Transcriptional Analysis of Genes Encoding Proteins Presented in the Extracellular Space of Endometrial Stromal Cells in Human

Hye Jin Park[a], Seung Tae Lee[a,b,*], Jung Im Yun[c,*]

[a]Department of Animal Life Science, [b]Department of Applied Animal Science, and [c]Institute of Animal Resources, Kangwon National University, Chuncheon 24341, Korea
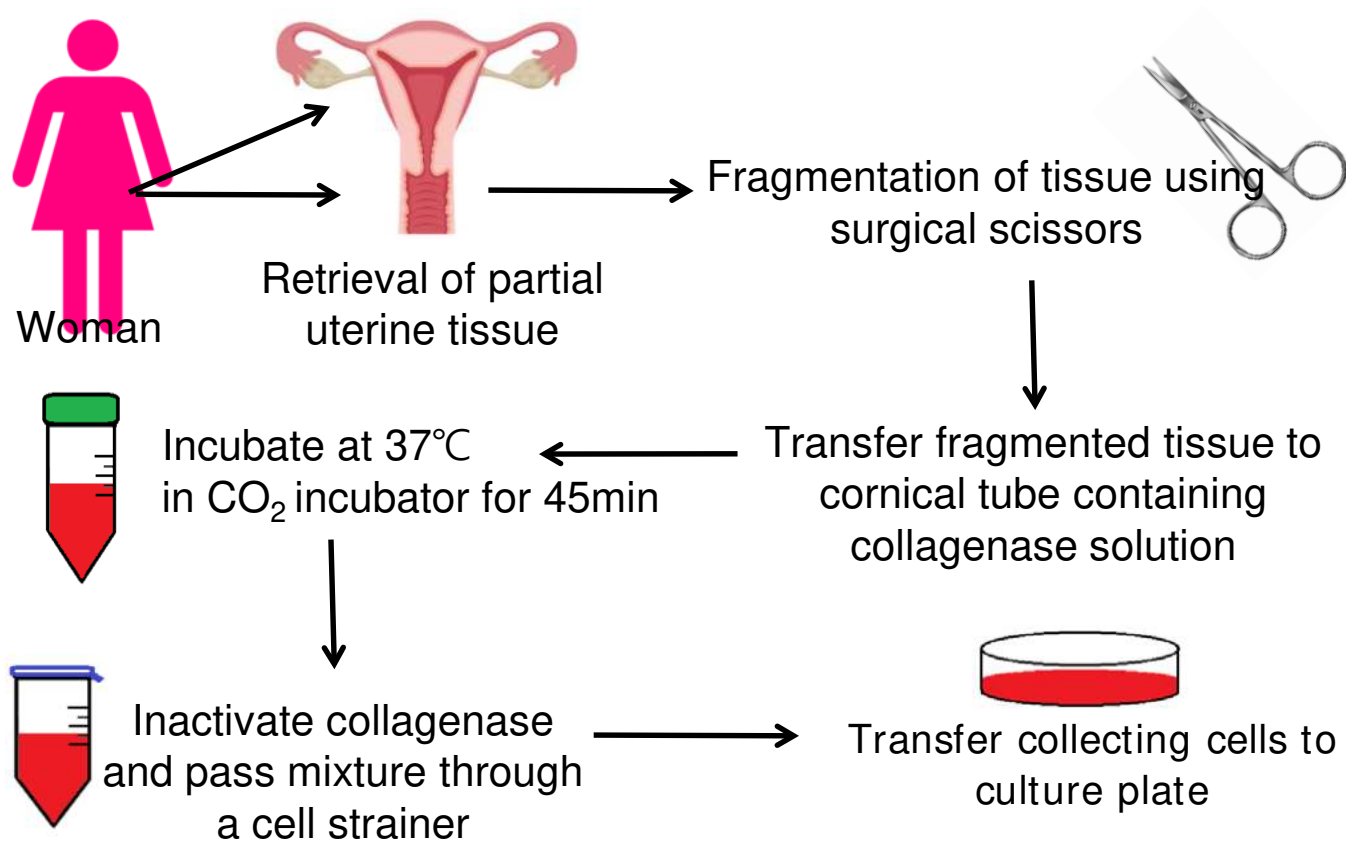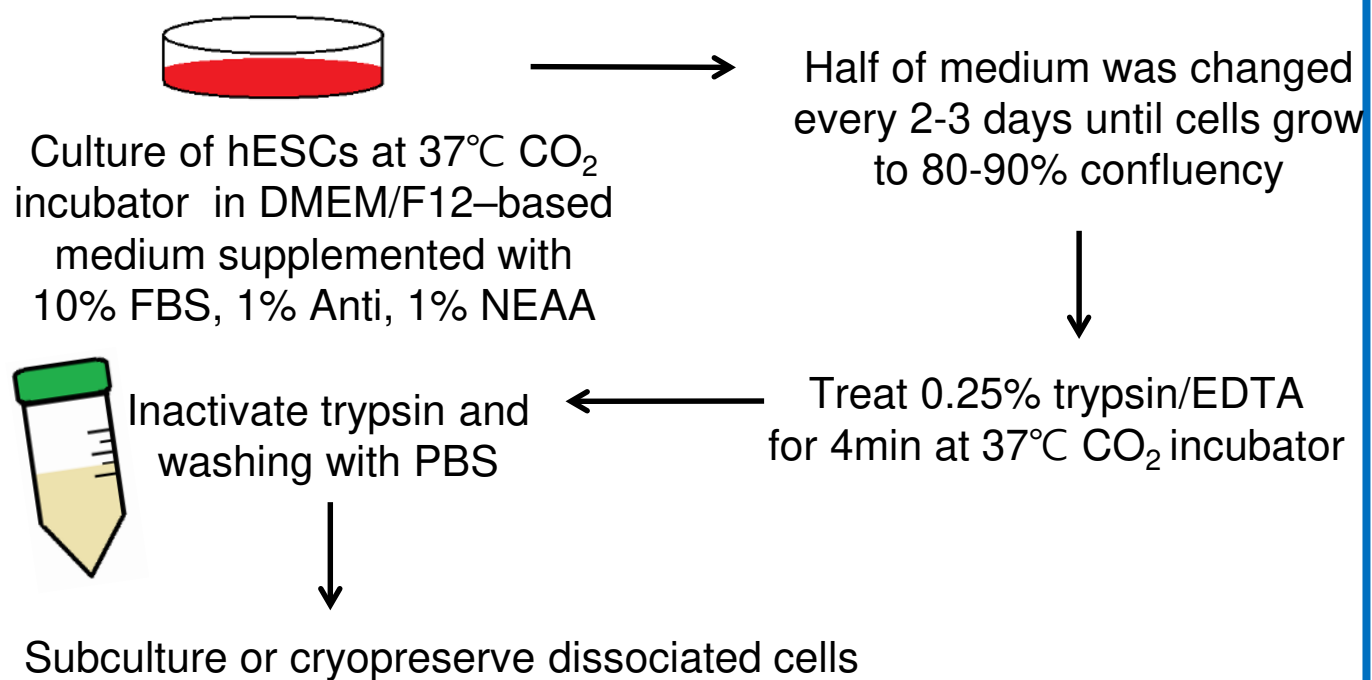
Introduction

