

(Decidualization)  
TGF- $\beta$  (Transforming Growth Factor- $\beta$ )

1, 2, 3, 2, 2, 2, 3, 2

**Transforming Growth Factor- $\beta$  is a Possible Paracrine Mediator  
in the Human Endometrial Decidualization**

**Dong Wook Park<sup>1,2</sup>, Dong Soon Choi<sup>1</sup>, Mi Ran Kim<sup>2</sup>, Kyung Joo Hwang<sup>2</sup>,  
Mi Yeong Jo<sup>2</sup>, Seong Hee Ahn<sup>2</sup>, Churl K Min<sup>3</sup>, Hee Sug Ryu<sup>2</sup>**

<sup>1</sup>Department Molecular Science and Technology, <sup>2</sup>Department of Obstetrics and Gynecology,  
School of Medicine, <sup>3</sup>Department of Biological Science, Ajou University, Suwon, Korea

**Objectives:** To investigate the role of TGF (Transforming growth factor- $\beta$ ) involved in the paracrine communication during decidualization between UEC (uterine epithelial cells) and USC (uterine stromal cells), we have employed a co-culture system composed of human endometrial epithelial and stromal cells in defined hormonal conditions.

**Design:** In the co-culture, endometrial epithelial cells cultured in the matrigel-coated cell culture insert are seeded on top of the endometrial stromal cells cultured within a collagen gel. The co-culture was maintained for 48 hours under the following hormonal conditions: progesterone dominant condition (100 nM P4 and 1 nM E2) or estrogen-dominant condition (100 nM E2 and 1 nM P4). 10 ng/ml HGF and/or 10 ng/ml TGF- $\beta$ 1 are added.

**Methods:** RT-PCR is utilized to detect mRNAs quantitatively. Enzyme-linked immunosorbent assay (ELISA) and immunohistochemical staining are utilized to detect proteins in the tissue.

**Results:** Prolactin mRNA is expressed in the co-cultured stromal cells under the progesterone dominant condition. TGF- $\beta$ 1 and its receptors are expressed in both the co-cultured epithelial and stromal cells irrespective of the steroid present, which is in contrast with no or negligible expression of TGF- $\beta$ 1 or its receptor in cells separately cultured. Both estrogen and progesterone significantly elevate the concentration of hepatocyte growth factor (HGF) in the conditioned medium of the co-culture with the value of 4,325 pg/ml in E2-dominant and 2,000 pg/ml in P4-dominant condition compare to 150 pg/ml in no hormone. In separately cultured stromal cells, administration of HGF induces the expression of TGF receptor 1 in both hormonal conditions, but induction of TGF receptor 2 is only manifest in the P4-dominant condition. Administration of TGF- $\beta$  and HGF directly induce the decidualization marker

: , ) 442-749 5  
Tel: (031) 219-5300, Fax: (031) 219-5245, e-mail: kmr5300@ajou.ac.kr  
: , ) 442-749 5

