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Expression and significance of CD133 and ABCG2 in endometriosis

S. Liu[1], J. Zhou[2], J. Wen[1]

[1] Department of Obstetrics and Gynecology, The Fourth Affiliated Hospital of Jiangsu University, Zhenjiang City
[2] Department of Oncology,Chinese Medicine Hospital of Changzhou, Changzhou City (China)

Summary

Background: Endometriosis is a common gynecological disease and exact pathogenesis is still unclear. Recently, an increasing interest has been given to the potential role of stem cells in the development of endometriosis. The aim of this study was to test the expression of stemness-related markers CD133 and ABCG2 in endometriosis. *Materials and Methods:* CD133 and ABCG2 protein expression in eutopic and ectopic endometrial tissue with endometriosis and endometrium tissue without endometriosis were examined by Western blot. *Results:* Eutopic endometrium showed high level of CD133 and ABCG2 protein when compared with ectopic endometrium (p = 0.042, p = 0.038) and control endometrium (p = 0.000, p = 0.000). The expression of CD133 protein in ectopic endometrium was positively correlated with R-AFS score of endometriosis (p = 0.000, r = 0.793) and no significant relation was noted between ABCG2 and R-AFS score (p = 0.563). Two of three patients with recurrence had much higher expression of ABCG2 protein than the patients without recurrence. *Conclusion:* Aberrant expression of CD133 and ABCG2 in eutopic and ectopic endometrial tissue with endometriosis suggests that they are probably associated with the pathogenesis of endometriosis and stem cells play a possible role in its development.

Key words: Endometriosis; Stem cells; CD133; ABCG2.

Introduction

Endometriosis is a chronic, progressive, and complex gynecological disease. It affects about 5% to 10% of women of reproductive age and has had a significant increase in case number and an earlier onset in recent years [1]. Endometriosis is characterized by the presence of functional endometrium-like tissue in ectopic sites and typically causes infertility and pain. Meuleman *et al.* reported that in a subset of infertile women, the prevalence of endometriosis was 47% and was also comparable to patients with (54%) and without (40%) pelvic pain [2].

At present the exact pathogenesis of endometriosis is still unclear. Many studies have focused on elucidating the immunological, endocrinological, environmental, and genetic factors involved in endometriosis, but none of them can completely explain the cause of all its types.

Recently, an increasing interest has been given to the potential role of stem cells in endometriosis development [3, 4] and growing evidences have suggested that it may arise from stem cells [4-8].

CD133, also known as Prominin-1, is a pentaspan, highly glycosylated, membrane glycoprotein that is associated with cholesterol in the plasma membrane. It is expressed in a wide range of somatic stem and progenitor cells [9-11]. ABCG2 is a member of the ATP binding cassette (ABC) transporters, which is widely expressed in stem cells, and also found to confer the side population phenotype, which is recognized as a universal marker of stem cells [12, 13]. Studies have shown that CD133 was expressed by epithelial cells of the endometrium [14] and ABCG2 positive cells were diffusely distributed in the stroma compartment of endometrium [15], but there are less data on CD133 and ABCG2 expression in endometriosis.

The present study aimed to determine whether CD133 and ABCG2 expression is altered in the human ectopic, eutopic endometrium, and to evaluate whether the expression of these two molecules correlates with the formation and progression of endometriosis.

Materials and Methods

Ethics statement

The research was approved by the Institutional Review Boards of the present hospital and the consent form was obtained before surgical procedure.

Tissue collection

Samples were obtained from endometrium in uterine cavity and ovarian endometriomas. A total of 22 eutopic endometrial and 22 ovarian endometriotic specimens were collected from patients with endometriosis, who underwent laparoscopic or laparotomic surgery at the present hospital between January 2011 and December 2011. Endometrial tissues from 20 patients without endometriosis, obtained during surgeries for other gynecol-

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ogical disease at the same period, served as controls. All of the patients had regular menstrual cycles and no other hormone-dependent diseases, did not receive hormone therapy during the three months before surgery, and were not pregnant or lactating during the study. The surgeries were performed at three to seven days after menstruations ended.The specimens were washed with saline to remove blood and immediately frozen in liquid nitrogen for Western blot analysis. All samples were histologically confirmed.

