of progestins. The impact of continuous or intermittent progestin treatment on survivin expression in Ishikawa cells was examined by the western blot. Survivin immunoreactivity was present in epithelial compartment of all pre-progestin-treated endometrial hyperplasia samples with mean nuclear indices 78 and cytoplasmic indices 114. In the 15 progestin responders, an average of 19.5-fold decrease of survivin expression was seen in epithelial nuclei ($P<0.001$) and 8-fold decrease in epithelial cytoplasm ($P<0.001$). In the eight non-responders, no significant changes in survivin expression were detected. With *in vitro* Ishikawa cells, survivin expression was effectively inhibited by either 72-h continuous treatment with 10 μM medroxyprogesterone acetate or 72 h after medroxyprogesterone acetate withdrawal. Our results indicated that dysregulation of survivin expression in hyperplastic endometrium may be part of the molecular mechanisms for progestin resistance. Intermittent, rather than continuous, progestin treatment may be more effective clinically for the treatment of endometrial hyperplasia.
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Endometrial hyperplasia is encountered most frequently in the perimenopausal period, when the balance of estrogen and progesterone in normal menstrual cycle is perturbed. It can also occur, however, in young women and teenagers, in whom anovulatory cycles are common. Endometrial hyperplasia represents a nonphysiological, noninvasive proliferation of the endometrium that is considered a precancerous lesions for estrogen-driven endometrial cancers.[1] Atypical hyperplasia has been most strongly associated with progression to endometrial carcinoma and the presence of concomitant endometrial carcinoma in association with endometrial hyperplasia.[2] Progestins have been widely used in women with endometrial hyperplasia that are appropriate for nonsurgical management.[3] However, up to 30% of patients fail to respond to progestin therapy, especially when atypical hyperplasia is present.[4]

Several studies have investigated the mechanisms involved in progestin resistance. Decreased availability of progestin receptors before treatment

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or alterations in progestin receptor regulatory function was thought to be one of the main mechanisms.[5,6] Other molecular mechanisms related to progestin resistance include dysregulation of transforming growth factor-α and epidermal growth factor receptors of the endometrial glandular cells,[7] upregulation of epidermal growth factor pathway[8] and insulin resistance.[9] In our earlier studies, we have shown that the apoptotic cascade seems to be blocked in progestin-resistant hyperplasia cases. Bcl-2, one of the important factors in apoptotic pathway was seen to be decreased after successful progestin treatment and Fas, a proapoptotic factor was upregulated in responders. These two factors, however, remained unchanged in cases not responsive to progestin treatment.[10–12] Despite all these findings, the true mechanism of progestin resistance in endometrial hyperplasia, and the key factor causing apoptotic pathway blockade in progestin resistance remains to be elucidated.

Evidence accumulated over the past 10 years suggests that survivin (encoded by BIRC5),[13] a small inhibitor of apoptosis (IAP) protein has multiple functions as an essential regulator of cell division, a modulator of apoptotic and nonapoptotic cell death and a promoter of angiogenesis.[14] The Survivin gene has been mapped to the chromosomal region 17q25, and encodes a 1.9-kb transcript, which contains four exons resulting in a 142-amino-acid (16.5 kDa) protein.[15] In the antiapoptotic network, survivin interacts with other adaptors or cofactors, and provides a heightened cell survival threshold and hinges on multiple signaling pathways.[14] Survivin has been found to be expressed during development and in proliferating cells, but has been largely undetectable in the most differentiated tissues. By contrast, survivin is highly expressed in most human tumors, including lung, breast and prostate cancer and so on.[16–18] In human endometrium, survivin is expressed in normal and proliferative endometrium and is overexpressed in hyperplastic and malignant endometrium.[19–21] The overexpression of survivin in neoplastic endometrium suggests that survivin may play an important role in the process of estrogen-dependent endometrial carcinogenesis.

In this study, we examined survivin expression *in vivo* in pre and postprogestin-treated endometrial hyperplasia samples to investigate whether survivin can serve as a biomarker of progestin resistance. To help further illuminate some of the molecular mechanisms underlying progestin resistance, we used the Ishikawa cell line (derived from a well-differentiated endometrioid adenocarcinoma) as an *in vitro* model to explore whether survivin is regulated by sex steroid hormones, all of which are aimed toward the continuing search for a better conservative therapeutic modality for patients with endometrial hyperplasia.