Tel: (031) 219-5300, Fax: (031) 219-5245, e-mail: kmr5300@ajou.ac.kr  
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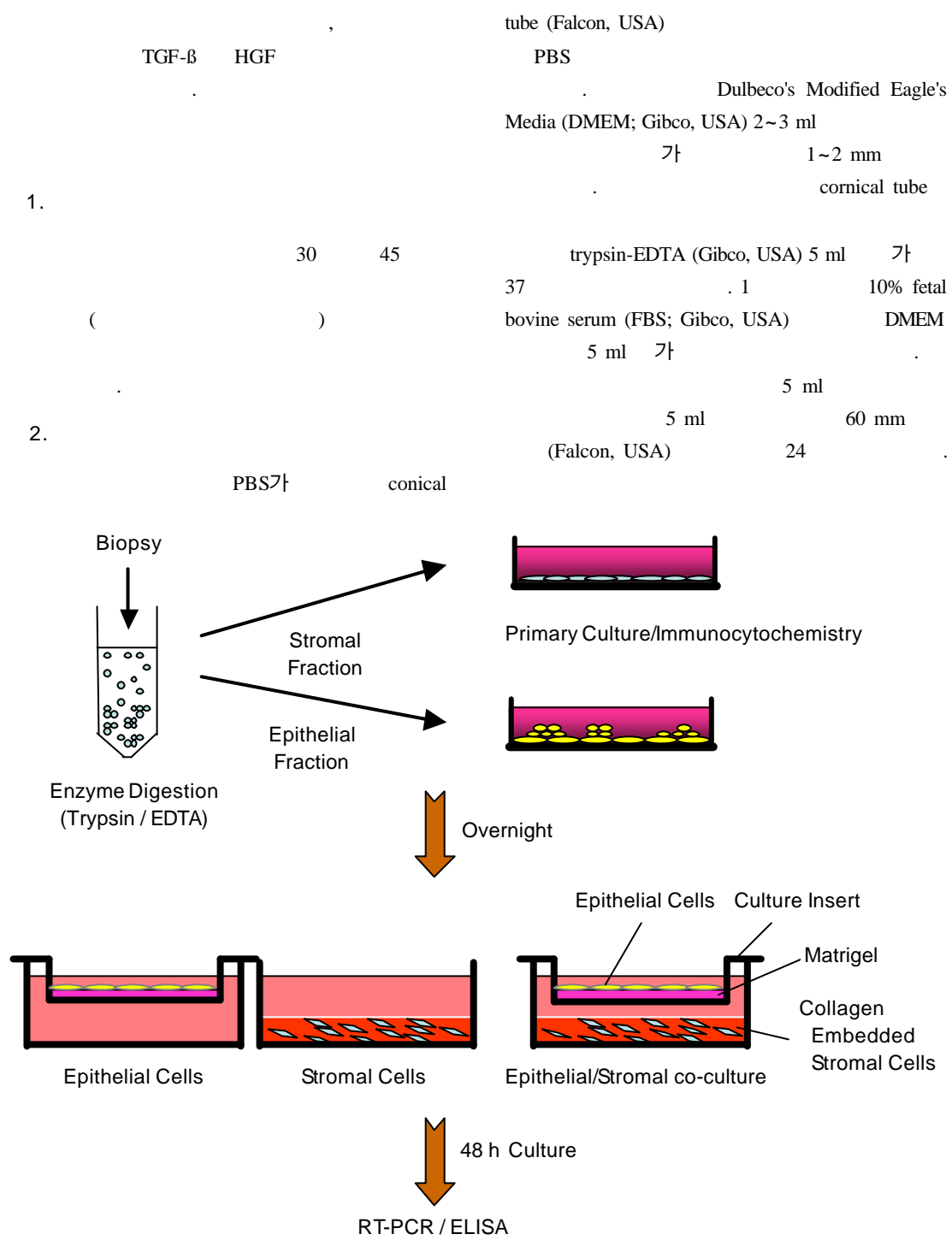
(01-PJ11-PC9-01NT00-0036 to C.K.M).

prolactin mRNA in separately cultured stromal cells.

**Conclusion:** It is likely that steroid hormones induces prolactin mRNA indirectly by promoting the cell to cell communication between the stromal and the epithelial cells. TGF- $\beta$  and HGF are two possible paracrine mediators in the human endometrial decidualization.