In *in-vivo* endometrium, endometrial cells experience dramatic alterations during the menstrual cycle by a variety of hormones secreted from sexual organs. These hormonal regulation make it difficult to study physiological and pharmacological aspects of endometrial biology. Therefore, organization of artificial uterine tissues through construction of non-cellular niche mimicking microenvironment enclosing endometrium is important for eliminating chaotic treatment effects detected in the implantation-related studies. Accordingly, as a step towards constructing a precisely defined three-dimensional microenvironment engineering extracellular signaling to regulate physiological characteristics of endometrial cells derived from human uterus, information on microenvironment factors presented in the extracellular space of human endometrial stromal cells (hESCs) should be requested
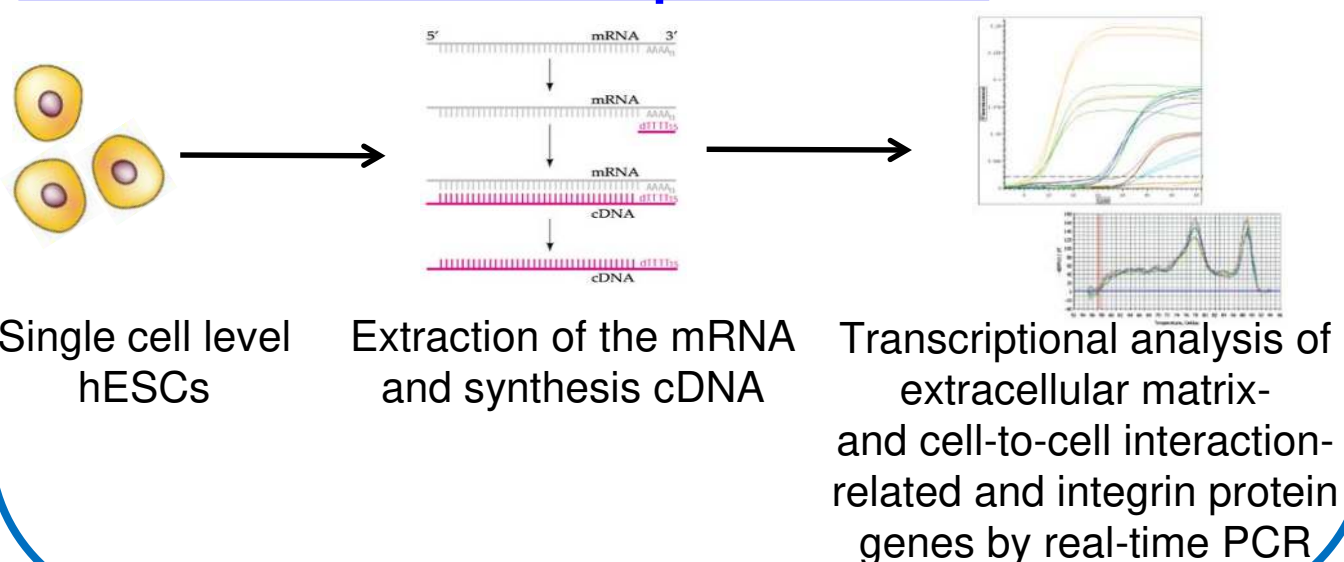
Materials and Methods

