Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 110 (2008) 222–229
Gynecologic Oncology
www.elsevier.com/locate/ygyno

Differential expression of clusterin according to histological type of endometrial carcinoma

Hee Jung Ahn [b,1], Jeehyeon Bae [c,1], Sunyoung Lee [a,*], Jung Eun Ko [a], Seongmin Yoon [c], Seung Jo Kim [a], Noriaki Sakuragi [d]

[a] Department of Obstetrics and Gynecology, College of Medicine, Pochon CHA University, Republic of Korea
[b] Department of Pathology, College of Medicine, Pochon CHA University, Republic of Korea
[c] Graduate School of Life Science and Biotechnology, College of Medicine, Pochon CHA University, Republic of Korea
[d] Department of Obstetrics and Gynecology, Hokkaido University School of Medicine, Japan

Received 5 January 2008
Available online 2 June 2008

Abstract

Objective. Clusterin expression has been associated with various malignancies. Endometrial carcinoma is divided into endometrioid and papillary serous type carcinoma according to the histological characteristics and regarding to the unopposed estrogenic stimulation. In this study, we investigated the expression profiles of clusterin according to the histological types and the effect of estrogen stimulation on its expression in endometrial carcinoma.

Method. Clusterin expression in endometrial carcinoma tissues was examined by RT-PCR, Western blot analysis, and immunohistochemistry. 63 endometrioid and 10 papillary serous types of fresh cases and 81 endometrioid and 7 papillary serous types of paraffin-embedded cases were studied. Regulation of clusterin expression by β-estradiol in HEC-1B and HEC-1A cells was investigated using RT-PCR and Western blot analysis. Cell proliferative function of clusterin was examined in 293T cells.

Results. We found higher expression of clusterin in endometrioid compared to papillary serous carcinoma using both immunohistochemistry ($p=0.033$) and Western blot analysis ($p=0.024$). The mRNA and protein expressions of clusterin in endometrioid carcinoma were higher than in benign endometrium ($p=0.002$). Forced expression of clusterin promoted 293T cell survival in a concentration-dependent manner, and estradiol treatment increased clusterin expression in HEC-1B but not in HEC-1A cells.

Conclusions. These data suggest that clusterin expression is related to endometrioid carcinoma of endometrium, in which estrogen is involved in the regulatory network of clusterin.

Keywords: Clusterin; Endometrial carcinoma; Endometrioid type; Papillary serous type; Estrogen

Clusterin (CLU), also known as apolipoprotein J, testosterone-repressed prostate message-2, and sulfated glycolprotein-2, has been implicated in various cell functions including apoptotic cell death, cell cycle regulation, cell adhesion, tissue remodeling, and immune system regulation involved in physiologic or pathologic process such as carcinogenesis and tumor progression [1–3]. CLU has two different but related protein isoforms, the glycosylated secretory CLU (sCLU) and the nuclear CLU (nCLU) [4]. In response to various types of cell damage, the ~55 kDa-sized nCLU is generated that induces apoptosis [4]. In contrast, a previous knockdown study indirectly indicated that the 75–80 kDa sCLU seems to act as an anti-apoptotic or a proliferative protein in certain cancer cells [3]. A recent study suggested that the role of CLU in tumor progression might be related to a pattern shift in its isoform production such that there is overexpression of sCLU and loss of nCLU [5]. However, it is still unclear what stimuli or molecules regulate the expression of sCLU and/or nCLU in malignant tumors.

* Corresponding author. Department of Obstetrics and Gynecology, College of Medicine, Pochon CHA University, Gynecologic Cancer Center, Bundang CHA Hospital, 351 Yatap, Bundang, Sungnam, Kyonggi, 463-712, Republic of Korea. Fax: +82 31 780 6194.
E-mail address: sunyoung_lee@cha.ac.kr (S. Lee).
[1] HJ. A. and J. B. contributed equally to this work.

0090-8258/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2008.04.010

Table 1
Stage, grades and CLU expression of the cases for Western blot analysis

	Endometrioid carcinoma (n=10)	Papillary serous carcinoma (n=4)	Normal endometrium (n=13)	P value*
Stage				P=0.834
1	8	3	NA	
2				
3	2	1		
Grade			NA	P=0.001
1	7			
2	3			
3		4		
Mean CLU expression ratio	2.83±2.23	0.38±0.24	0.62±0.56	P=0.005

*Kruskal–Wallis test.

Overexpressed sCLU has been observed in malignant tumors in various organs, including prostate [6], kidney [7], lung [8], ovary [9], breast [10] and colon [5], suggesting that the primary function of sCLU in cancer cells seems to be anti-apoptotic. However, decreased expression of sCLU has also been reported in malignancies such as prostate [11], esophageal [12], and pancreatic carcinomas [13], and neuroblastoma [14]. These contradictory reports suggest that sCLU expression is likely to be regulated by different mechanisms in different histological types of carcinoma.