**Key Words:** Decidualization, TGF- $\beta$ , HGF, Co-culture, Paracrine

first trimester decidua villous  
TGF- $\beta$ 가  
(endometrial stromal cell; ESC) (placentation) 가  
HGF (Hepatocyte growth factor)  
(decidualization)  
(blastocyst) .<sup>16</sup> HGF  
(functional layer) (lumen formation)  
,<sup>17</sup> (trophoblast)  
,<sup>18</sup> ,  
HGF 가  
Transformin growth factor- $\beta$  (TGF- $\beta$ ) 25 kD  
homodimeric <sup>5</sup> <sup>6</sup> <sup>19</sup>  
,<sup>7</sup> <sup>8</sup> 가  
,<sup>9</sup> ,  
TGF- $\beta$  mRNA Irwin <sup>20</sup>  
progesterone estrogen 가 decidualization  
TGF- $\beta$  progesterone 가  
,<sup>10</sup> progesterone (10 )  
matrix metalloproteinase (in vivo decidualization)  
,<sup>11</sup> , <sup>2</sup>  
TGF- $\beta$  MMP  
paracrine mediator 가 <sup>21</sup> Bentin-Ley <sup>22</sup> 2  
TGF- $\beta$  (latent pro- collagen gel  
form) plasmin prote- <sup>3</sup>  
olytic cleavage <sup>13</sup> , <sup>3</sup>  
TGF- $\beta$  가  
TGF- $\beta$  type 2  
type 1 type TGF- $\beta$  mRNA  
1 Smad-2 -3 RT-PCR , <sup>3</sup>  
Smad-4 가  
HGF 가 ELISA  
,<sup>14</sup> Ando <sup>15</sup> TGF- $\beta$  subtype  
(TGF- $\beta$ 1,  $\beta$ 2,  $\beta$ 3) type-1, type-2 mRNA가



**Figure 1.** A schematic drawing of the culture system. Endometrial biopsy was minced and separated into respective epithelial and stromal fractions. Each fraction was cultured overnight. In the co-culture, endometrial epithelial cells cultured in the matrigel-coated cell culture insert are seeded on top of the endometrial stromal cells cultured within a collagen gel. The cultures were maintained for 48 hours under each hormone condition.

3.

Cytokeratin, vimentin Met

matrigel (Biocoat, USA) coating cell culture

insert (Sigma, USA) 6 well

(Nunc A/S, Denmark) 10% FBS가

24

trypsin-EDTA 2 ml 가 10

5 ml 가 400 rpm 5

cells/ml collagen (Biocoat, USA) 5 × 10<sup>6</sup>

6 well (Nunc A/S, Roskilde, Denmark)

500 µl 37 1 col-

lagen . 3

10% FBS (Gibco, USA)가 가

24

48

(Figure 1).

progesterone 100

nM progesterone (Sigma, USA), 1 nM estrogen (Sigma, USA) 가 , estrogen

100 nM estrogen, 1 nM progesterone

. 10 nM/ml TGF-β1 (Sigma, USA) 10 nM/

ml HGF (Sigma, USA)

PBS 3

paraformaldehyde 가 4% PBS

4% H<sub>2</sub>O<sub>2</sub> 5

peroxydase

PBS 1/400 cyto-

keratin (Anti-human mouse mono-

clonal; Santa Cruz Biotechnology Inc., USA), vimentin

(Anti-human goat polyclonal; Santa

Cruz Biotechnology, USA) Met

(Anti-human rabbit polyclonal; Santa Cruz Biotechno-

logy Inc., USA) 1 cham-

ber LSAB-kit (DAKO A/S, Den-

mark) 15

PBS diaminobenzidine

(DAB; DAKO A/S, Denmark)

4. RT - PCR

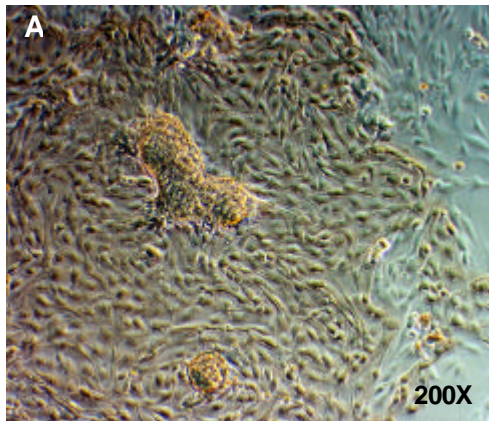
TRIzol reagent (Invitrogen life technology, Nether-