Materials and methods

Selection of Matched Cases

Twenty-three paired pre and postprogestin-treated endometrial hyperplasia samples were studied. These cases were derived from our earlier studies on the mechanisms of progestin resistance.[10–12] The method of collection was previously described. [10,12] Clinically, the 23 cases were comprised of 15 progestin responders (successfully treated) and 8 nonresponders (failures to response to progestin treatment). The patients' age, treatment regimen and the pretreatment findings are summarized in Table 1. The age of responders ranged from 25 to 50 years (median of 42), and those who failed treatment ranged from 23 to 49 years of age (median of 38). Patients in both groups were each treated with one of a variety of progestin-based medications, including megestrol acetate, medroxyprogesterone acetate, norethindrone or depo-medroxyprogesterone acetate. Duration of therapy ranged from 2 to 9 months among the group of successfully treated cases, and 2–12 months among the patients who experienced treatment failure. Among the responders, there were three with an initial diagnosis of simple hyperplasia without atypia, one simple hyperplasia with atypia, seven with complex hyperplasia without atypia and four with complex hyperplasia with atypia. The nonresponders included one case of simple hyperplasia with atypia, three cases of complex hyperplasia without atypia and four case of complex hyperplasia with atypia before the initiation of progestin treatment. Pathological diagnoses of the above endometrial lesions were made by a gynecologic pathologist (WZ) on the basis of WHO classification.[22]

Tissue Processing

All human endometrial tissue samples were fixed in 10% buffered formalin and processed routinely for paraffin embedding. Tissue obtained before treatment included 11 endometrial biopsies and 12 suction curettage specimens. Tissue samples obtained after therapy included 8 endometrial biopsies, 10 suction curettage specimens and 5 hysterectomy specimens. In all Five micron sections for immunohistochemistry (IHC) were cut and placed on positively charged glass slides. A section on each case was stained with hematoxylin and eosin (H&E) and examined microscopically to confirm the diagnosis (OF and WZ). The study was approved by the Human Investigation Committee.

Histologic Evaluation of Post-treatment Samples

Hematoxylin and eosin sections of endometrial samples obtained 2–12 month after a trial of progestin administration were assessed for therapeutic

Table 1 Endometrial hyperplasia patients treated with progestins

Case no.	*Age (years)*	*Progestin dose*	*Treatment duration (months)*	*Diagnosis*	*Postprogestin findings*
Responders					
1	25	MA 40 mg qd	5	ACH	Progestin effects
2	33	MA 40 mg qd	6	ACH	Progestin effects, SM
3	39	MPA 20 mg qd	3	ASH	Progestin effects
4	42	Norethindrone acetate 1 mg qd	5	ACH	Progestin effects, SM
5	45	MPA 20 mg qd	6	ACH	Progestin effects
6	25	MA 40 mg qd	3	CH	Progestin effects, SM
7	32	MPA 10 mg qd (days 1–10)	9	CH	PE, Progestin effects, SM
8	40	Norethindrone acetate 1 mg qd	2	CH	Progestin effects
9	42	MPA 10 mg qd (days 1–10)	6	CH	PE, Progestin effects
10	45	MPA 20 mg qd	4	CH	Progestin effects
11	48	MPA 10 mg qd (days 1–10)	3	CH	Progestin effects
12	50	MPA 20 mg qd	3	CH	Progestin effects
13	41	MA 40 mg qd	3	SH	Progestin effects
14	44	MPA 20 mg qd	2	SH	Progestin effects, SM
15	45	MPA 10 mg qd (days 1–10)	3	SH	Progestin effects
Non-responders					
16	37	MPA 20 mg qd	6	ACH	Focal ACH, progestin effects
17	39	MPA 20 mg qd	3	ACH	ACH, SM
18	41	MPA 20 mg qd	2	ACH	ACH, SM
19	49	MPA 10 mg qd	3	ACH	Focal ACH, PE, progestin effects
20	24	Norethindrone 1 mg qd	3	CH	focal CH, progestin effects
21	27	Progesterone 200 mg qd (4–6 months)	12	CH,	focal CH, progestin effects, SM
22	39	MPA 10 mg qd	6	CH	CH
23	23	MPA 10 mg qd	6	SH	SH, PE

ACH, complex hyperplasia with atypia; ASH, simple hyperplasia with atypia; CH, complex hyperplasia; MA, megestrol acetate; MPA, medroxyprogesterone acetate; PE, proliferative endometrium; SH, simple hyperplasia; SM, squamous metaplasia.
qd, once a day, days 1–10, taking the progestin from days 1 to day 10 in menstrual cycle.

response to treatment. Patients were considered to have complete regression of hyperplasia (responders) if postprogestin treatment samples showed no histologic evidence of hyperplasia, and the histologic changes were confined to those typical of benign proliferative or secretory endometrium and/or progestin effect with stromal decidualization and endometrial epithelia with cuboidal to attenuated changes. If more than 10% of the entire endometrial sample contained endometrial hyperplasia after progestin treatment, the patient was considered to have persistent disease (nonresponder).

