Integrins functioning in uterine endometrial stromal and epithelial cells in estrus

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Abstract

Here, as a basic study in the construction of a non-cellular niche that supports artificial organization of three-dimensional endometrial tissue, we defined the types of integrin heterodimers that are expressed transcriptionally, translationally and functionally in endometrial stromal (ES) and endometrial epithelial (EE) cells isolated from the mouse uterus in estrus. Gene and protein expression of integrin subunits were analyzed at the transcriptional and translational level by real-time PCR and fluorescent immunoassay, respectively. Moreover, the functionality of integrin heterodimers was confirmed by attachment and antibody inhibition assays. Itga2, Itga5, Itga9, Itgav, Itgb1, Itgb3 and Itgb5 in ES cells, and Itga2, Itga5, Itga6, Itga7, Itga9, Itgav, Itgb1, Itgb3, Itgb4, Itgb5 and Itgb6 in EE cells showed significantly higher transcriptional levels than the other integrin subunits. Furthermore, translational expression of the total integrin α and β subunit genes that showed increased transcription was determined in ES and EE cells. ES cells showed significantly increased adhesion to collagen I, fibronectin and vitronectin, and functional blocking of integrin α₂, α₅ or αᵥ significantly inhibited adhesion to these molecules. Moreover, EE cells showed significantly increased adhesion to collagen I, fibronectin, laminin and vitronectin, and functional blocking of integrin α₂, α₅ or αᵥ significantly inhibited adhesion to these molecules. Accordingly, we confirmed that integrin α₂β₁, α₅β₁, α₂β₃, α₅β₃ and/or α₂β₅, and integrin α₂β₁, α₅β₁, α₂β₃ and/or α₂β₅, actively function on the surface of ES and EE cells from mouse uterus in estrus phase, respectively.

Introduction

Uterine endometrial stromal (ES) and endometrial epithelial (EE) cells undergo dynamic periodic alterations during the reproductive period (Chan et al. 2004, Schwab et al. 2005). This is particularly evident during the estrus cycle, when the endometrium undergoes histological and functional remodeling (Tang et al. 2005, Arai et al. 2013), resulting in an increase in the receptivity of the endometrium to embryos (Ponsuksili et al. 2012, Chadchan et al. 2016). Therefore, during estrus, the endometrial microenvironment plays an important role in successful embryo implantation and pregnancy maintenance.

Endometrial tissue is structured as a three-dimensional (3D) microenvironment in vivo that consists of extracellular matrix (ECM) components and a number of distinct cell populations (Jokimaa et al. 2002, Yamada et al. 2002). The specificity of cells that constitute endometrial tissue at each stage of the estrus cycle is regulated by the alteration of 3D communication networks formed by the integration of cell-to-cell and cell-to-ECM contacts (Burghardt et al. 2002). To date, the endometrial microenvironment has been primarily constructed on two-dimensional culture plates rather than in 3D (Arnold et al. 2001), making it difficult to evaluate the effects of specific stimulations on endometrial tissue in vitro. Therefore, an in vitro mimic of the 3D endometrial microenvironment equivalent to endometrial tissue at each stage of the estrus cycle in vivo will be important for future studies.

The ECM, a non-cellular 3D macromolecular network composed of diverse fibrous ECM proteins, proteoglycans and glycoproteins (Kim et al. 2011, Theocharis et al. 2016) provides not only a physical scaffold to structure the 3D microenvironment but also signals a variety of cellular responses (Strauss 2013, Leppert et al. 2014). In particular, ECM-derived signals are transported to the cytoplasm through integrins that directly recognize components of the ECM (Rosso et al. 2004, Byron & Frame 2016), resulting in cytological alterations (Gronthos et al. 2001,
Vitillo et al. 2016). Therefore, the stimulation of ECM protein-derived signals through integrins makes it possible to accurately regulate the specificity of endometrial cells at each stage of the estrus cycle, which requires detailed information on the integrins that are functionally expressed on the membranes of cells that make up the endometrium.