land) RNA

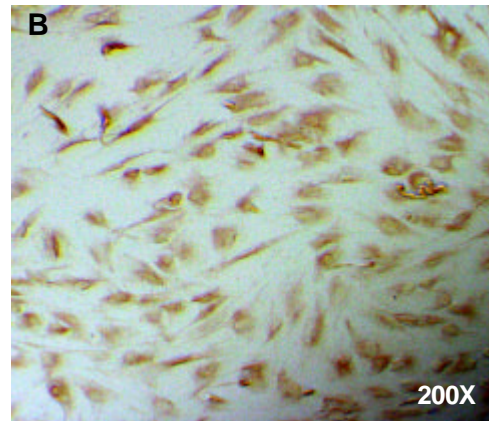
RNA 260 nm 280 nm

**Table 1.** PCR primers

| Product     | Sequence  | Product size |
|-------------|---|--------------|
| TGF-β1      | Forward : 5'-CACGTGGAGCTGTACCAGAA-3'<br>Backward: 5'-GTCCAGGCTCCAAATGTAGG-3'  | 500 bp       |
| TGF- 1      | Forward : 5'-ACGGCGTTACAGTGTCTG-3'<br>Backward: 5'-GGTGTGGCAGATATAGACC-3'     | 358 bp       |
| TGF- 2      | Forward : 5'-AGCAACTGCAGCATCACCTC-3'<br>Backward: 5'-TGATGTCTGAGAAGATGTCC-3'  | 688 bp       |
| Integrin-β3 | Forward : 5'-CGCTTCAGCTGATGTGTGTT-3'<br>Backward: 5'-CATCTCCCACCCTAGTCCAA-3'  | 224 bp       |
| Prolactin   | Forward : 5'-GCCCCCTTGCCCATCTGTCC-3'<br>Backward: 5'-AGAAGCCGTTTGGTTTGCTCC-3' | 386 bp       |
| β-actin     | Forward : 5'-CTCTCCAGCCTTCCTCCT-3'<br>Backward: 5'-CTCGTCATACTCCTGCTTGCT-3'   | 275 bp       |