Immunohistochemistry for Survivin Expression in Endometrial Samples

Detection of survivin expression was performed using a mouse monoclonal IgG containing antihuman survivin (Santa Cruz Biotechnology Inc., Irvine, CA, USA). The appropriate dilutions were determined in preliminary experiments. IHC was performed as described previously.[11,23] Parallel sections were incubated with antisurvivin (1:100 dilution) (Dako, Carpinteria, CA, USA) in a moisture chamber for 2 h followed by a 45-minute incubation with biotinylated secondary antibody (Vector, Burlingame, CA).

Expression of survivin was assessed using a graded scale on glandular immunoreactivity. Evaluation was performed without referenced knowledge as to the state of treatment or the response to therapy. Cellular reactivity for survivin was based on the presence of distinct cytoplasmic and nuclear staining. Glandular survivin reactivity was graded on a score ranging from 0 to 300 based on the product of staining intensity (0–3) and percentage (0–100) of the cells stained. Score of 0 represented negative expression. An intensity score of 1–3 represented weak, moderate and strong staining, respectively. A total of 500 cells were evaluated in the determination of glandular immunoreactivity. All IHC slides were reviewed independently by two investigators (WZ, OF). Human normal proliferative endometrium and endometrial cancer tissue sections were utilized as positive controls for survivin IHC. Omitted primary antibodies or those preabsorbed with recombinant survivin were used as negative controls.

Cell Culture and Hormone Treatment

The Ishikawa cell line, which was derived from a well-differentiated endometrioid carcinoma, was generously provided by Dr Masato Nishida, Tsukuba University, Tsukuba City, Japan. The culture conditions were described previously.[12] The cells were

plated in 96-well microtest plates in 200 μl of culture medium per well at a cell density of 1×10^5 cells/ml. At 80% confluence, the media was changed to 1% FBS, and incubation was initiated with medroxyprogesterone acetate (MPA) or β-estradiol (Sigma-Aldrich, St Louis, MO, USA) or with cell media only. Dose–response analysis of the Ishikawa cells was performed with the steroid hormone treatment before the hormone-induced survivin expression experiments. The doses tested for MPA were 0.1, 1 and 10 μM. The doses tested for β-estradiol were 0.001, 0.01 and 0.1 μM. Selective progestin receptor or estrogen receptor antagonist Mifepristone (RU486) or Fulvestrant (ICI 182 780; both purchased from Sigma-Aldrich) were used as MPA or β-estradiol inhibitors. For MPA withdrawal assay, 10 μM concentration was selected because this dose showed maximal inhibition of the Ishikawa cell growth *in vitro*. Each treatment condition was performed in triplicate. The hormones were dissolved in DMSO and prepared in culture media with a final DMSO concentration not exceeding 0.1%. MPA was added to the cultures for 24 h, and then removed for another 24, 48 and 72 h before western blot analysis for survivin expression. Cells cultured with culture media were used only as negative control in all the experiments. DMSO concentration was kept the same in all experimental and control groups.

Western Blot Analysis

Proteins were extracted from Ishikawa cell in lysis buffer as described previously.[11] Nucleoprotein was extracted by Nuclear-Cytosol Extraction Kit (Applygen, Beijing, China) according to the manufacture's instruction. The protein concentration was determined by a detergent compatible protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Western blot was performed as described previously.[11] Samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% polyacrylamide gels and transferred to nitrocellulose membranes. The blots were incubated with antibody for survivin (monoclonal antibody at 1:200 dilution, Dako) or PR (Boster, Wuhan, China) for 2 h. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ). β-Actin (Sigma-Aldrich) and H2AFX (Protein Tech Group Inc. Chicago, IL, USA) were used as loading control for total and nucleoprotein, respectively. The relative levels of survivin expression were estimated on the basis of the ratio of survivin/β-actin or PR/H2AFX. All the experiments were repeated at least three times, and the intensity of the signal was analyzed using a digital imaging analysis system (1D Image Analysis Software, Scientific Imaging Kodak Company).

Statistical Analysis

Pre- and post-treatment measurements for survivin expression were each analyzed with respect to treatment outcome using the paired and unpaired Student's *t*-test. The Student's *t*-test was also used for assessing the change in survivin regulations in the Ishikawa cells after the designated treatment periods with MPA StatView system (Abacus, Berkeley, CA, USA) and SPSS Version 14 (SPSS, Chicago, IL, USA) were used for the analysis. All *P*-values were two-sided, and a *P*-value less than 0.05 was considered as significant.