Western blot analysis

Tissues were grinded in RIPA buffer (150 mM NaCl, 1mM EGTA, 0.1%SDS, 1mM NaF,1mM Na_3VO_4, one mg/ml aprotinin, and one mg/ml leupeptin in ten mM Tris, pH 7.4) containing one mM phenylmethanesulfonyl fluoride. Then the tissue homogenate was centrifugated at 1,200 r/min for 15 minutes at 4°C and the supernatant was removed for protein analysis. Protein concentration was determined by the BCA assay kit (CWBIO). Total protein was fractionated on 10% SDS–polyacrylamide gel, then was transferred to polyvinylidene fluoride membranes, blocked with 5% skim milk, and blotted against primary antibodies and secondary antibody sequential y. Primary antibodies were follows: GAPDH (CWBIO), CD133 (PTG), and ABCG2 (PTG).

Table 1. — *The relative gray scale value of CD133 and ABCG2 in eutopic, ectopic, and control endometrium.*

	Eutopic endometrium	Ectopic endometrium	Control endometrium
CD133	830.2±257.8[ab]	662.0±275.0	327.0±179.0
ABCG2	884.9±221.6[cd]	727.6±264.7	397.5±187.5

[a]: $p = 0.042$ compared with ectopic endometrium
[b]: $p = 0.000$ compared with control endometrium
[c]: $p = 0.038$ compared with ectopic endometrium
[d]: $p = 0.000$ compared with control endometrium

Western blot analysis was performed by a crescendo Western HRP substrate.

Statistical analysis

The relative gray scale value of the target protein band was shown as the mean ± SD. Statistical analysis was plotted by using SPSS 17.0. The results were analyzed using t-test and Pearson correlation coefficient was used for correlation analysis. The values of $p < 0.05$ were considered statistically significant.

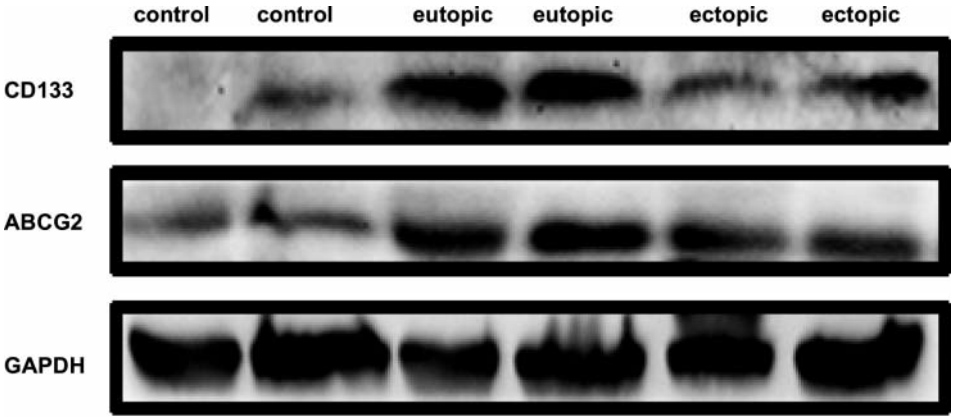

Figure 1. — Expressions of CD133 and ABCG2 in eutopic, ectopic, and control endometrium.