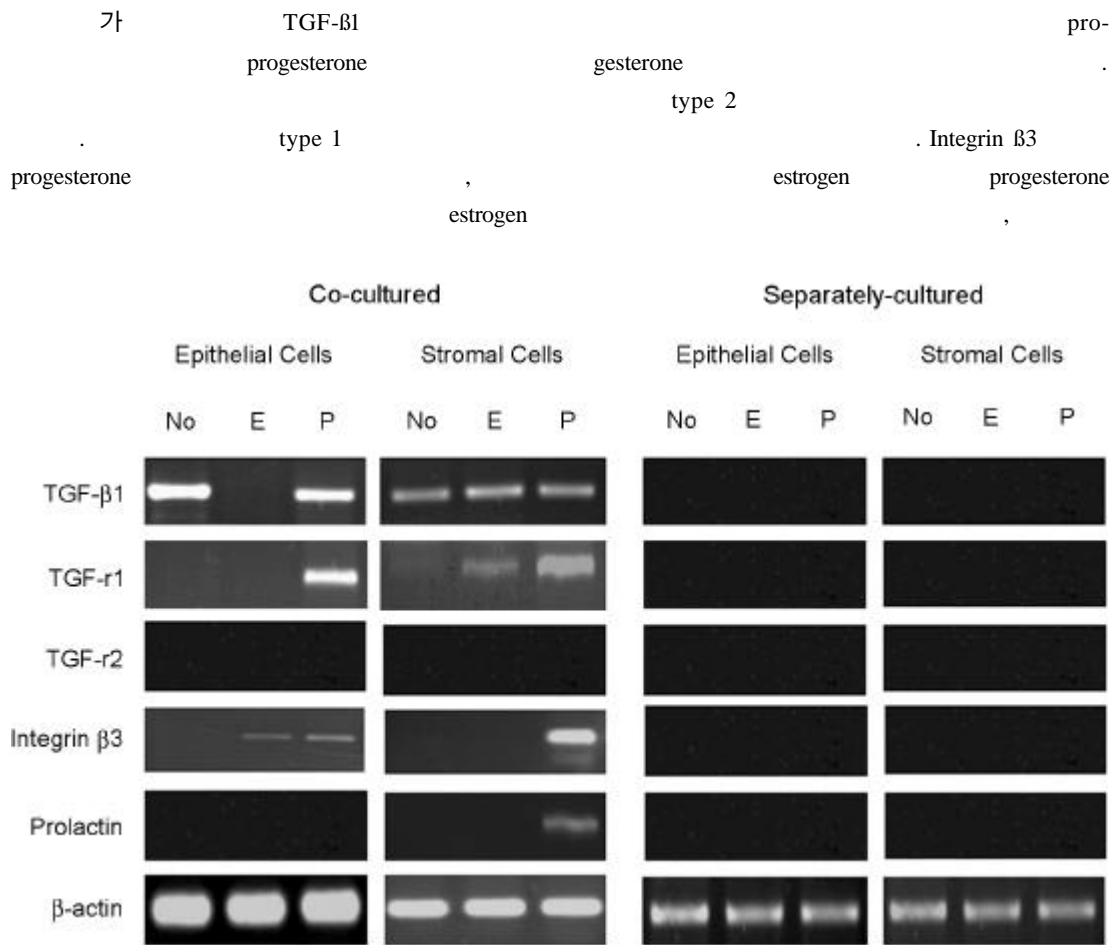
Cytokeratin



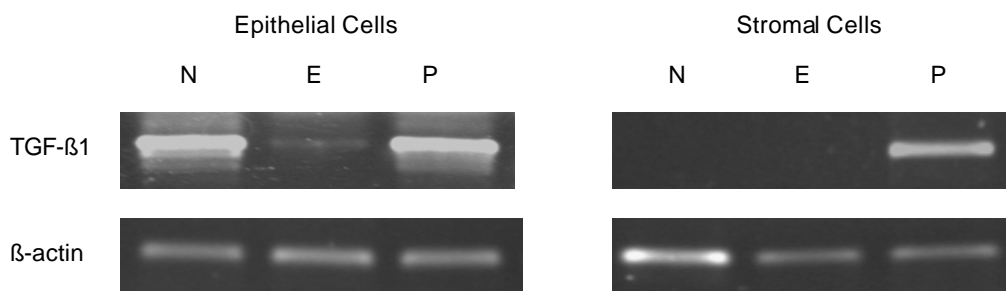
Vimentin

**Figure 2.** Immunocytochemical staining of primary cultures of human endometrial epithelial (A) and stromal (B) cells. Epithelial cells and stromal cells were separated as described. Epithelial cells were cytokeratin-positive while stromal cells were vimentin-positive.

가 1.9 190 (Molecular device, USA) 450 nm  
 . cDNA M-MLV reverse  
 transcriptase kit (Bioneer corporation, Korea) 3  
 . primer primer  
 designing software Primer3 (available at steve@genome.  
 wi.mit.edu)  
 primer PCR  
 (Table 1). primer  
 PCR Accupower  
 PCR premix kit (Bioneer, Korea),  
 PCR . 94  
 3 1 cycle, 94 30 35 cycle, 72  
 1 1 cycle, 72 5 1 cycle .  
 PCR 3  
 가  
 5. HGF  
 HGF  
 (No hormone, progesterone  
 , estrogen ) 48  
 -75  
 Quantikine human HGF immunoassay kit (R&D systems,  
 Inc., USA) , SpectraMAX RT-PCR mRNA  
 1. cytokeratin vi -  
 mentin  
 cytokeratin  
 vimentin  
 95% 가 cytokeratin  
 (Figure 2A).  
 , 95% 가 vimentin  
 (Figure 2B) 가  
 2. TGF - $\beta$ 1,  
 1, 2, integrin  $\beta$ 3, prolactin mRNA