Results

Endometrial Changes after Progestin Treatment

In 15 (65%) of 23 patients, total regression of hyperplasia was attained after a mean treatment duration of 4. 2 months (2–months; Table 1). The patients' median age was 42 years. Of these 15 cases, 9 showed endometrial changes consistent with progestin effect, 1 showed proliferative endometrium with progestin effect, 4 had squamous metaplasia associated with progestin effect and 1 showed proliferative endometrium and squamous metaplasia associated with progestin effect. In 8 (35%) of 23 patients, persistent endometrial hyperplasia was present at post-treatment evaluation. In this group of patients, progestin administration ranged from 2 to 12 months with an average duration of 5.1 months. The median age of these patients was 38 years. Among the eight patients with persistent disease post-treatment, four had evidence of progestin effect adjacent to hyperplastic endometrium and three had changes of squamous metaplasia accompanying persistent disease.

Survivin Expression in Pre- and Postprogestin-Treated Endometrial Samples

The patterns of survivin expression in endometrial glands were both cytoplasmic and nuclear. Endometrial stromal cells stained sporadically for survivin. Survivin immnoreactivity was present in epithelial compartment of all preprogestin-treated endometrial hyperplasia samples with nuclear indices that ranged from 20 to 135 (mean = 78) and cytoplasmic indices that ranged from 70 to 200 (mean = 114). Survivin reactivity was found in stromal cells of the majority of preprogestin samples with nuclear score indices ranging from 3 to 40 (mean = 18) and cytoplasmic indices ranging from 0 to 90 (mean = 49). In postprogestin cases, an average of 19.5-fold decrease of survivin expression was seen in epithelial nuclei ($P<0.001$), and 8-fold decrease in epithelial cytoplasm ($P<0.001$) in responders. Compared with preprogestin samples, survivin expression in stromal cells of both nuclear and cytoplasmic compartments was also

Table 2 Comparisons of survivin expression between responders and nonresponders

	Preprogestin Mean (range)	*Postprogestin Mean (range)*	*Post–pre difference Mean (s.d.)*	P-*values*
Responders				
Epithelial cells index (range)				
Nuclear	78 (20–135)	4 (0–25)	−74 (34.6)	<0.001
Cytoplasmic	114 (70–200)	14 (0–60)	−100 (42.9)	<0.001
Stromal cells index (range)				
Nuclear	18 (3–40)	3 (0–15)	−15 (11.2)	0.01
Cytoplasmic	49 (0–90)	10 (0–30)	−39 (28.5)	0.02
Non-responders				
Epithelial cells index (range)				
Nuclear	70 (30–135)	75 (40–120)	5 (42.9)	0.8
Cytoplasmic	128 (70–180)	89 (50–110)	−39 (36.1)	0.06
Stromal cells index (range)				
Nuclear	18 (7–30)	12 (3–25)	−6 (8.7)	0.2
Cytoplasmic	56 (20–90)	36 (20–70)	−20 (30.1)	0.2

*Survivin staining index was measured on the basis of the morphological nonresponding areas only.

significantly reduced ($P=0.01$ and 0.02, respectively) in responders after progestin treatment. However, in nonresponders, significant changes of survivin expression were detected neither in epithelial nor in stromal compartment compared with pretreatment samples. The data are summarized in Table 2, and representative pictures of survivin immunostainings are presented in Figure 1.

Survivin Expression after β-Estradiol and MPA Treatment

As endometrial hyperplasia is associated with unopposed estrogen stimulation and the survivin expression may be associated with abnormal hormone levels, we studied hormonal regulation of survivin expression in endometrial cancer cells. With three different doses of β-estradiol, the concentration of 0.001 μM represented the optimal dose for survivin induction (25% increase) in Ishikawa cells in the first 48 h of treatment ($P=0.002$ for 24 h, $P=0.001$ for 48 h). The stimulating effect of β-estradiol on survivin expression faded with prolongation of treatment. No statistical difference in survivin expression was observed among groups treated by different β-estradiol doses in 72 h. The representative western blots were presented in Figure 2a.

In contrast to β-estradiol, survivin expression in Ishikawa cells was significantly inhibited by MPA treatment. The inhibition was the most prominent when the cells were treated by 10 μM MPA for 72 h ($P=0.008$). Representative western blots are presented in Figure 2b. As expected, the inhibitory or stimulatory effects of MPA or β-estradiol on survivin expression were eliminated when specific antagonist Mifepristone (RU486) or Fulvestrant (ICI 182 780) were used to block progestin receptor or estrogen receptor separately. Representative western blots are presented in Figure 2c.