Isolation of hESCs from Endometrium

Woman → Retrieval of partial uterine tissue → Fragmentation of tissue using surgical scissors → Transfer fragmented tissue to cornical tube containing collagenase solution → Incubate at 37℃ in CO_2 incubator for 45min → Inactivate collagenase and pass mixture through a cell strainer → Transfer collecting cells to culture plate

Culture of hESCs

Culture of hESCs at 37℃ CO_2 incubator in DMEM/F12–based medium supplemented with 10% FBS, 1% Anti, 1% NEAA → Half of medium was changed every 2-3 days until cells grow to 80-90% confluency → Treat 0.25% trypsin/EDTA for 4min at 37℃ CO_2 incubator → Inactivate trypsin and washing with PBS → Subculture or cryopreserve dissociated cells

Measurement of Transcriptional levels

Single cell level hESCs → Extraction of the mRNA and synthesis cDNA → Transcriptional analysis of extracellular matrix- and cell-to-cell interaction-related and integrin protein genes by real-time PCR

Results

Figure 1. Transcriptional levels of the extracellular matrix- and cell-to-cell interaction-related protein genes in the hESCs. The hESCs were isolated by enzymatic method and mRNA level was measured by real-time PCR. Total 12 extracellular matrix-related protein genes were quantified, and *COLI* and *COLIII* showed significantly higher expression levels than other genes. By contrast *FN*, *NID*, *LN*, *TN*, *ELN*, *COLIV* and *COLV* were weakly transcribed (A). Among 11 cell-to-cell interaction-related protein genes, *CDH2* showed significantly the strongest transcription compared to the other genes. By contrast *CDH1*, *CDH3*, *DSG2*, *ICAM1*, *HSPG2*, *VCAN* and *SDC1* were weakly transcribed (B). All data shown are means $\pm$ SD of 3 independent experiments. [*,**]$p<0.05$. ND=not detected.
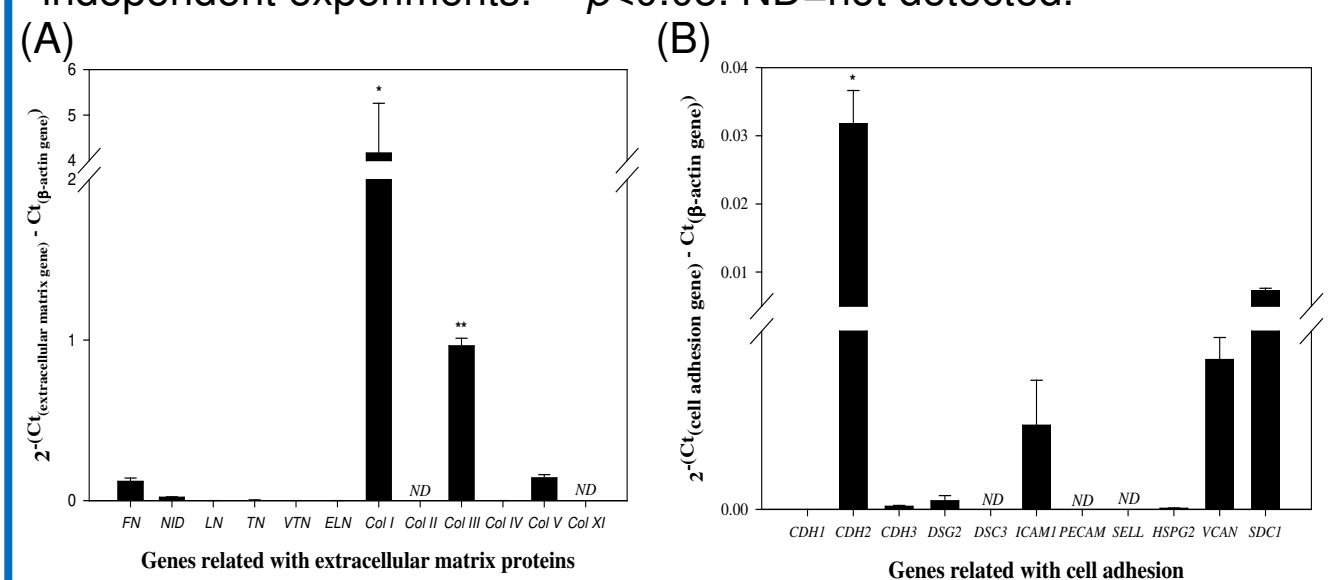