**Figure 3.** Expression of TGF-β1, its receptor 1, 2, integrin β3 subunit and prolactin mRNAs in co-cultured or separately cultured epithelial and stromal cells in three different hormonal conditions. TGF-β1, its receptor 1 (TGF-r1), integrin β3 mRNAs were detected in the co-cultred epithelial and stromal cells. Prolactin mRNA was shown in the co-cultured stromal cells under the progesterone dominant condition. In contrast, there was no significant expression of these mRNAs in separately cultured cells.



**Figure 4.** HGF administration induces TGF-β1 mRNA expression in separately cultured epithelial and stromal cells. HGF (10 nM/ml) was administrated in separately cultured epithelial and stromal cells. In epithelial cells, TGF-β1 mRNA was detected in all hormone conditions. In stromal cells, TGF-β1 mRNAs were detected only in the progesterone dominant condition.

progesterone  
Prolactin  
(Figure 3).

3. HGF  
TGF-β mRNA  
RT-PCR mRNA  
HGF 가 TGF-β1  
estrogen  
TGF-β1, mRNA progesterone  
(Figure 4).

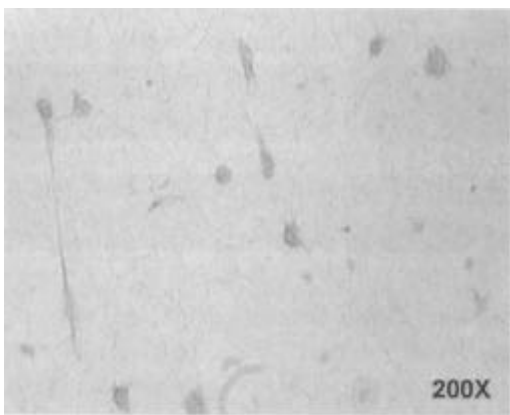
4. Met  
HGF Met

5. HGF  
가 HGF  
ELISA HGF  
150 ±24 pg/ml (n=3), estrogen  
4,325 ±436 pg/ml (n=3), progesterone  
2,000 ±540 pg/ml (n=3)  
(Table 2).

6. HGF TGF-β  
가 TGF-β mRNA  
HGF TGF-β1  
가 TGF type 1  
mRNA가 TGF type 2 mRNA  
HGF 가 progesterone  
, TGF-β1 가  
가 estrogen  
가 progesterone  
(Figure 6).  
HGF TGF-β1 가  
mRNA  
(data not shown).

7. HGF TGF-β  
beta 가 prolactin mRNA  
HGF TGF-β1

(Figure 5). Met  
(data not shown).