Survivin and Progestin Receptor Expression after MPA Withdrawal

As hormone withdrawal usually causes 'breakdown' of the endometrium, we studied whether the removal of MPA may change survivin expression level *in vitro*. As shown in Figure 2, the optimal dose of MPA was 10 μM. The reduction of survivin expression turned apparent after 48 h of MPA removal and was most pronounced in 72 h after MPA withdrawal, with survivin expression reduced to 7.5% as that of control ($P=0.002$). Interestingly, instead of being downregulated, the expression of progestin receptor increased after MPA withdrawal, the effect was most significant after 48 h of MPA treatment with progestin receptor expression increased about 74% compared with control ($P=0.019$). Representative western blots are presented in Figure 3.

Discussion

As one of the 'node proteins' involving cell division and apoptosis network, survivin is thought to play an important role in tumorigenesis and drug/radiation resistance in various cancer cells.[24–26] Survivin has been reported to be highly expressed in endometrial cancer.[21,27,28] Interfering with survivin expression by siRNA could effectively reduce cell proliferation and induce apoptosis in endometrial cancer cells by downregulating cyclin D1 and phosphorylated RB, and activating caspase-3 and caspase-8.[20] These findings suggest that, like in malignant tumors from other organs, survivin might also play an important role in endometrial cancer development. The overexpression of survivin in