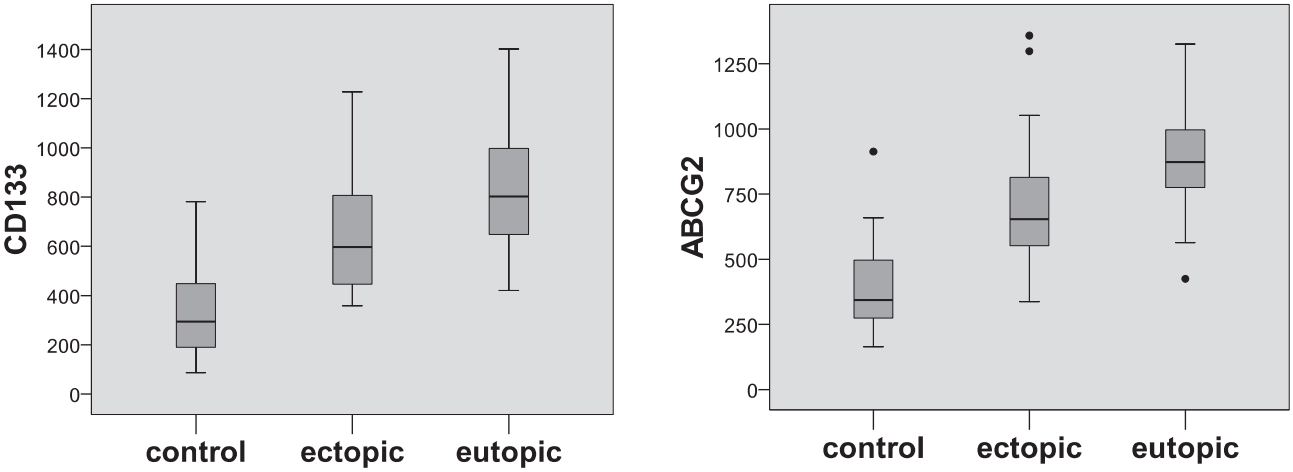

Figure 2. — Expression level of CD133 and ABCG2 in endometriosis and control.

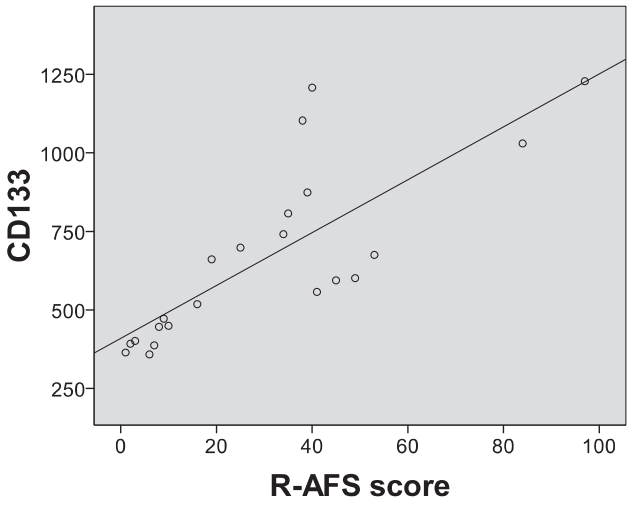

Figure 3. — The correlation between CD133 protein expression level in ectopic endometrium and R-AFS score of endometriosis.

Results

There were no statistically significant differences between age and body mass index groups ($p > 0.05$). To determine the expression level of CD133 and ABCG2 protein in endometriosis, the authors performed Western blot assay with GAPDH as an internal control.

The results showed CD133 and ABCG2 protein were expressed in eutopic, ectopic endometrium of endometriosis group, and endometrium of control group. The relative gray scale value of CD133 in eutopic, ectopic, and control endometrium was 830.2 ± 257.8, 662.0 ± 275.0, and 327.0 ± 179.0, respectively, and the relative gray scale value of ABCG2 in eutopic, ectopic, and control endometrium was 884.9 ± 221.6, 727.6 ± 264.7, and 397.5 ± 187.5, respectively. As shown in Table 1, Figure 1, and Figure 2, the eutopic endometrium showed a high level of CD133 and ABCG2 protein when compared with ectopic ($p = 0.042$, $p = 0.038$), and control endometrium ($p = 0.000$, $p = 0.000$). In the endometriosis group, the extent of disease was staged according to the Revised American Fertility Society (R-AFS) classification (renamed later American Society for Reproductive Medicine's classification—ASRM classification) [16]. The expression of CD133 protein in ectopic endometrium was positively correlated with R-AFS score of endometriosis ($p = 0.000$, r = 0.793) (Figure 3), no significant relation was noted between ABCG2 and R-AFS score ($p = 0.563$).