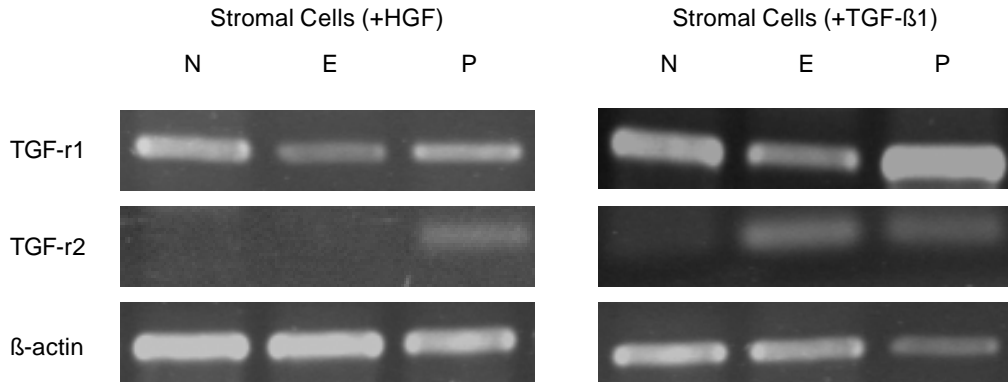


**Figure 5.** Immunocytochemical staining of 3-dimensionally cultured human endometrial stromal cells. Stromal cells were separated and 3-dimensionally cultured in collagen gel as described. Met positive stromal cells were shown brown color.

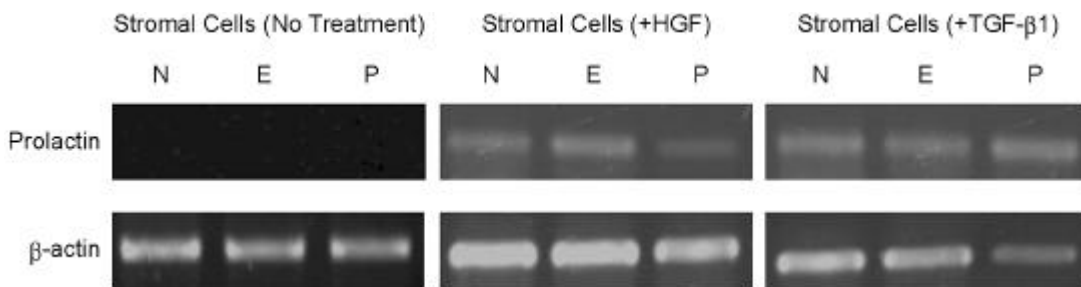
**Table 2.** The concentration of HGF in conditioned medium was determined by enzyme-linked immunosorbent assay (ELISA). Cells grown under either the estrogen dominant or the progesterone dominant condition (n=3; 4325 ±436 pg/ml in the estrogen dominant; 2000 ±540 pg/ml in the progesterone dominant) revealed higher concentrations of HGF than no hormone-treated groups (n=3; 150 ±24 pg/ml) (Mean ±S.D.)

| Hormone conditions    | HGF concentration (pg/ml) |
|-----------------------|---------------------------|
| No hormone            | 150 ±24                   |
| Estrogen dominant     | 4,325 ±436                |
| Progesterone dominant | 2,000 ±540                |

Note. Results are expressed as mean concentration of three different conditioned medium (n=3; Mean ±S.D.)



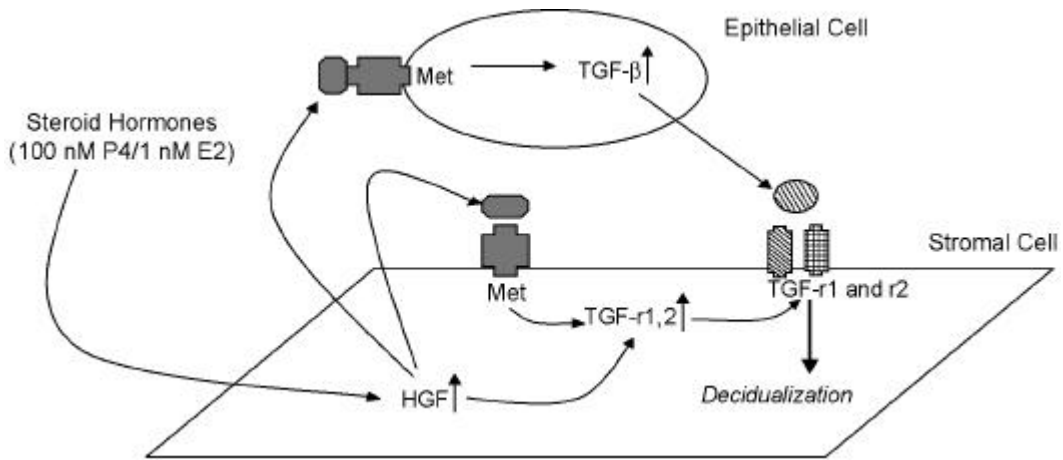
**Figure 6.** HGF and TGF-β1 administration induces TGF-r1 and -r2 mRNA expression in separately cultured stromal cells. Administration of HGF at 10 nM/ml induces TGF-r1 expression in all hormone conditions, while TGF-r2 was expressed in the progesterone dominant condition. TGF-β1 administration (10 nM/ml) induces TGF-r1 and -r2 expression in all hormone conditions except the expression of TGF-r2 is detected in no hormone condition.