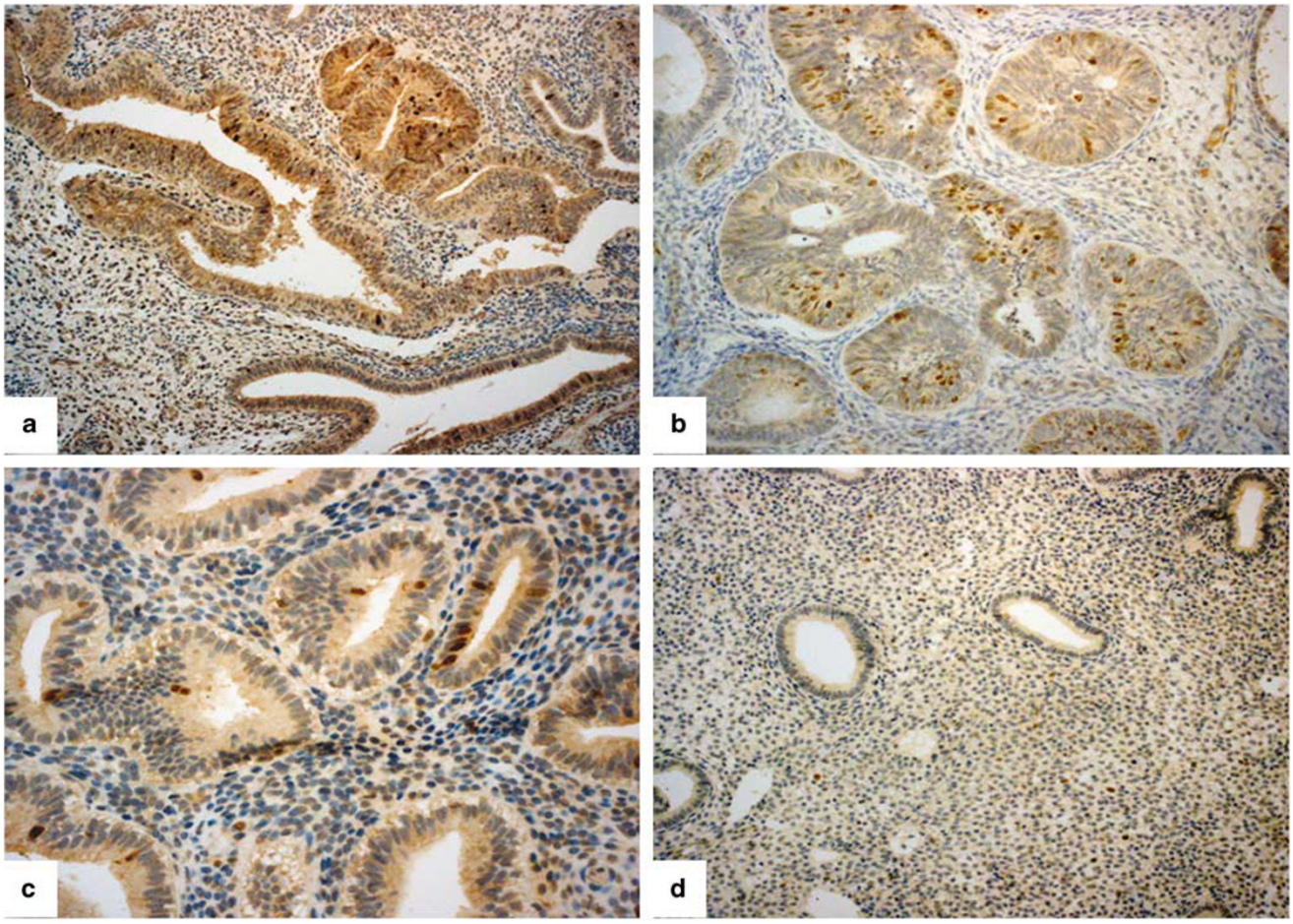

Figure 1 Immunohistochemical staining of survivin expression in hyperplastic endometrium pre- and postprogestin treatment. (**a**) Complex hyperplasia of the endometrium before progestin treatment (case 20). Both cytoplasmic and nuclear stainings of survivin are seen in the hyper plastic endometrial glands. (**b**) Area of atypical complex hyperplasia from case 20 after norethindrone 1 mg qd treatment for 3 months (nonresponder). Although cytoplasmic survivin reactivity was reduced, the nuclear survivin expression remained prominent. (**c**) Complex hyperplasia of the endometrium before progestin treatment (case 6). Both cytoplasmic and nuclear stainings of survivin are seen in hyperplastic endometrial glands. (**d**) A complete response to progestin treatment was seen after 3 months of megestrol acetate treatment (case 6). No cytoplasmic or nuclear survivin expression was seen (responder; **a**, **b**, **c**: magnification $\times 200$, **d**: magnification $\times 100$).

endometrial hyperplasias, as shown in this study, suggests that aberrant expression of survivin may be an early event of type I endometrial tumorigenesis, given the probable precancerous status of endometrial hyperplasia. Our findings are consistent with those of another previously reported study in which survivin was found to be expressed significantly more in hyperplastic endometrium than in normal controls.[21]

Endometrial hyperplasia and most endometrial cancers are clearly associated with unopposed estrogen stimulation. Survivin expression has been shown to be regulated by menstrual-related hormones. Huang *et al*[29] reported that follicle-stimulating hormone enhanced survivin expression in ovarian cancer cells. Studies in breast cancer cells have shown that estradiol treatment could upregulate survivin expression in estrogen receptor-positive MCF-7 human breast cancer cells.[30] Sayeed *et al*[31] reported that estrogen receptor-α binds to P53 and inhibits P53-mediated transcriptional depression of survivin.[31] Formby *et al*[32] reported that progesterone could inhibit survivin expression and induce apoptosis in breast cancer cells. Our findings in endometrial cancer are broadly similar to those of the aforementioned studies. We showed that survivin was upregulated by estradiol and downregulated by MPA in endometrial cancer cells. The regulation of survivin expression might be mediated by estrogen and progestin receptors as we found the effect of estradiol and MPA on survivin was abolished when we blocked estrogen or progestin receptors by selective estrogen or progestin receptor downregulators, Fulvestrant or Mifepristone. Our findings support the idea that the overexpression of survivin in endometrial hyperplasia and in most endometrial cancer is the result of prolonged unopposed estrogen stimulation and might be part of the mechanism in estrogen-related endometrial carcinogenesis.