The two histologically different types of carcinoma of the endometrium, endometrioid and papillary serous, are differentially regulated by hormones [15]. Most endometrioid adenocarcinoma occurs in association with endometrial hyperplasia, and its clinical characteristics are consistent with unopposed estrogenic stimulation such as anovulation or estrogen-only therapy. In contrast, uterine papillary serous carcinoma develops in atrophic endometria, and there is no evidence that unopposed hyper estrogenic stimulation is one of its clinical factors. The serous type responds badly to conventional therapies and has a higher recurrence rate than endometrioid carcinoma [16]. Thus, clinicians need to determine different management guidelines based on the different carcinogenic mechanisms of these two distinct histological types. This is difficult at present as our knowledge of these molecular mechanisms is limited.

Therefore, we attempted in this study to establish whether CLU expression levels are different in the two distinct histological types of carcinomas, and further to determine their association with unopposed estrogenic stimulation in endometrial carcinoma.

Materials and methods

Clinical specimens

Paraffin-embedded tissue sections of endometrial carcinoma were obtained from 88 patients undergoing hysterectomy at Bundang CHA Hospital, Gynecologic Cancer Center between 2001 and 2006. The carcinoma cases for immunohistochemistry consisted of 81 endometrioid and 7 serous types. The mean age of endometrioid carcinoma group was 47.71±11.90 and that of serous group was 61.14±4.71. Among these carcinoma cases, 54 samples of fresh frozen tissue were used for the mRNA expression study. In addition, 36 samples of RNA extracted from endometrial carcinoma were obtained from patients of Hokkaido University, who were treated from December 1993 to May 1999. None of the patients had received preoperative chemotherapy or radiation at the time of sampling. For the mRNA study, 79 endometrioid, 11 serous types, and 36 cases of normal endometrial tissues that were obtained from patients with benign gynecological disease, were used. All clinical specimens were collected under protocols approved by the institutional review boards (IRBs) of Bundang CHA hospital and Hokkaido University. Fresh tissues and extracted total RNA were stored at −70 °C until use. Stages and grades of endometrial carcinoma were determined according to the International Federation of Gynecology and Obstetrics standards and summarized in Tables 1, Tables 2, Tables 3.

Western blot analysis

Protein was isolated using PRO-PREP Protein Extraction Solution (C/T) (Intron Biotechnology, South Korea) from fresh tissues. A protein of about 36 kDa was detected by Western blot analysis using mouse monoclonal anti-clusterin antibodies (clusterin 41D, 1:5000; Upstate Biotechnology, USA). 20 μg total protein were loaded on a 10% SDS-PAGE gel and transferred to nitrocellulose membranes (Amersham Bioscience, UK). The membranes were blocked in tris-buffered saline with 0.1% Tween 20 (TBST) containing 5% nonfat milk powder for 1 h at room temperature. The membranes were incubated overnight at 4 °C with the primary antibodies and then with secondary antibody (goat anti-mouse IgG-Horse radish peroxidase, Santa Cruz Biotechnology, USA) for 1 h at room temperature. The signals were detected using an ECL Western blotting detection system (Amersham Bioscience, UK) and quantified by Luminescent Image Analyzer LAS-3000 (Fuji, USA).

Immunohistochemistry

Immunolocalization of the clusterin was performed on 5 μm formalin-fixed and paraffin-embedded tissue sections of 88 endometrial carcinoma cases and 19 cases of normal endometrial tissues. Antigen retrieval with Tris–EDTA (pH 9.0) was performed in a pressure cooker at 95 °C for 3 min. After blocking with 2% normal horse serum for 30 min, the sections were incubated with the mouse monoclonal anti-clusterin antibodies (clusterin 41D) for 90 min at room temperature. As

Table 2
Stage, grades and CLU expression of the cases for immunohistochemical study

	Endometrioid carcinoma (n=81)	Papillary serous carcinoma (n=7)	Normal endometrium (n=19)	P value*	Correlations with CLU IHC scores**
Stage			NA	P=0.132	P=0.254
Unknown	10	0			
1	63	5			
2	4	0			
3	3	0			
4	1	2			
Grade			NA	P=0.000	P=0.379
1	46	0			
2	35	0			
3	0	7			
CLU IHC scores				P=0.033	
0	38	6	10		
1	9	1	6		
2	6	0	3		
3	28	0	0		

*Kruskal–Wallis test.
**Spearman's correlation test.

Table 3
Stage, grades and CLU expression of the cases for RT-PCR study

	Endometrioid carcinoma ($n=63$)	Papillary serous carcinoma ($n=10$)	Normal endometrium ($n=36$)	P value	Correlations with CLU mRNA expression***
Stage			NA	*$P=0.485$	$P=0.812$
Unknown	1	1			
1	48	6			
2	1	0			
3	12	3			
4	1	0			
Grade			NA	*$P=0.007$	$P=0.670$
1	36	3			
2	19	0			
3	8	7			
Mean CLU mRNA expression ratio	0.96±0.77	0.64±0.41	0.48±0.47	**$P=0.41$	

*Kruskal–Wallis test.
**Mann–Whitney test; p value indicates the significance of differences between endometrioid vs. serous carcinoma.
***Spearman's correlation test.

negative controls, the sections were incubated with PBS. After the incubation with the primary antibodies, 5% hydrogen peroxide was used for 10 min to block the endogenous peroxidase before incubation with the secondary antibody. The biotinylated anti-goat IgG and biotin/avidin system of the Vectastain elite ABC kit (Vector Laboratories, USA) was used for this immunoperoxidase procedure and diaminobenzidine tetrahydrochloride (DAB) solution (Vector Laboratories) was used as a substrate, according to the manufacturer's recommendations. Nuclei were counterstained with hematoxylin and the sections were dehydrated in ethanol, cleared in xylene, and mounted in SP15-500 Permount (Fisher Scientific, USA).