As a step toward developing a defined non-cellular niche that uses integrin signaling to construct artificial and stereoscopic 3D endometrial tissues in mice, we examined the types of integrin heterodimers expressed on the membranes of ES and EE cells derived from the mouse uterus in estrus. Integrin subunits expressed in ES and EE cells were identified at the transcriptional and translational level, and the combinations of α and β integrin subunits were determined with functional assays.

Materials and methods

Animals

Six- or seven-week-old female ICR mice in estrus identified through vaginal cytological evaluation described previously (McLean et al. 2012) were used as uterus endometrium cell donors. All animal housing, handling and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Kangwon National University (IACUC approval No. KW-150327-3) and conducted according to the Animal Care and Use Guidelines of Kangwon National University.

Isolation of ES and EE cells from uterus

Uterine horns washed with Hank’s balanced salt solution (HBSS; Invitrogen) supplemented with 2% (v/v) antibiotic–antimycotic solution (Welgene Inc., Daegu, Korea) were split longitudinally and the tissue fragmented into fine pieces by surgical scissors was digested using 1.5 mg/mL collagenase (Sigma-Aldrich) in HBSS at 37°C for 45 min. Then, the digested cells were filtered through 100μm nylon mesh (SPL, Pocheon, Korea). A sedimentation step collected cell clumps in the tube bottom after separating the filtrated cells under unit gravity by incubating in a 15 mL tube at room temperature for 15 min. This procedure was repeated three times to remove debris included in cell clump. Subsequently, an adherent step separated attached ES and suspended EE cells by incubating the cell clumps on a 100 mm culture plate at 37°C for 10 min that was repeated twice. Subsequently, two types of cells were isolated and counted using a hemocytometer.

Real-time polymerase chain reaction

According to the manufacturer’s instructions, the Dynabeads mRNA Direct Kit (Ambion) was used for extracting total mRNA from the cells and cDNA was synthesized using the ReverTra Ace qPCR RT Master Mix with gDNA remover kit (Toyobo, Osaka, Japan). Then, the quantification of the specific gene expression was conducted using a THUNDERBIRD SYBR qPCR Mix (Toyobo) under the 7500 Real-time PCR system (Applied Biosystems), and melting curve date was analyzed for identifying PCR specificity. The mRNA level was presented as 2−ΔCt, where...
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Ct = threshold cycle for target amplification, ΔCt = Ct_target gene (specific genes for each sample) − Ct_internal reference (β-actin for each sample). Design of primer sequences by Primer3 software (Whitehead Institute/MIT Center for Genome Research) was performed with information of cDNA sequences obtained from GenBank for mouse, and Table 1 shows general information and sequences of primers.

**Immunocytochemistry**

The cells fixed with 4% (v/v) paraformaldehyde (Junsei, Tokyo, Japan) for 10 min were washed with Dulbecco’s phosphate-buffered saline (DPBS; Welgene), and the fixed cells were stained for 16 h at 4°C with fluorescence-conjugated anti-integrin antibodies diluted in DPBS. Table 2 describes the detailed information and dilution rate of the used antibodies. After rinsing with DPBS, the stained cells counterstained with mounting medium for fluorescence with DAPI (Vector Laboratories, Inc., Burlingame, CA, USA) were monitored under fluorescence microscope (BX53, Olympus).

**Fluorescence immunoassay**

The fixed cells were produced by incubating living cells in 4% (v/v) paraformaldehyde for 10 min. After washing twice with DPBS, they were stained for 2 h at room temperature with fluorescence-conjugated anti-integrin antibodies diluted in DPBS. Table 2 describes the detailed information and dilution rate of the used antibodies. Subsequently, the stained cells was washed with DPBS and fluorescence intensity was measured using SoftMax Pro 6.2.2. (Molecular Devices Cooperation, Sunnyvale, CA, USA) after adding 100 µL DPBS to the stained cells.

**Table 2** Antibodies used in experiments.

<table>
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<tr>
<th>Antibody name</th>
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<th>Company</th>
<th>Application</th>
<th>Dilution rate</th>
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*FI, fluorescence immunoassay, AIA, antibody inhibition assay, ICC, immunocytochemistry.