Figure 2. Transcriptional levels of the integrin α and β subunit genes in the hESCs. The hESCs were isolated by enzymatic method and mRNA level was measured by real-time PCR. Total 25 integrin subunit genes were quantified and integrin α_1, α_3, α_4, and α_v (A) and integrin β_1, β_3, and β_5 (B) subunit genes showed significantly higher expression levels than other subunits. By contrast, integrin α_2, α_5, α_6, α_7, α_8, α_9, α_{10}, α_{11}, α_L, and α_X (A) and integrin β_2, β_4, and β_8 (B) subunit genes were weakly transcribed. All data shown are means $\pm$ SD of 3 independent experiments. [*,***]$p<0.05$. ND=not detected.
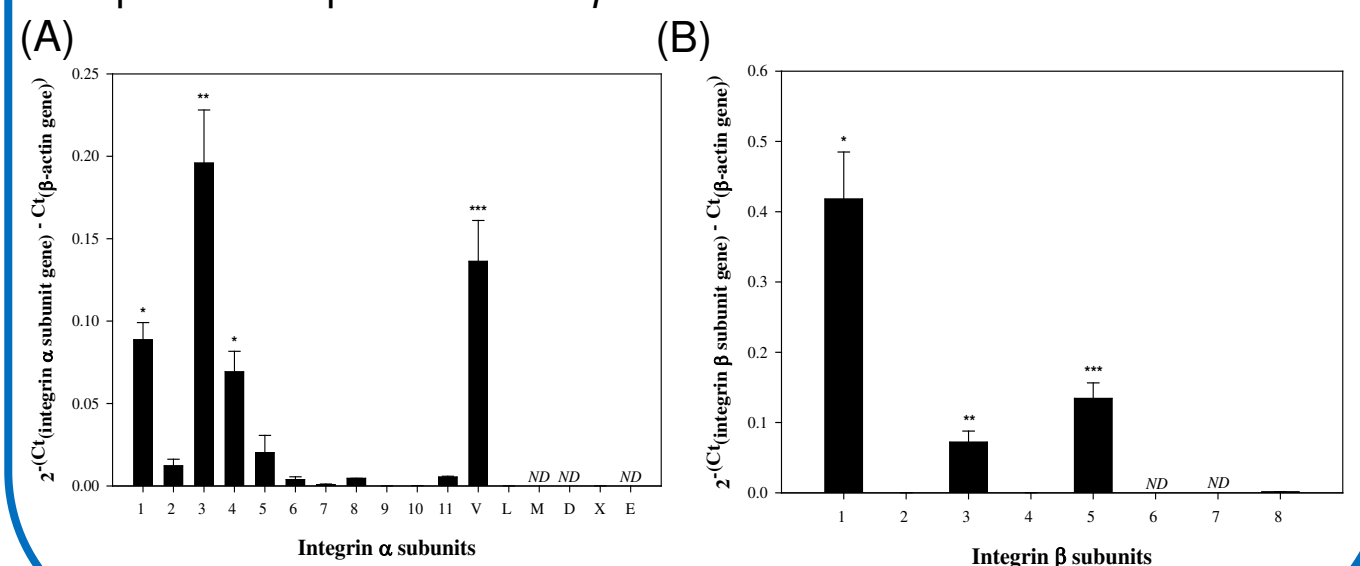
Conclusion

In conclusion, we could identify types of extracellular matrix- (*COLI* and *COLIII*), cell-to-cell interaction- (*CDH2*), and integrin-related (*ITGA1*, *ITGA3*, *ITGA4*, *ITGAV*, *ITGB1*, *ITGB3* and *ITGB5*) protein genes significantly up-regulated transcriptionally in hESCs. Moreover, these results will contribute greatly to develop non-cellular niche mimicking endometrium

Acknowledgement