**Figure 7.** HGF and TGF-β1 administration induces prolactin mRNA expression in separately cultured stromal cells. Prolactin mRNA was induced by either HGF or TGF-β1 administration in all hormone conditions.

가  
 prolactin mRNA가  
 conditioned medium  
 가  
 prolactin mRNA  
 (Figure 7).  
 in vitro  
 in vivo  
 3  
 decidualization  
 1  
 TGF-β1  
 type 1 mRNA  
 Wegner 23  
 mRNA  
 TGF-β





**Figure 8.** A proposed mechanism for the stromal decidualization. Progesterone induces HGF expression in stromal cells, then HGF plays a paracrine role in epithelial cells by inducing TGF- $\beta$  expression and an autocrine role in stromal cells by inducing the expression of TGF- $\beta$  receptor 1 and 2. Consequently, HGF and TGF- $\beta$  induce the stromal decidualization under the progesterone dominant condition in vitro.

|                                 |   |  |            |  |
|---------------------------------|---|--|------------|--|
| TGF- $\beta$                    | mRNA가<br>(data not shown).                                  | 가 paracrine factor   | 가          | . 가                                      |
| TGF- $\beta$ 1                  |   | HGF  | Met        | HGF                                      |
| Integrin $\beta$ 3<br>window    | implantation<br><sup>24</sup>                               | HGF  | Met        | Met                                      |
| integrin $\beta$ 3<br>gesterone | mRNA가<br>pro-   |  |            | . ELISA                                  |
|                                 | . Prolactin<br><sup>25</sup> Prolactin mRNA<br>progesterone | 150 $\pm$ 24 pg/ml (n=3), estrogen<br>436 pg/ml (n=3), progesterone<br>540 pg/ml (n=3) |            | 4,325 $\pm$<br>2,000 $\pm$               |
|                                 |   | HGF  |            |  |
|                                 |   | HGF 가가<br>TGF- $\beta$ mRNA  |            |  |
| HGF                             |   | ml HGF 가<br>$\beta$ 1 estrogen<br>mRNA   |            | 10 nM/<br>TGF-                           |
|                                 | <sup>17</sup><br><sup>18</sup>                              |  |            | , TGF- $\beta$ 2<br>mRNA<br>progesterone |
| 가                               | 가   |  | 가 cytokine |  |
|                                 | <sup>19</sup>   | HGF  | HGF 가가     | TGF mRNA                                 |

HGF 가 TGF type 1 mRNA  
TGF type  
2 progesterone  
type 2 (Figure  
3) type 2 type 1  
TGF-β TGF  
type 2 TGF-β가 TGF  
type 1  
. 9 HGF TGF  
type 2 progesterone TGF-  
β  
HGF TGF-β1 가가  
pro-  
lactin mRNA  
TGF-β HGF가  
decidualization  
Makrigiannakis 26 progesterone  
decidualization progesterone  
CRH (corticotrophin-releasing hormone)가  
progesterone  
3 48 proge-  
sterone decidualization maker pro-  
lactin mRNA  
HGF HGF  
TGF-β TGF type 1, 2  
(Figure 8).  
, progesterone deci-  
dualization progesterone  
HGF TGF  
가

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