The immunohistochemical results for clusterin were scored as follows: 0, no staining; 1, less than 10% cytoplasmic positive stained cells; 2, 10–50% cytoplasmic positive stained cells; 3, cytoplasmic positive stained cells in more than 50% of the population. Two independent observers analyzed the immunohistochemistry and the mean value of results from both observers was used. When differences of scoring between observers occurred, both investigators reinvestigated the slides, and the final decision was made by consensus.

Semi-quantitative RT-PCR

The Easy-spin total RNA Extraction Kit (Intron Biotechnology) was used according to the manufacturer's instructions to isolate total RNA from 44 samples of freshly frozen tissues of endometrial carcinoma and 36 samples of fresh normal endometrium tissues with 29 cases of total RNA obtained from Hokkaido University were also used. 18S rRNA was used as a loading control. Reverse transcription reactions were performed on 2 μg of total RNA using Maxime™ RT PreMix (Intron Biotechnology) as suggested by the manufacturer. PCR reactions were performed with Taq DNA polymerase (COSMO Gene Technology, South Korea) together with cDNA, clusterin forward (5′-GGCGTGCAAAGACTCCAGAA-3′) and reverse primers (5′-ACATCCAG-CATGTGCGTCTG-3′) or with 18S rRNA forward (5′-TACCTACCTGGTT-GATCCTG-3′) and reverse primers (5′-GGGTTGGTTTTGATCTGATA-3′). Thermal cycling conditions were 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and dissociation at 72 °C for 10 min. Electrophoresis of the RT-PCR products was performed on a 2% agarose gel. The image of the ethidium bromide (0.1 μg/ml) stained gel was digitalized by a video image analyzer. The software Multi Gauge (Science Lab, USA) was used for quantification of the PCR products.

Cell viability assay

Cell viability was measured after transfecting increasing concentrations of pCMV-clusterin (0, 30, 300, and 500 ng) onto 293T cells (2×10^5). The cells were also electroporated along with 50 ng of pEGFP-N1 to identify transfected cells. The empty pCMV vector was used as a control. After 24 h of incubation, GFP-positive cells were counted under fluorescence microscopy, and data were expressed as % of control.

Cell culture and estrogen treatment

The human endometrial cancer cell lines, estrogen receptor (ER) positive HEC-1B and ER-negative HEC-1A were obtained from the American Type Culture Collection (Manassas, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium with essential amino acid solution (GIBCO, USA) without phenol red and supplemented with 1% charcoal-stripped fetal bovine serum plus 1% penicillin–streptomycin (Welgene, South Korea) in a humidified 5% CO_2 atmosphere at 37 °C. The cells (2.5×10^6) were seeded in 10 cm culture dishes with 10^{-8}, 10^{-7}, or 10^{-6} M β-estradiol (E2758; Sigma, St. Louis, MO) and incubated for 48 h. Control cells received 1% DMSO (Sigma) as a vehicle control.

Statistics

Statistical analyses were performed using SPSS version 13.0 software. Both Kruskal–Wallis and Mann–Whitney tests were used to compare expression levels among different groups. In addition, bivariate correlation analysis was determined by Spearman coefficients. The level of significance was assigned at $p<0.05$.

Results

Quantitative analysis and immune localization of clusterin in two subtypes of endometrial carcinoma

Western blot analysis of sCLU was performed on 10 representative samples of endometrioid carcinoma and 4 of serous carcinoma tissues and 13 normal controls. As previously described [4], two protein bands were observed on the SDS-PAGE gel, representing the non-glycosylated full-length uncleaved 60 kDa protein and the ~36 kDa cleaved alpha and beta peptide bands from the 75–80 kDa glycosylated protein. In this study, the predominant form of expressed CLU in endometrium tissue was the 36 kDa smeared band, identified as sCLU by Western blot analysis (Fig. 1). Semi-quantification with GAPDH expression showed that sCLU expression ratios were significantly higher in the endometrioid type than in the serous type ($p=0.024$) and in normal control endometrium ($p=0.002$) (Fig. 3A).