Each patient received intensive follow-up for two years and there were three patients with recurrence, two of which had much higher expression of ABCG2 protein than the patients without recurrence and the gray scale values were 1,298 and 1,358, respectively.

Discussion

In the present study, the authors have shown that the ectopic endometrium expressed the proteins CD133 and ABCG2, which are generally considered markers of stem cells. The advanced stage patients had increased expression of CD133 in ectopic endometrium compared with the early stage patients and the level of CD133 was positively correlated with R-AFS score of endometriosis. It is known that self-renewal, differentiation, and high proliferative capacity are key functional properties of adult stem cells [17]. Therefore, there are probably a subset of stem cells inside the endometriosis lesions, which may be the reservoir cells that allow the ectopic endometrial cells to remain active and promote the lesions' progression. The presence of stem cell markers in endometriotic lesions suggests the possible involvement of stem cells in the pathogenesis and the ectopic stem cells might lead to the establishment and progression of endometriosis. In addition, the monoclonal origin and the long-term culturing properties of cell clones established from endometriotic lesions, also support the stem cell hypothesis of endometriosis [18]. At present, many study groups have identified, isolated, and characterized endometrial stem/progenitor cells through a variety of methods such as clonogenicity, label-retaining cells, "side-population" cells, undifferentiation markers, and cellular differentiation [19-22]. The concurrent expressions of CD133 and ABCG2 in both eutopic and ectopic endometrium are circumstantial evidence that ectopic endometrial stem cells probably source from eutopic endometrial stem cells. Stem cells that are inappropriately shed during retrograde menstruation may contribute to the pathogenetic process, because their immense regenerative capacity promotes rapid clonal expansion [23, 24]. However, Du and Taylor used murine models to investigate the contribution of non-endometrial stem cells to endometriosis found that ectopic lesions also probably derived from ectopic differentiation of bone marrow stem cells [25].

Liu and Lang demonstrated that there are fundamental abnormal changes within the eutopic endometrium of women with endometriosis compared to normal endometrium of women without and thought that the character of eutopic endometrium determines the fate of the backward-flowing endometrial tissue [26]. The present study showed the expression of CD133 and ABCG2 in eutopic endometrium was significantly higher than in the endometrium of the control, hence they suppose that the endometrial stem cells with endometriosis have probably some specialities that are distinct from the endometrial stem cells without endometriosis, which cause them to shed more easily from uterine cavity and have increasing abilities to spread and attach in an ectopic site resulting in endometriotic lesions. In other words, aberrant expressions of CD133 and ABCG2 probably cause aberrant stem cell function, which may contribute to the pathogenesis of endometriosis.

Endometriosis is a benign disease but has a high recurrence rate even after excision and prevention by medical management [27]. In the present study two of three patients with recurrence after surgery combined with the administration of drugs for six months had significant stronger expression of ABCG2 in ectopic lesions than the patients without recurrence. Many investigations indicated that the stem cells resistant to chemotherapy or radiation is often characterized by an elevated expression of the stem cell surface marker ABCG2 [28, 29]. The present authors proposed that the ABCG2 positive ectopic endometrial cells are also probably resistant to medication and can still remain active and grow, form lesions again after treatment is concluded, which can explain the higher recurrence rate of endometriosis. Because the present sample size was too small, further study is needed to prove the relationship between ABCG2 and the recurrence of endometriosis.

In addition, endometriosis is considered a neoplastic disease with malignant potential [30,31] and the stem cell-like ability is one of the features shared by endometriosis and cancer [32]. Hence the present authors suggest that CD133 and ABCG2 positive cells transformation may be the underlying mechanism leading to the progression from endometriosis to ovarian cancer.

To conclude, the present results demonstrated that the expression of CD133 and ABCG2 in ectopic lesions indicates the existence of putative stem cells, which are probably sourced from the eutopic endometrial stem cells with aberrant functions, and can induce the development of endometriosis and also have relationship with the recurrence and the progression to ovarian cancer. CD133 and ABCG2 have the potential to be utilized as novel markers for predicting recurrence and malignancy and CD133 and ABCG2 positive cells have the possibility of becoming new therapeutic targets of endometriosis. In the future the present authors will isolate cells CD133+/ABCG2+ to show their biological characteristics and abilities associated with endometriosis, in order to check their expressions from the other stemness-related markers, to identify their colony-forming potential, self-renewal capacity, and multipotency, and to examine their functions in an animal model of endometriosis initiation or progression, and so on.