**Attachment assay**

In order to prepare ECM substrate for cell adhesion, 96-well tissue culture plates were, respectively, coated with following concentrations of the purified ECM proteins: 0, 5 and 10 µg/mL collagen I (Sigma-Aldrich) interacting with integrin α1β1, α1β3, (White et al. 2004; Znoyko et al. 2006; Heino 2007); 0, 40 and 80 µg/mL fibronectin (Millipore) interacting with integrin α5β1, α5β3, α5β1 and α5β3 (Sanchez-Aparicio et al. 1994; Muller et al. 1995; Su et al. 2016; Veqa & Schwarzbauer 2016); 0, 200 and 400 µg/mL laminin (Sigma-Aldrich) interacting with integrin α6β1, α6β3, α6β1 and α6β3 (Kikkawa et al. 2000; Nishiuchi et al. 2006); 0, 20 and 40 µg/mL tenascin C (R&D Systems) interacting with integrin α9β1, α9β3 and α9β5 (Yokosaki et al. 1994; Fiorilli et al. 2008); and 0, 5 and 10 µg/mL vitronectin (R&D Systems) interacting with integrin α5β1, α5β3, and β5 (Bodary & McLean 1990; Wayner et al. 1991; Delannet et al. 1994, Horton 1997) overnight at 4°C. Subsequently, for inhibiting non-specific binding of cells, each well was blocked with 1% (w/v) bovine serum albumin (BSA; Sigma-Aldrich) at 4°C for 1 h and washed three times with DPBS. Dulbecco’s modified Eagle’s medium: nutrient mixture F-12 (DMEM/F12; Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Welgene) and 1% (v/v) antibiotic–antimycotic solution was used as the culture medium to resuspend 5 × 10^4 cells. The resuspended cells were plated on to each well. After incubating at 37°C for 2 h, the removal of non-adherent cells were conducted by washing sufficiently each well and adherent cells were fixed in 4% (v/v) paraformaldehyde at room temperature for 10 min. Then, the fixed adherent cells were stained with 0.1% (w/v) crystal violet (Sigma-Aldrich) in 20% (v/v) methanol (Sigma-Aldrich) for 5 min. After washing twice with distilled water, the amount of adherent cells were quantified at 570 nm using a microplate reader (Epoch Microplate Spectrophotometer; BioTek Instruments Inc.) after

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adding 50μL of 0.2% (v/v) triton X-100 (Biopure, Cambridge, MA, USA) diluted with distilled water.

**Antibody inhibition assay**

Each well of 96-well tissue culture plate was coated with 5 μg/mL collagen I, 40 μg/mL fibronectin, 200 μg/mL laminin or 5 μg/mL vitronectin overnight at 4°C, and the coated wells was blocked with 1% (w/v) BSA for 1 h at 4°C. Subsequently, function of integrins was inhibited by incubating 5 × 10⁴ cells in DMEM/F12 culture medium including anti-integrin α₂ (HMA2), anti-integrin α₅ (5H10-27 (MFR5)), anti-integrin α₆ (NK1-GoH3) or anti-integrin α₅ (RMV-7) blocking antibody for 2 h at 37°C, and the detailed information regarding the used antibodies is described in Table 2. Next, the functionally blocked cells was plated on the each well and incubated at 37°C for 8 h. The non-adherent cells were removed by washing extensively with DPBS, the adherent cells were fixed in 4% (v/v) paraformaldehyde for 10 min at room temperature and the fixed adherent cells was stained with 0.1% (w/v) crystal violet in 20% (v/v) methanol for 5 min. Finally, the wells washed twice with distilled water were supplemented with 50 μL 0.2% (v/v) triton X-100 diluted with distilled water and the amount of dye was measured at 570 nm using a microplate reader.

**Statistical analysis**

The Statistical Analysis System (SAS) program was used for analyzing statistically all the numerical data shown in each experiment. Comparison among treatment groups were performed by the least-square or DUNCAN method, when significance of the main effects through variance (ANOVA) analysis was detected in the SAS package. Moreover, significant differences among treatments were determined when P value was less than 0.05.