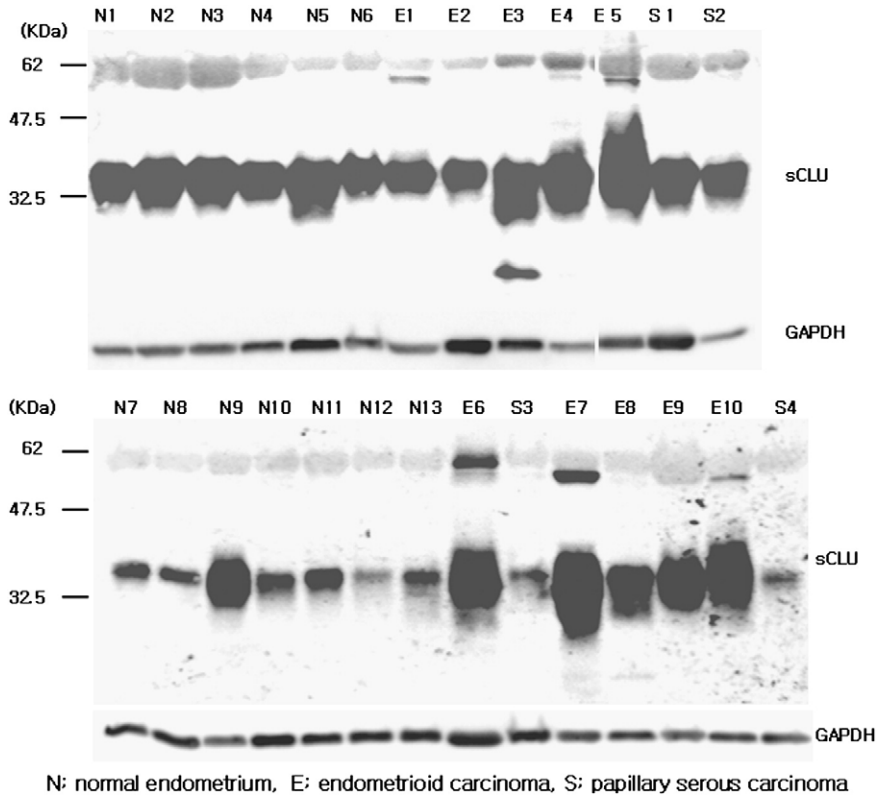

Fig. 1. CLU expression in endometrial carcinoma using Western blot analysis.

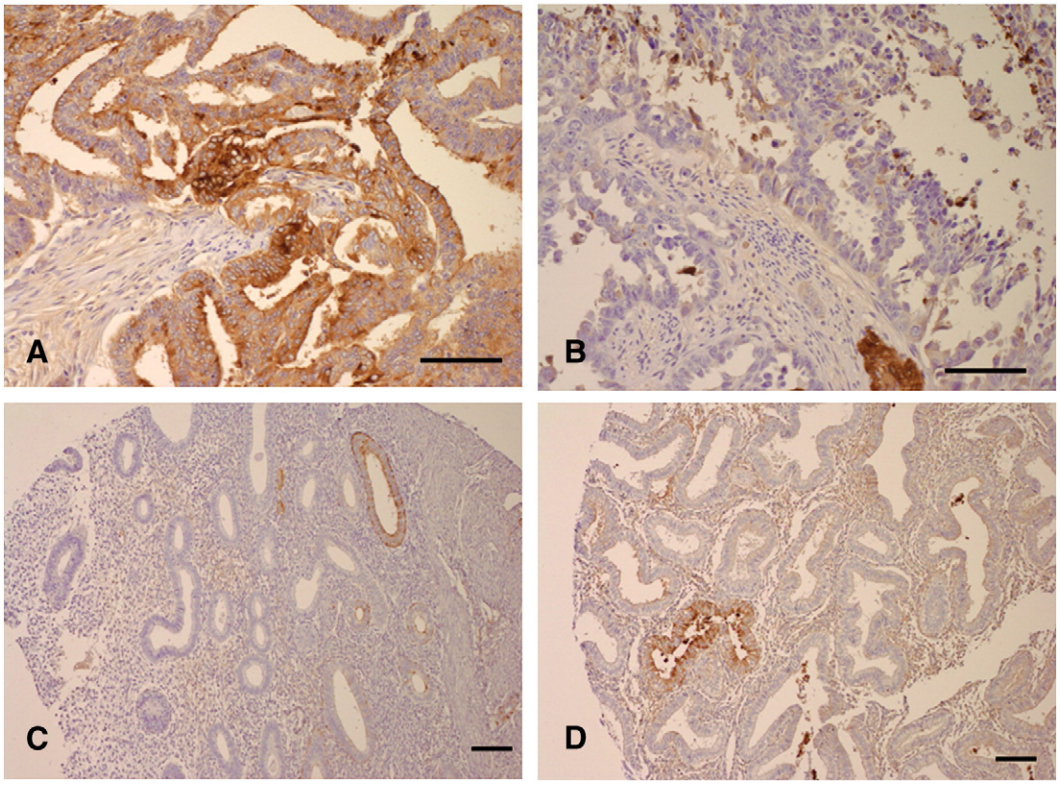

Fig. 2. Immunohistochemical staining shows cytoplasmic CLU expression in adenocarcinoma and normal endometrium; (A) Endometrioid carcinoma; (B) Papillary serous carcinoma; (C) Normal proliferative and (D) Normal secretory endometrium. Scale bar = 10 μm.