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References

[1] Paris K., Aris A.: "Endometriosis-associated infertility: a decade's trend study of women from the Estrie region of Quebec, Canada". *Gynecol. Endocrinol.*, 2010, *26*, 838.

[2] Meuleman C., Vandenabeele B., Fieuws S., Spiessens C., Timmerman D., D'Hooghe T.: "High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners". *Fertil. Steril.*, 2009, *92*, 68.

[3] Oliveira F.R., Dela Cruz C., Del Puerto H.L., Vilamil Q.T., Reis F.M., Camargos A.F.: "Stem cells: are they the answer to the puzzling etiology of endometriosis?" *Histol. Histopathol.*, 2012, *27*, 23.

[4] Maruyama T., Yoshimura Y.: "Stem cell theory for the pathogenesis of endometriosis". *Front. Biosci.*, 2012, *4*, 2854.

[5] Silveira C.G., Abrão M.S., Dias J.A. Jr., Coudry R.A., Soares F.A., Drigo S.A., *et al.*: "Common chromosomal imbalances and stemness-related protein expression markers in endometriotic lesions from different anatomical sites: the potential role of stem cells". *Hum. Reprod.*, 2012, *27*, 3187.

[6] Zeng B., Hu J., Yuan R., Hu L., Zhong L., Kang K.: "Increased expression of importin13 in endometriosis and endometrial carcinoma". *Med. Sci. Monit.*, 2012, *18*, CR361.

[7] Pacchiarotti A., Caserta D., Sbracia M., Moscarini M.: :Expression of oct-4 and c-kit antigens in endometriosis". *Fertil. Steril.*, 2011, *95*, 1171.

[8] Kao A.P., Wang K.H., Chang C.C., Lee J.N., Long C.Y., Chen H.S., *et al.*: "Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells and the development of an in vivo endometriotic invasion model". *Fertil. Steril.*, 2011, *95*, 1308.

[9] Corbeil D., Karbanová J., Fargeas C.A., Jászai J.: "Prominin-1 (CD133): molecular and cellular features across species". *Adv. Exp. Med. Biol.*, 2013, *777*, 3.

[10] Meregalli M., Farini A., Belicchi M., Torrente Y.: "CD133(+) cells isolated from various sources and their role in future clinical perspectives". *Expert Opin. Biol. Ther.*, 2010, *10*, 1521.

[11] Meng J., Chun S., Asfahani R., Lochmüller H., Muntoni F., Morgan J.: "Human skeletal muscle-derived CD133+ cells form functional satellite cells after intramuscular transplantation in immunodeficient host mice". *Mol. Ther.*, 2014, *22*, 1008. doi: 10.1038/mt.2014.26. Epub 2014 Feb 26.

[12] Ding X.W., Wu J.H., Jiang C.P.: "ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy". *Life Sci.*, 2010, *86*, 631.

[13] Hirata N., Nakagawa M., Fujibayashi Y., Yamauchi K., Murata A., Minami I., *et al.*: "A chemical probe that labels human pluripotent stem cells". *Cell Rep.*, 2014, *6*, 1165.

[14] Schwab K.E., Hutchinson P., Gargett C.E.: "Identification of surface markers for prospective isolation of human endometrial stromal colony-forming cells". *Hum. Reprod.*, 2008, *23*, 934.

[15] Hu F.F., Jing Xu, Cui Y.G., Qian X.Q., Mao Y.D., Liao L.M., *et al.*: "Isolation and characterization of side population cells in the postpartum murine endometrium". *Reprod. Sci.*, 2010, *17*, 629.

[16] American Society for Reproductive Medicine: "1997 Revised American Society for Reproductive Medicine classification of endometriosis". *Fertil. Steril.*, 1997, *67*, 817.