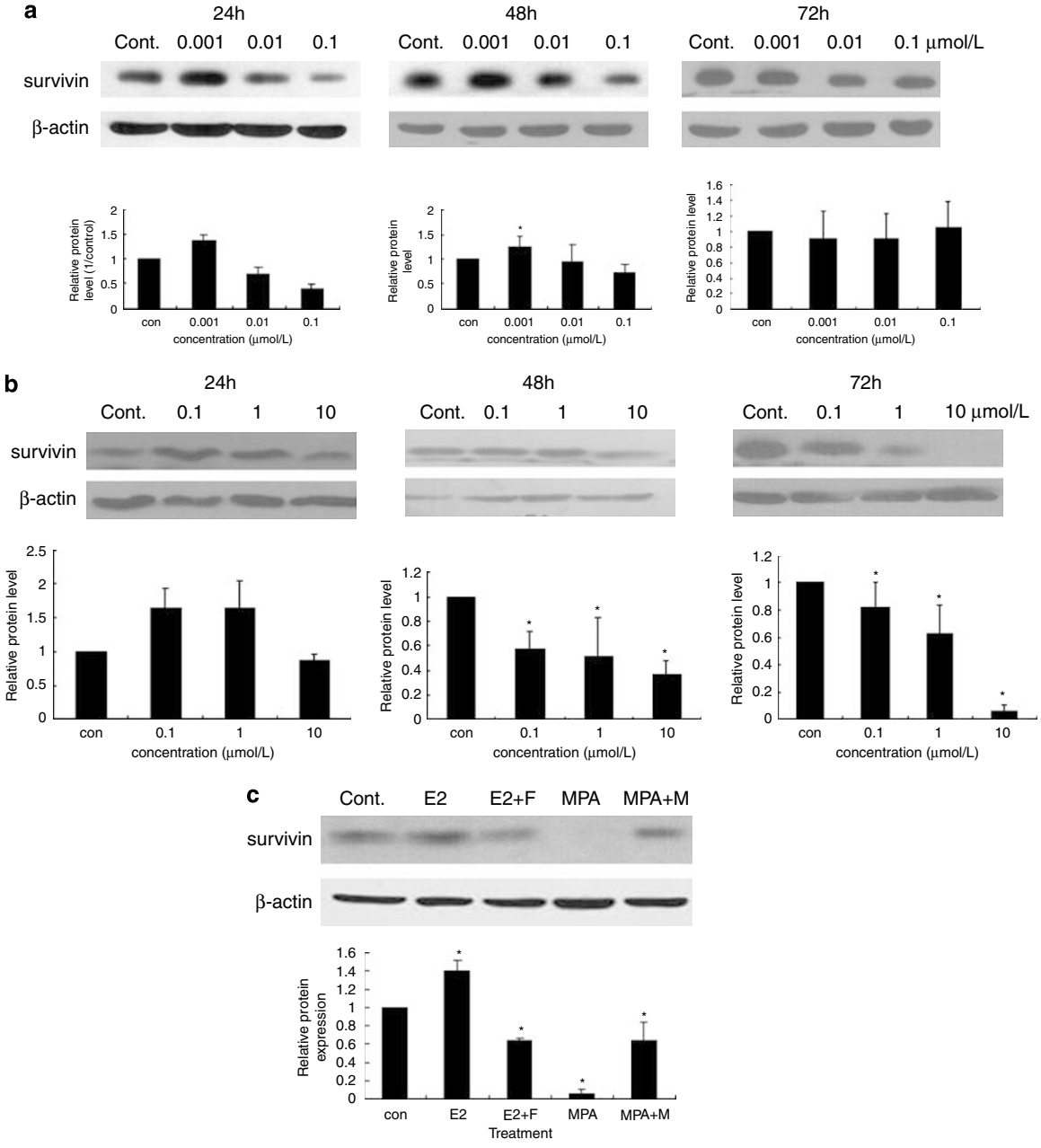

Figure 2 Survivin expression regulated by estrogen and progestogen. (**a**) Survivin expression in Ishikawa cells after β-estradiol (E2) stimulation. Maximum survivin was seen in a dose of 0.001 μM β-estradiol, and the effect was most significant in the first 48 h ($P=0.002$ for 24 h, $P=0.001$ for 48 h). (**b**) Survivin expression in Ishikawa cells after medroxyprogesterone acetate (MPA) treatment. The inhibition effect on survivin expression was most significant by 10 μmol/l MPA after 72 h treatment ($P=0.008$). (**c**) Mifepristone or Fulvestrant blocked MPA or β-estradiol (E2) effect on survivin expression in Ishikawa cells. Ishikawa cells were treated by 0.001 μM β-estradiol, 0.001 μM β-estradiol + 1 μM Fulvestrant (E2 + F) for 24 h; 10 μM medroxyprogesterone acetate, 10 μM medroxyprogesterone acetate + 1 μM Mifepristone (MPA + M) for 72 h. The stimulatory or inhibitory effect of β-estradiol or MPA was abolished by their selective receptor antagonists, respectively. *$P<0.05$ by student's *t*-test.

Long-term progestin treatment has long been used as an effective treatment regimen for endometrial hyperplasia as well as for endometrial cancer in those patients that opt for fertility preservation or for whom surgery is contraindicated or suboptimal. However, the 30% failure rate remains one of the biggest drawbacks associated with this treatment option, for both patients and their physicians.[33] In spite of extensive investigative efforts, the precise mechanisms underlying progestin resistance in some patients remain unclear. In this study, 8 out of 23 patients were found to be progestin-resistant. Interestingly, we found that all the progestin-resistant patients unanimously showed persistent survivin expression after progestin treatment. In contrast, survivin expression was significantly