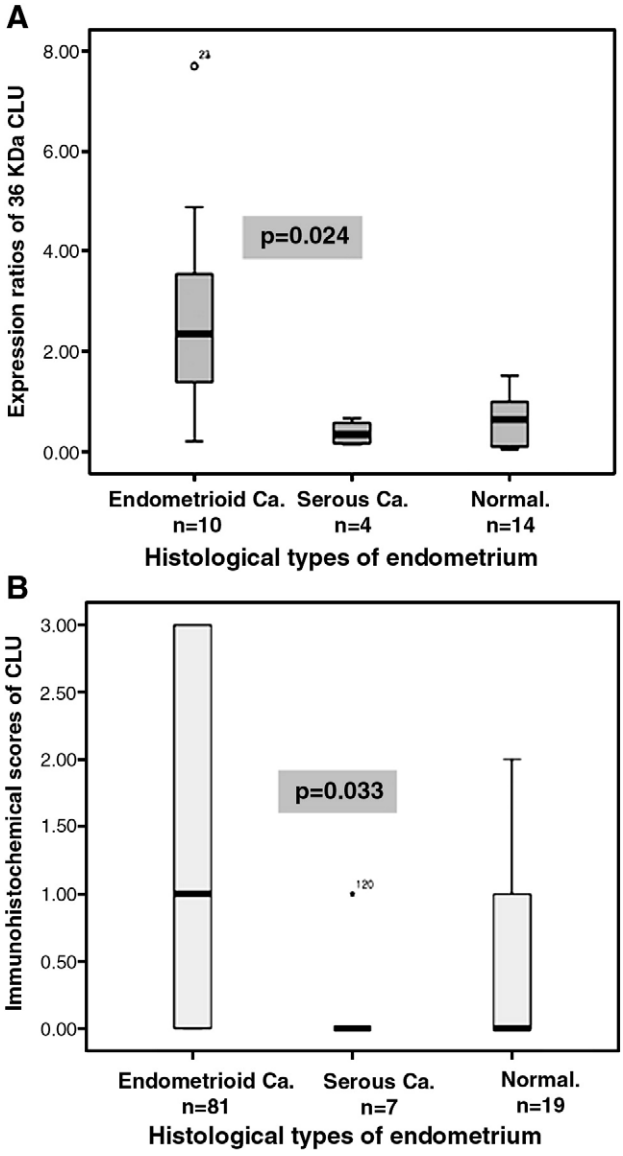

Fig. 3. Box plot graph showing differential expression of CLU protein in two subtypes of endometrial carcinoma and normal endometrium. (A) Result from Western blot analysis. (B) Result from immunohistochemical staining. *p* values indicate the significance of differences between endometrioid vs. serous carcinoma.

We examined 81 cases of endometrioid carcinoma, 7 cases of serous carcinoma and 19 cases of normal control endometrium by immunohistochemical staining (IHC). CLU was localized only in the cytoplasm with moderate to strong staining intensity in 53% of endometrioid carcinoma samples (Fig. 2A). Of the 7 cases of serous carcinoma, we could find only one case of weakly stained carcinoma (Fig. 2B). CLU staining was of weak intensity in 47% of 19 normal endometrium tissues, and the scores were not significantly different when secretory and proliferative phases of the endometrium were compared ($p=0.44$) (Figs. 2C and D). The IHC scores of cytoplasmic CLU were significantly higher in endometrioid than in serous carcinoma ($p=0.033$) (Fig. 3B). However, the correlation between IHC scores and histological grade and stage was not significant among the endometrioid carcinoma cases ($p=0.79$, $p=0.50$).

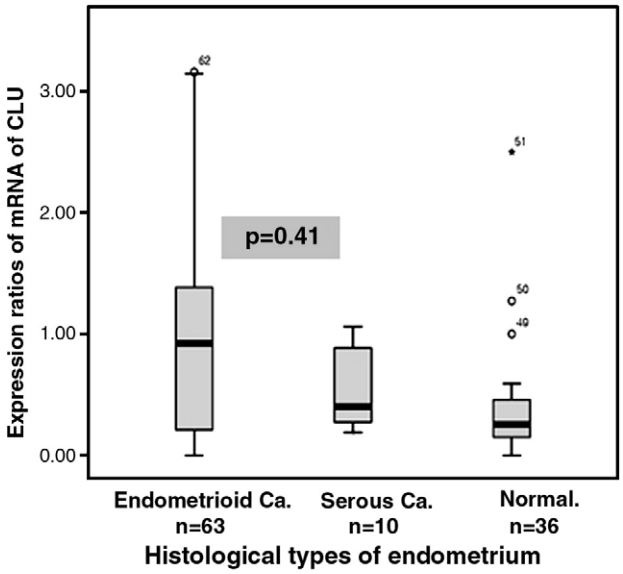

Fig. 4. Box plot graph showing differential expression of CLU mRNA in two subtypes of endometrial carcinoma and normal endometrium. *p* value indicates the significance of differences between endometrioid vs. serous carcinoma.

Clusterin mRNA expression in endometrial carcinoma

Semi-quantitative RT-PCR analysis was also used to evaluate CLU mRNA in the different histological types of endometrial carcinoma. 63 samples of endometrioid type, 10 samples of serous type and 36 samples of normal endometrium were examined. The mRNA expression ratio of CLU in endometrioid carcinoma was significantly higher than in benign endometrium ($p=0.002$). The mean mRNA expression ratio of CLU in endometrioid carcinoma was higher than that of serous type, though this did not reach statistical significance (0.96 ± 0.77 vs. 0.64 ± 0.41, $p=0.41$) (Fig. 4).