[17] Dittmer J., Leyh B.: "Paracrine effects of stem cells in wound healing and cancer progression (Review)". *Int. J. Oncol.*, 2014, *44*, 1789. doi: 10.3892/ijo.2014.2385. Epub 2014 Apr 11.

[18] Chan R.W., Ng E.H., Yeung W.S.: "Identification of cells with colony-forming activity, self-renewal capacity, and multipotency in ovarian endometriosis". *Am. J. Pathol.*, 2011, *178*, 2832.

[19] Gargett C.E., Schwab K.E., Zil wood R.M., Nguyen H.P., Wu D.: "Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium". *Biol. Reprod.*, 2009, *80*, 1136.

[20] Rossignoli F., Caselli A., Grisendi G., Piccinno S., Burns J.S., Murgia A., *et al.*: "Isolation, characterization, and transduction of endometrial decidual tissue multipotent mesenchymal stromal/stem cells from menstrual blood". *Biomed. Res. Int.*, 2013, *2013*, 901821. doi: 10.1155/2013/901821. Epub 2013 Mar 31.

[21] Miyazaki K., Maruyama T., Masuda H., Yamasaki A., Uchida S., Oda H.: "Stem cell-like differentiation potentials of endometrial side population cells as revealed by a newly developed in vivo endometrial stem cel assay". *PLoS One*, 2012, *7*, e50749.

[22] Gargett C.E., Nguyen H.P., Ye L.: "Endometrial regeneration and endometrial stem/progenitor cells". *Rev. Endocr. Metab. Disord.*, 2012, *13*, 235.
[23] Pittatore G., Moggio A., Benedetto C., Bussolati B., Revelli A.: "Endometrial adult/progenitor stem cells: pathogenetic theory and new antiangiogenic approach for endometriosis therapy". *Reprod. Sci.*, 2014, *21*, 296.
[24] Alickson J.G., Sanchez A., Yefimenko N., Borlongan C.V., Sanberg P.R.: "Recent studies assessing the proliferative capability of a novel adult stem cell identified in menstrual blood". *Open Stem Cell J.*, 2011, *3*, 4.
[25] Du H., Taylor H.S.: "Contribution of bone marrow-derived stem cells to endometrium and endometriosis". *Stem Cells*, 2007, *25*, 2082.
[26] Liu H., Lang J.H.: "Is abnormal eutopic endometrium the cause of endometriosis? The role of eutopic endometrium in pathogenesis of endometriosis". *Med. Sci. Monit.*, 2011, *17*, RA92.
[27] Koga K., Osuga Y., Takemura Y., Takamura M., Taketani Y.: "Recurrence of endometrioma after laparoscopic excision and its prevention by medical management". *Front. Biosci.*, 2013, *5*, 676.
[28] To K.K., Ren S.X., Wong C.C., Cho C.H.: "Reversal of ABCG2-mediated multidrug resistance by human cathelicidin and its analogs in cancer cells". *Peptides*, 2012, *40C*, 13.
[29] Mo W., Zhang J.T.: "Human ABCG2: structure, function, and its role in multidrug resistance". *Int. J. Biochem. Mol. Biol.*, 2012, *3*, 1.
[30] Munksgaard P.S., Blaakaer J.: "The association between endometriosis and ovarian cancer: A review of histological, genetic and molecular alterations". *Gynecol. Oncol.*, 2012, *124*, 164.
[31] Sova H., Kangas J., Puistola U., Santala M., Liakka A., Karihtala P.: "Down-regulation of 8-hydroxydeoxyguanosine and peroxiredoxin II in the pathogenesis of endometriosis-associated ovarian cancer". *Anticancer Res.*, 2012, *32*, 3037.
[32] Pollacco J., Sacco K., Portelli M., Schembri-Wismayer P., Calleja-Agius J.: "Molecular links between endometriosis and cancer". *Gynecol. Endocrinol.*, 2012, *28*, 577.

Address reprint requests to:
S. LIU, M.D.
Department of Obstetrics and Gynecology
The Fourth Affiliated Hospital of Jiangsu University
20 Zhengdong Street
212001 Zhenjiang City (China)
e-mail: leo423923@163.com