**Results**

**Characterization of the integrin subunits expressed on the membranes of ES and EE cells derived from the mouse uterine endometrium in estrus**

To determine the types of integrin heterodimers expressed on the membranes of ES and EE cells in the uterine endometrium in estrus, we investigated the

![Figure 1](https://www.reproduction-online.org)
transcriptional and translational expression of integrin subunits. Transcriptional analysis of the genes encoding 17 α and 8 β integrin subunits revealed that ES cells showed significantly higher expression of integrin α2, α5, α6, α9 and αv (Fig. 1A), and integrin β1, β3 and β5 (Fig. 1B) subunit genes. EE cells showed significantly higher expression of integrin α4, α7, α9, and αv (Fig. 1C), and integrin β1, β3, β4, β5 and β6 (Fig. 1D) subunit genes. The minimum level of expression was detected for the integrin subunit genes α1, α2, α4, α7, α9, α10, α11, α5, αγ, αγ, αθ, αρ, αφ, αθ, αμ and ακ (Fig. 1A), and β2, β3, β5, β7 and β8 (Fig. 1B) in ES cells, and for α1, α2, α4, α7, α9, α10, α11, α5, αγ, αθ, αφ, αμ and ακ (Fig. 1C), and β2 and β7 (Fig. 1D) in EE cells. Moreover, in EE cells, the expression of the integrin α7 and α9 subunit genes with no significant difference from integrin α2 subunit gene was higher than the other integrin subunit genes with minimum expression (Fig. 1C). No transcription of the subunits α6, α8 (Fig. 1C) or β8 (Fig. 1D) was detected in EE cells.

We also examined translational regulation of the α and β integrin subunits that showed increased transcription. In ES cells, the expression of integrin α2, α5, α6, α9, αν, β1, β3 and β5 subunit proteins was observed on the surface of cells (Fig. 2A, B, C, D, E, F, G and H), and the integrin subunit proteins α5 (Fig. 3A) and β3 (Fig. 3B) had the strongest expression among the five α (α2, α5, α6, α9 and αν) and three β (β1, β3 and β5) integrin subunits. In addition, among the α (α2, α5, α6, α9 and αν) and β (β1 and β3) subunits with significantly weaker expression than α5 and β3, significantly stronger expression was detected in integrin α9 and αν subunit proteins (Fig. 3A) compared with α5 and αν, and no significant difference in the expression of β1 and β3 subunits was observed (Fig. 3B). In EE cells, the localization of integrin α2, α5, α6, α9, αν, αν, β1, β3 and β5 subunit proteins was identified on the surface of cells (Fig. 2I, J, K, L, M, N, O, P, Q and R). The strongest expression among the six α (α2, α5, α6, α9, αν, and αν) and four β (β1, β3, β5, and β6) integrin subunits was detected for α5 (Fig. 3C) and β3 (Fig. 3D), whereas significantly lower expression was observed for the other integrin α (α2, α5, α6, α9, and αν) and β (β1, β3, and β5) subunits. The expression of α integrin subunits did not show any significant differences among the α2, α5, α6, α9, αν and β3 subunits (Fig. 3C), and the integrin β3 subunit

Figure 2 Translational expression of α and β integrin subunit genes highly expressed in ES and EE cells of mouse uterine tissue. Using a sedimentation-adherence method, ES and EE cells were isolated from endometrial cells retrieved enzymatically from uterus derived from ICR mice. Translational expression of α and β integrin subunit genes in the sorted ES and EE cells were identified by immunocytochemistry. As a result, integrin α2, α5, α6, α9, αν, β1, β3 and β5 subunit proteins (A, B, C, D, E, F, G and H) were localized on the surface of ES cells, and the surface of EE cells showed the expression of integrin α2, α5, α6, α9, αν, β1, β3 and β5 subunit proteins (I, J, K, L, M, N, O, P, Q and R). All figures are representative immunocytochemistry images of integrin subunit proteins expressed on the surface of ES and EE cells, respectively. n=3. Scale bars represent 20μm.
showed the highest significant expression among the β1, β4 and β5 subunits (Fig. 3D). These results indicate that ES cells present α2, α5, α6, α9 and αv, and β1, β3 and β5 integrin subunits, and EE cells present α2, α5, α6, α9 and αv, and β1, β3, β4 and β5 integrin subunits on the cell surface.