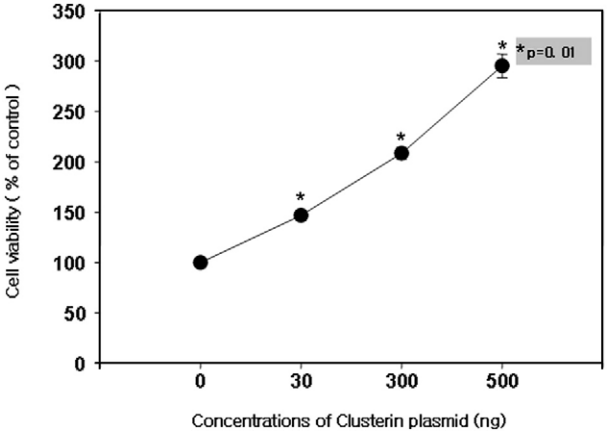

Fig. 5. Clusterin promotes the survival of 293T cells. Control cells were transfected with 500 ng of the empty vector. Cell viability was normalized with control and expressed as % of control. Asterisks indicate significant values compared to the control ($p=0.01$).

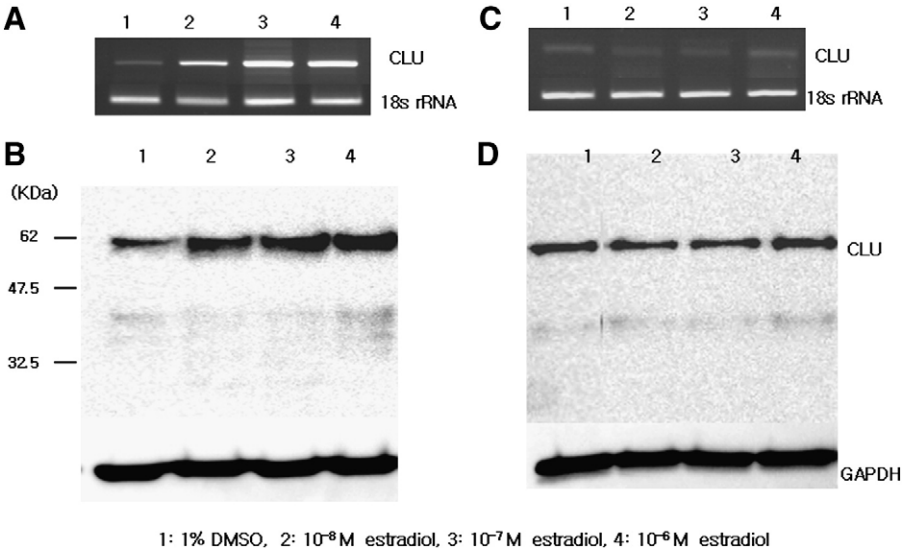

Fig. 6. The mRNA and the protein expressions of CLU in estrogen-treated cell lines. (A) RT-PCR result showing the increased expression of CLU mRNA by β-estradiol treatment in HEC-1B cells. (B) Western blot analysis showing the increased expression of CLU precursor after β-estradiol in HEC-1B cells. (C) RT-PCR result showing no effect of β-estradiol on the expression of CLU mRNA in HEC-1A cells. (D) Western blot analysis showing no effect of β-estradiol on the expression of CLU precursor in HEC-1A cells.

Functiontional analysis of CLU in human cells

To examine the functional output of clusterin expression *in vivo*, we over-expressed clusterin in human cells, and the cell viability was determined. Ectopic expression of clusterin significantly increased 293T cell survival ($p=0.01$) in a concentration-dependent manner (Fig. 5).

Estrogenic regulation of CLU in HEC-1B and HEC-1A cells

Although both HEC-1A and -B cells had a low basal level of CLU mRNA expression, β-estradiol treatment dose-dependently increased CLU mRNA levels only in ER-positive HEC-1B cells (Fig. 6A), while no change of CLU mRNA levels in ER-negative HEC-1A cells after β-estradiol treatment was observed (Fig. 6C). In contrast to the findings of the carcinoma tissue experiment, the predominant form of CLU expressed in the cells was the 62 kDa, uncleaved, full-length, and non-glycosylated precursor form of CLU. The glycosylated 36 kDa CLU peptide bands were very indistinctly observed in the cells. The expression levels of the precursor CLU was increased by β-estradiol treatment in a dose-dependent manner in HEC-1B (Fig. 6B), but not in HEC-1A cells (Fig. 6D).

Discussion

We have demonstrated here for the first time that sCLU is over-expressed in adenocarcinoma of the endometrium. Furthermore, sCLU expression was histological type-specific; it was highly expressed in endometrioid type but not in papillary serous type compared to normal endometrium.

The biological role of over expression of sCLU in the endometrioid carcinoma might be anti-apoptotic or pro-survival, similar to the situation in other carcinomas [30–33]. Humphreys et al. suggested that sCLU has chaperone-like activity similar to that of small heat shock protein, and that this activity may contribute to cytoprotection [30]. Miyake et al. demonstrated that paclitaxel-induced apoptotic cell death was inhibited by sCLU [33]. In addition, Xie et al. reported that sCLU expression levels were associated with tumor metastasis of ovarian carcinoma [34]. Although, we were unable to find any significant relationship between sCLU expression and tumor stage or grade in the current study, we demonstrated that the forced expression of clusterin promoted cell survival. Therefore, it is possible that sCLU helps at the initiation step of endometrioid carcinoma development rather than its progression or metastasis.