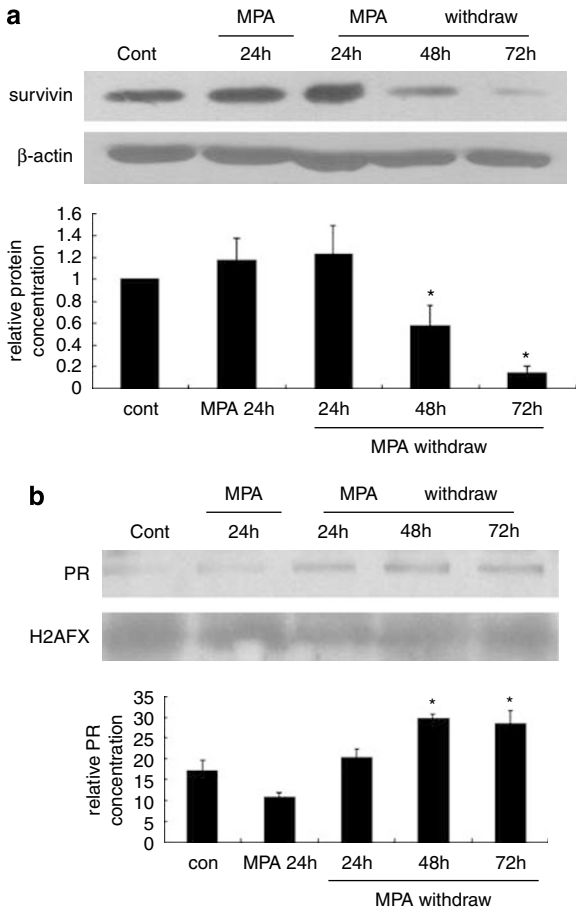

Figure 3 Effect of medroxyprogesterone acetate (MPA) withdrawal on survivin and progesterone receptor (PR) expression. (**a**) Survivin expression was significantly downregulated 72 h after the withdrawal of MPA. (**b**) Progesterone receptor (PR) expression in Ishikawa cells after MPA withdrawal. Progesterone receptor (PR) expression in Ishikawa cells was significantly upregulated 48 and 72 h after MPA withdrawal. $^*P<0.05$ by student's *t*-test.

downregulated after successful progestin treatment, suggesting survivin may play a role in progestin resistance in these precancerous endometrial lesions.

Survivin has been shown to play an important role in the drug-resistant phenotype of human cancer cells. Enforced high survivin expression could effectively suppress apoptosis induced by chemotherapeutic agents and increase drug resistance in various cancer cell lines, such as cervical, ovarian and prostate cancer cell lines.[34–36] In the aspect of sex-steroid hormone resistance, Zhang *et al*[37] reported that survivin mediates resistance to antiandrogen therapy. No studies regarding progestin resistance and survivin have been reported previously.

It is noteworthy that all of the aforementioned studies showed that survivin expression level was positively correlated with drug resistance in cancer. Cancer cells expressing lower survivin level were more sensitive to drug treatment than those with high survivin expression. However, in our study, the survivin levels were largely similar in the hyperplastic lesions before progestin treatment, but they were remarkably discrepant in treatment-sensitive and treatment-resistant groups after progestin treatment. This phenomenon suggests that there might be other mechanisms by which survivin mediate progestin resistance in endometrial cancer. It is well accepted that insulin resistance is correlated to endometrial cancer.[38,39] The resulted compensatory hyperinsulinaemia leads to increased bioavailability of insulin-like growth factor-I (IGF-I), which plays an important role in cancer cell proliferation, adhesion, migration and apoptosis.[40] In the study of prostate cancer, Zhang *et al*[37] found that IGF-I could stimulate survivin expression through AKT signaling pathway even during androgen blockade or in cells without androgen receptor. This finding might help us postulate that in progestin-resistant hyperplasia cases, the activated IGF-I pathway caused by insulin resistance might stimulate survivin expression, which antagonize progestin-induced apoptosis leading to progestin resistance in the end. Further research is on the way testing this hypothesis.

The downregulation of progestin receptor after long-term continuous progestin treatment is thought to be one of the main mechanisms of progestin resistance.[20,41,42] We, thus, investigated whether cyclic progestin treatment could achieve the same therapeutic effect as continuous therapy while avoiding the unfavorable effects of progestin receptor downregulation. Our results showed that instead of being downregulated, progestin receptor level could be effectively upregulated after MPA withdrawal. Meanwhile, the inhibitory effect of MPA on survivin was similar irrespective of whether cells were treated by continuous or cyclic MPA regimen. This is similar to our previous progestin withdrawal experiment on Fas–Fas ligand.[11] The promising results of withdrawal test on progestin receptor study as well as on survivin and Fas–Fas ligand study[11] suggests that cyclic progestin therapy may be more efficient than continuous modality in the management of endometrial hyperplasia by increasing the rate of apoptosis and reducing the possibility of progestin resistance. A clinical trial of comparing cyclic and continuous modalities is currently being carried out by the Gynecologic Oncology Group (GOG) in the United States.

In summary, our study shows that survivin is highly expressed in endometrial hyperplasia. The expression of survivin in the endometrium is upregulated by estrogen and downregulated by progestin. Persistence survivin expression may be associated with progestin resistance in endometrial hyperplasia cases, and as such may potentially be used as a marker for predicting the effect of conservative progestin treatment. Cyclic progestin therapy may have a better result to treat patients with endometrial hyperplasia.

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