**Functional identification of integrin heterodimers expressed on the membranes of ES and EE cells derived from mouse uterine endometrium in estrus**

Based on the identification of the α and β integrin subunits expressed on the membranes of ES and EE cells derived from mouse uterine endometrium in estrus, we surmised that ES cells possess α2β1, α5β1, α6β1, α9β1, αvβ1, α2β3, α9β3, and αvβ3, and EE cells possess α2β1, α5β1, α6β1, α2β6, α9β6, α3β1, α6β1, α9β1, and αvβ3 as active forms of integrin heterodimers, as described previously (Lessey 1988, Reddy & Mangale 2003). The presence of these integrin heterodimers was investigated by estimating levels of adherent ES and EE cells cultured on purified ECM proteins that interact specifically with each integrin heterodimer. Post-culture adherent levels of ES and EE cells treated with antibodies specifically blocking the function of each integrin were determined.

Compared with purified ECM-protein-free culture plates, ES cells showed significantly improved adhesion to collagen I- (Fig. 4A), fibronectin- (Fig. 4B) and vitronectin-coated (Fig. 4E) culture plates, and EE cells to collagen I- (Fig. 4F), fibronectin- (Fig. 4G), laminin- (Fig. 4H) and vitronectin-coated (Fig. 4I) culture plates. Moreover, no significant differences in adhesion levels were observed in ES cells cultured on laminin- (Fig. 4C) or EE cells cultured on tenasin C-coated (Fig. 4J) culture plates. Rather, ES cells cultured on tenasin C-coated culture plates showed a significant decrease in the level of adhesion, compared to those cultured on purified ECM-protein-free culture plates (Fig. 4D). These results suggest the presence of the collagen I-specific integrin α2β1, fibronectin-specific integrin α5β1 and vitronectin-specific integrin αvβ1, α6β1 or α9β1 on the cell membrane of mouse ES cells, with the presence of individual integrin subunits α2 and α5. Simultaneously, we speculate the presence of the collagen I-specific integrin

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**Reproduction** (2017) **153** 351–360

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α₂β₁; fibronectin-specific integrin α₅β₁; laminin-specific integrins α₆β₁, α₆β₄ and/or α₇β₁; and vitronectin-specific integrins αᵥβ₁, αᵥβ₃ and/or αᵥβ₅ on the cell membrane of mouse EE cells, with the presence of the individual integrin subunit α₉.

These specific integrin function-blocked mouse ES or EE cells were incubated on 5 μg/mL collagen I, 40 μg/mL fibronectin, 200 μg/mL laminin or 5 μg/mL vitronectin as the minimum concentration among those seen in the ECM showing significantly improved adhesion of ES or EE cells (Fig. 5). Significantly lower adhesion was detected in ES cells with blockage of the integrin subunit α₂ (Fig. 5A), α₅ (Fig. 5B) or αᵥ (Fig. 5C), and in EE cells with blockage of the integrin subunit α₂ (Fig. 5D), α₅ (Fig. 5E), α₆ (Fig. 5F) or αᵥ (Fig. 5G), compared with cells without blockage of those integrin heterodimers.

From these results, we could confirm that the mouse endometrial ES cells derived from the uterus in estrus

Figure 4 Identification of integrin heterodimers that interact with fibronectin, laminin and vitronectin on the membrane of ES and EE cells derived from the mouse uterus. A 96-well tissue culture plate was coated with collagen I, fibronectin, laminin, tenascin C, vitronectin, and ES or EE cells resuspended in culture medium were plated in each well. Subsequently, adherent cells were stained with crystal violet, and the level of adhesion was quantified using a microplate reader. The percentage of maximum adhesion is represented as the optical density of cells plated on extracellular matrix (ECM) protein-free plates. Both mouse ES and EE cells cultured on collagen I- (A, F), fibronectin- (B, G) and vitronectin-coated (E, I) culture plates showed significantly higher levels of adhesion than those on ECM protein-free culture plates. However, no significant difference or significant decrease in adhesion level was detected in ES cells cultured on laminin- (C) and tenascin C-coated (D) culture plates, respectively. In addition, EE cells cultured on laminin- (H) or tenascin C-coated (I) culture plates showed significant improvement, or no significant difference, in adhesion levels compared to those on ECM protein-free culture plates. All data shown are mean ± s.d. of three independent experiments. *, **P < 0.05.