Eighty percent of endometrial carcinoma is the endometrioid type. In contrast, uterine papillary serous carcinomas account for only 3% of cases. This type usually recurs and metastasizes distantly even after the surgical treatment of carcinoma at an early stage [17,18]. Information about the molecular factors that regulate the development of different phenotypes of endometrial carcinoma would help successful management of the two distinct carcinomas. Even though several molecules have been shown to be associated with endometrial carcinogenesis, including PTEN, K-RAS2, CTTNB1, DNA mismatch repair genes, p53, Her-2/neu, β-catenin, and TGF-beta [19], the molecular factors that are differentially expressed in the two phenotypes of endometrial carcinomas have not been identified. Our results suggest that sCLU is an important factor that can distinguish the two different types of carcinogenesis.

A previous study showed that sCLU in endometrium is associated with tissue remodeling by mediating clearance or neutralizing the tissue debris at the late secretory phase when the endometrium undergoes dynamic growth and involution [20]. However, in our study, the expression level of sCLU in endometrioid carcinoma tissue was higher than in normal secretory phase endometrium. Therefore, our data suggest that the regulatory

mechanism and role of sCLU are likely to be different in carcinoma and normal endometrium.

We also demonstrated in this study that sCLU expression was related to estrogen treatment in HEC-1B cells. Usually, estrogenic effects are mediated by the estrogen receptor (ER). Estrogen binds to the ligand-binding domain (LBD) of ER, which leads to its dimerization and subsequent binding to an estrogen response element (ERE) located in the promoters of target genes. This triggers activation or repression of many downstream target genes [21,22]. Estrogen also acts indirectly through the activation of early response genes such as *jun* and *fos* which contain the ERE [23,24]. So far, however, no ERE has been identified in the promoter region of *clusterin* [25], although there are several sites of androgen/glucocorticoid responsive elements within the first intron and two functional AP-1 sites located in the proximal promoter of the *clusterin* gene [26]. According to our current data regarding the comparison of ER-positive and -negative cell lines, estrogen seems to affect clusterin via ER. But, we still need to investigate whether an unidentified ERE is present in the promoter region of *clusterin*. The *clusterin* promoter region also has heat shock element consensus sequences that can bind to heat shock transcription factors [27]. Estrogen up-regulates these heat shock transcription factors in the endometrium [28], and some of the heat shock proteins are differentially expressed among the various histological types of endometrial carcinoma [29], suggesting that estrogen-induced up-regulation of clusterin could be also mediated by heat shock transcription factors. Although further mechanistic studies are required, the present study shows that estrogen can stimulate the clusterin expression of endometrial cells and suggests that the over-expressed sCLU can affect the tumorigenesis of endometrial epithelium retaining ERs.

Our data suggest that the expression of sCLU can be regulated by different factors in the histologically different types of endometrial carcinomas, and one of the important regulatory factors of sCLU could be estrogenic stimulation.

Conflict of interest statement

No conflict of interest.

References

[1] Rosenberg ME, Silkensen J. Clusterin: physiologic and pathophysiologic considerations. Int J Biochem Cell Biol 1995;27:633–45.

[2] Trougakos IP, Gonos ES. Clusterin: apolipoprotein J in human aging and cancer. Int J Biochem Cell Biol 2002;34:1430–48.

[3] Trougakos IP, So A, Jansen B, Gleave ME, Gonos ES. Silencing expression of the clusterin: apolipoprotein j gene in human cancer cells using small interfering RNA induces spontaneous apoptosis, reduced growth ability, and cell sensitization to genotoxic and oxidative stress. Cancer Res 2004;64:1834–42.

[4] Leskov KS, Klokov DY, Li J, Kinsella TJ, Boothman DA. Synthesis and functional analyses of nuclear clusterin, a cell death protein. J Biol Chem 2003;278:11590–600.

[5] Pucci S, Bonanno E, Pichiorri F, Angeloni C, Spagnoli LG. Modulation of different clusterin: isoforms in human colon tumorigenesis. Oncogene 2004;23:2298–304.

[6] Steinberg J, Oyasu R, Lang S, Sintich S, Rademaker A, Lee C, et al. Intracellular levels of SGP-2 (clusterin): correlate with tumor grade in prostate cancer. Clin Cancer Res 1997;3:1707–11.

[7] Parczyk K, Pilarsky C, Rachel U, Koch-Brandt C. Gp80 (clusterin; TRPM-2) mRNA level is enhanced in human renal clear cell carcinomas. J Cancer Res Clin Oncol 1994;120:186–8.

[8] July LV, Beraldi E, So A, Fazli L, Evans K, English JC, et al. Nucleotide-based therapies targeting clusterin: chemosensitize human lung adenocarcinoma cells both in vitro and in vivo. Mol Cancer Ther 2004;3:223–32.

[9] Hough CD, Cho KR, Zonderman AB, Schwartz DR, Morin PJ. Coordinately up-regulated genes in ovarian cancer. Cancer Res 2001;61:3869–76.

[10] Redondo M, Villar E, Torres-Munoz J, Tellez T, Morell M, Petito CK. Overexpression of clusterin in human breast carcinoma. Am J Pathol 2000;157:393–9.

[11] Scaltriti M, Brausi M, Amorosi A, Caporali A, D'Arca D, Astancolle S. Clusterin (SGP-2, ApoJ): expression is downregulated in low- and high-grade human prostate cancer. Int J Cancer 2004;108:23–30.

[12] Zhang LY, Ying WT, Mao YS, He HZ, Liu Y, Wang HX, et al. Loss of clusterin: both in serum and tissue correlates with the tumorigenesis of esophageal squamous cell carcinoma via proteomics approaches. World J Gastroenterol 2003;9:650–4.

[13] Xie MJ, Motoo Y, Su SB, Mouri H, Ohtsubo K, Matsubara F, et al. Expression of clusterin: in human pancreatic cancer. Pancreas 2002;25:234–8.

[14] Santilli G, Aronow BJ, Sala A. Essential requirement of apolipoprotein J (clusterin): signaling for IkappaB expression and regulation of NF-kappaB activity. J Biol Chem 2003;278:38214–9.

[15] Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983;15:10–7.

[16] Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R, Hendrickson M, et al. Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. Am J Surg Pathol 1982;6:93–108.

[17] Goff BA, Kato D, Schmidt RA, Ek M, Ferry JA, Muntz HG, et al. Uterine papillary serous carcinoma: patterns of metastatic spread. Gynecol Oncol 1994;54:264–8.

[18] Silva EG, Jenkins R. Serous carcinoma in endometrial polyps. Mod Pathol 1990;3:120–8.

[19] Goodfellow PJ, Mutch DG. Molecular genetics of endometrial cancers. In: Gershenson DM, Mcguire WP, editors. Gynecologic Cancer: Controversies in management. St.Louis: Elsevier; 2004. p. 229–39.

[20] Brown TL, Moulton BC, Baker VV, Mira J, Harmony JA. Expression of apolipoprotein J in the uterus is associated with tissue remodeling. Biol Reprod 1995;52:1038–49.

[21] Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994;63:451–86.

[22] Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 1997;389:753–8.

[23] Webb DK, Moulton BC, Webb DK, Moulton BC, Khan SA. Estrogen induces expression of *c-jun* and *jun*-B protooncogenes in specific rat uterine cells. Endocrinology 1993;133:20–8.

[24] Hyder SM, Nawaz Z, Chiappetta C, Yokoyama K, Stancel GM. The prooncogene *c-jun* contains an unusual estrogen-inducible enhancer within the coding sequence. J Biol Chem 1995;270:8506–13.

[25] Tenniswood M, Wang Z, Lakins J, Morrissey C, O'Sullivan J, Tang H. Clusterin: in the male reproductive tract. J Androl 1998;19:508–16.

[26] Wong P, Pineault J, Lakins J, Taillefer D, Leger J, Wang C, et al. Genomic organization and expression of the rat TRPM-2 (clusterin) gene, a gene implicated in apoptosis. J Biol Chem 1993;268:5021–31.

[27] Michel D, Chatelain G, North S, Brun G. Stress-induced transcription of the clusterin: ApoJ gene. Biochem J 1997;328:45–50.

[28] Yang X, Dale EC, Diaz J, Shyamala G. Estrogen dependent expression of heat shock transcription factor: implications for uterine synthesis of heat shock proteins. J Steroid Biochem Mol Biol 1995;52:415–9.

[29] Nanbu K, Konishi I, Komatsu T, Mandai M, Yamamoto S, Kuroda H, et al. Expression of heat shock proteins HSP70 and HSP90 in endometrial carcinomas. Correlation with clinicopathology, sex steroid receptor status, and p53 protein expression. Cancer 1996;77:330–8.

[30] Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR, Humphreys DT, Carver JA. Clusterin has chaperone-like activity similar to that of small heat shock proteins. J Biol Chem 1999;274:6875–81.

[31] Cervellera M, Raschella G, Santilli G, Tanno B, Ventura A, Mancini C, et al. Direct transactivation of the anti-apoptotic gene apolipoprotein J (clusterin) by B-MYB. J Biol Chem 2000;275:21055–60.
[32] Sintich SM, Steinberg J, Kozlowski JM, Lee C, Pruden S, Sayeed S, et al. Cytotoxic sensitivity to tumor necrosis factor-alpha in PC3 and LNCaP prostatic cancer cells is regulated by extracellular levels of SGP-2 (clusterin). Prostate 1999;39:87–93.
[33] Miyake H, Hara I, Kamidono S, Gleave ME, Eto H. Resistance to cytotoxic chemotherapy-induced apoptosis in human prostate cancer cells is associated with intracellular clusterin expression. Oncol Rep 2003;10:469–73.
[34] Xie D, Lau SH, Sham JS, Wu QL, Fang Y, Liang LZ, et al. Up-regulated expression of cytoplasmic clusterin in human ovarian carcinoma. Cancer 2005;103:277–83.