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Figure 5 Functional analysis of integrin heterodimers on the membrane of ES and EE cells derived from the mouse uterus. Mouse ES and EE cells incubated in the absence or presence of anti-integrin α₂, anti-integrin α₅, anti-integrin α₆ or anti-integrin αᵥ blocking antibodies were plated on 5 μg/mL collagen I, 40 μg/mL fibronectin, 200 μg/mL laminin or 5 μg/mL vitronectin-coated wells, and incubated for 2 h at 37°C. After staining adherent cells with crystal violet, the level of adherence was quantified using a microplate reader. As the parameter of functional blocking by antibodies, the percentage of maximum adhesion, represented by the optical density of cells plated on each ECM protein-coated well in the absence of blocking antibodies was determined. Mouse ES cells treated with integrin α₂ (A), α₅ (B) and αᵥ (C) subunit blocking antibodies showed significantly lower rates of attachment to collagen I, fibronectin and vitronectin compared with those without blocking antibodies, respectively. Moreover, compared with those not treated with blocking antibodies, functional blocking of integrin α₂ (D), α₅ (E), α₆ (F) and αᵥ (G) subunits in EE cells significantly decreased the rates of attachment to collagen I, fibronectin, laminin and vitronectin, respectively. All data shown are mean ± s.d. of three independent experiments. *P < 0.05.
simultaneously express the functional integrins αβ₁, αβ₃, αβ₅, αβ₇, and/or αβ₉ on the cell membrane, and integrins αβ₁, αβ₅, αβ₁, and/or αβ₅, and αβ₁, αβ₁, and/or αβ₁ on the membrane of mouse uterine endometrial EE cells.

Discussion

Knowledge of the ECM-derived signals that mediate intracytoplasmic transduction through cell surface receptors that belong to the integrin family will be essential in the construction of an artificial microenvironment that accurately reflects the in vitro organization of the endometrium, including in terms of ES and EE cells. Identification of the integrins expressed in ES and EE cells will be particularly important. Here, we report the types of integrin heterodimers expressed on the surface of ES and EE cells derived from the mouse endometrium during estrus. Transcriptional analysis of 17 α and 8 β integrin subunits, followed by confirmation of their expression at the translational level, attachment to ECM proteins and inhibition with blocking antibodies revealed the presence of integrin αβ₁, αβ₁, αβ₃, αβ₅, and/or αβ₇, as heterodimers, and αβ₆ and αβ₉ as individual subunits on the ES cell membrane. EE cells expressed the integrin heterodimers αβ₁, αβ₃, αβ₁, and/or αβ₅, and αβ₁, αβ₁, and/or αβ₅, and the individual subunits αβ and αβ on the cell membrane. These results suggest that the collagen I-specific integrin αβ₁, fibronectin-specific integrin αβ₁ and vitronectin-specific integrins αβ₁ or αβ₁ in ES cells, and the collagen I-specific integrin αβ₁, fibronectin-specific integrin αβ₁, laminin-specific integrins αβ₁, αβ₁, or αβ₁ and vitronectin-specific integrins αβ₁, αβ₁, or αβ₁ in EE cells may be important for the transmission of extracellular signals that organize endometrial tissue. Moreover, collagen I, fibronectin, laminin and vitronectin analogs will be important for the construction of niches customized to the organization of endometrial tissue. In addition, we speculate that the integrin subunits αβ₁, αβ₁ in ES cells, or αβ₁ and αβ₁ in EE cells, may play an important role in the cytological changes of ES or EE cells generated during the estrus cycle.

Generally, functional integrins are organized as heterodimers of α and β subunits (Multhaupt et al. 2016, Pan et al. 2016). These heterodimeric transmembrane receptors are activated by direct interaction with a binding motif embedded in ECM proteins (Brizzi et al. 2012, Seguin et al. 2015) and they induce a variety of biological responses (Hynes 2009, Schaefer & Reinhardt 2016). Therefore, despite the transcriptional and translational expression of the αβ₁ and αβ₁ integrin subunit genes in ES cells (Figs 1, 2 and 3), the lack of a significant increase in the adhesion of ES cells to laminin (Fig. 4C) or tenascin C (Fig. 4D) was observed. These results indicate that αβ₁ or αβ₁ are localized in the inactive form as an individual subunit, and not as the active heterodimer, on the surface of the cell membrane. Furthermore, EE cells with the αβ₁ and αβ₁ subunits that recognize laminin exhibited a significant decrease in adhesion resulting from blockade of the αβ₁ subunit (Fig. 5F), indicating that αβ₁ is present as a heterodimer and αβ₁ as an individual subunit. There was also no significant difference in the adhesion level of EE cells to tenascin C (Fig. 4I), indicating that αβ₁ is present as an individual subunit on the cell membrane. These results are strongly supported by previous studies reporting the absence of laminin in ES tissue (Faber et al. 1986) and tenascin C in both ES (Julian et al. 1994, Michie & Head 1994) and EE tissue (Michie & Head 1994).

Interestingly, integrins αβ₁ and αβ₁ heterodimers that interact specifically with laminin were only observed on the surface of EE cells in estrus, and αβ₁ identified as an individual subunit in the inactive form, are localized on the membrane of ES cells in estrus. This suggests that laminin-specific integrin heterodimers may play an important role in endometrial receptivity for implantation, which is supported by reports on the expression of laminin in the trophectoderm (Klaffky et al. 2006) and failed pregnancy in the laminin-deficient mouse (Zhang et al. 2000). Furthermore, the expression of laminin-specific integrin heterodimers in mouse EE cells has also been shown in humans (Koks et al. 2000, Park et al. 2000). Accordingly, at the clinical level, the presence of functional integrin heterodimers with the αβ₁ subunit may be important for the diagnosis of healthy uterine epithelium.

The uterine endometrium experiences physical and physiological alterations during implantation (Kao et al. 2002). The presence of inactive integrin subunits prior to implantation of the embryo can induce dramatic changes in the uterine endometrium through post-adhesion activation of embryos. In this study, specific integrin subunits (αβ₁ and αβ₁ in ES cells, and αβ₁ and αβ₁ in EE cells) were present in an inactive form on the cell membrane, indicating that signals derived from laminin (interacting with heterodimers with integrin αβ₁ or αβ₁ subunits) and tenascin C (interacting with heterodimers with integrin αβ₁ subunits) may be important in the remodeling of ES and EE tissue following embryo contact with EE cells. This is supported by the endometrial identification of laminin and tenascin C during pregnancy (Michie & Head 1994, Kaloglu & Onalıoğlu 2010). Accordingly, research on the expression of inactive integrin subunits in specific cells may be useful in the generation of specific microenvironments.

In conclusion, the co-expression of integrin αβ₁, αβ₁, αβ₁, αβ₁, and/or αβ₁ and integrin αβ₁, αβ₁, αβ₁, and/or αβ₁ on the surface of ES and EE cells derived from mouse endometrium in estrus, respectively, was confirmed. Integrin αβ₁ and αβ₁ or αβ₁ and αβ₁ were also identified as individual subunits on the membranes of ES or EE cells. Identification of the integrin heterodimers or subunits expressed on ES...
and EC cells will be useful for determining the type of ECM analogs that specifically activate each integrin heterodimer to generate synthetic niches to represent endometrial tissue in vivo. Moreover, the generation of synthetic endometrial tissue based on this knowledge will contribute to our understanding of the mechanism of implantation, and aid in the study of uterine receptivity and endometrial hormonal responses.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research was supported by a grant of the Korea Health Technology R&D Project (HI12C1404(A121515)), Ministry of Health and Welfare, Republic of Korea.

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Received 20 September 2016
First decision 6 October 2016
Revised manuscript received 23 November 2016
Accepted 19 December 2016