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석사학위 청구논문

Characterization of Extracellular matirx protein 1 (ECM1) expression during mouse preimplantation stages in uterus

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이 논문을 석사학위논문으로 제출함

2016년 12월

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Submitted in partial fulfillment of the

requirements for the degree of master.

December, 2016

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Graduated School

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Certificate of Committee Approval

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requirements for the degree of:

Master of Science

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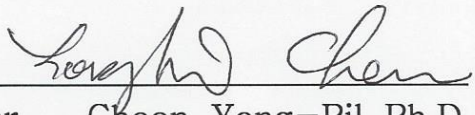

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논 문 개 요

하얀 털 Bax 유전자 제거 생쥐에서 임신 7일 자궁에서 발현하는 유전자를 마이크로어레이 방법을 이용하여 발현 변화가 유의하게 있는 전사체를 찾아 착상과 관련있는 것으로 추정할 수 있는 후보군은 찾았으며, 그 중 하나가 extracellular matrix protein 1 (ECM1)이었다. ECM1은 연골이 뼈로 변화하기 위한 mineralization을 음성조절하여 뼈의 형성을 억제하고, 세포 증식과 혈관생성을 촉진하며, 암 발생시 혈관 주변을 중심으로 발현하는 것으로 알려져왔다. 사람에서 ECM1의 돌연변이는 지질단백증이라는 상염색체 열성 피부 질환의 원인이 된다. 사람의 ECM1은 모든 엑손을 포함한 ECM1a과 선택적 이어맞추기 된 것인 (alternative splicing form) ECM1b, ECM1c, ECM1d의 네 가지로 발현하는데 ECM1a는 대부분의 조직에서 발현되는 것으로 알려져 있으며 특히 혈관이 많이 분포하는 곳에서 높은 발현양을 보인다. ECM1b는 피부 각질층 등에서 나타나며, ECM1c는 표피의 기저막에서, ECM1d는 지질단백증이 있는 사람에게서 발견되었다. 생쥐의 경우, 엑손 전체를 포함하는 ECM1a와 선택적 이어 맞추기 형인 ECM1b가 있는 것으으로 알려져 있다. ECM1이 세포외기질과 연관된 일부 기능이 제안되어 왔으나 세포외 기질의 변화가 왕성한 것으로 알려진 착상시기의 자궁에서의 기능에 대해서는 아직 밝혀진 것이 없다. 따라서 본 연구에서는 ECM1이 uterus에 미치는 영향을 알아보고자 하였다. ECM1이

착상시기 전후로 어떻게 발현되는지 알기위해 PCR을 통해 mRNA의 발현을 확인하고 ECM1의 splicing form을 확인하기 위해 sequencing을 진행했다. 그 후 Western blot을 통해 단백질의 발현유형을 확인하고, 면역화학염색 방법으로 발현하는 세포와 조직을 확인했다. PCR 결과에서 ECM1은 선택적 이어맞추기 된 것이 발현하는 것을 확인하였으며 이를 각각 그 벤드 크기에 따라 mECM1a, mECM1b, mECM1s라 각각 명명하였다. 염기서열 분석 결과 mECM1a는 기존의 mECM1a, mECM1b는 mECM1b와 일치하였다. 한편 mECM1s는 기존에 밝혀지지 않은 엑손 9, 10, 11로 구성된 새로운 것이었다. mRNA 수준에서 mECM1a, mECM1b의 발현은 1일, 7일에서 확인되고 mECM1s는 실험한 모든 단계에서 발현되었다. IHC결과 ECM1은 착상 이전시기인 1-4일 에는 자궁내막상피, 내막선상피에 표지되었다. 착상이 진행되면서 일차 탈락막과 2차 탈락막 세포의 세포질에 표지되었다. . 이러한 결과를 통하여 임신 초기에 ECM1은 기저수준으로 발현하고 착상이후에 탈락막을 중심으로 발현양이 증가함을 알 수 있었다. 따라서 ECM1에 대한 가능의 자세한 연구는 향후 제작하고자하는 ECM1 conditional knockout 생쥐를 이용하여 확인해야하나, 이전에 알려진 사실과 우리 결과를 바탕으로 ECM1이 착상과 관련된 자궁 분화에 중요한 역할을 할 것으로 사료된다.

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INTRODUCTION

The development of embryo and differentiation of uterus are simultaneous in early pregnancy for successful implantation (Psychoyos, 1973; Paria et al., 1993). In rodents, blastocyst attaches to antimesometrial region of uterine at first. By the invasion of blastocyst to uterus, uterine stromal cells beginning to proliferate and differentiate, so endometrila fibroblasts get an epithelioid phenotype. These cells called decidual cell. Decidualization is start surround implantation site. In rodent, decidualization is started in antimesometrial side of uterus, and then mesometrial region begin to forming the decidua (Abrahamsohn et al., 1993; Luciane et al., 2007; Cheon et al., 2002).

The extracellular matrix protein 1 (Ecm1) is first indentified secreted glycoprotein from murine osteogenic stromal cell line MN7 (Mathieu et al., 1994). Four splice variants of ECM1 have been described in human: ECM1a is consist of 540 amino acid (aa), expressed in various tissues, especially highly vascularized tissues such as heart and placenta (Smits et al., 1997; Sander et al., 2006); ECM1b has 415 aa of lacking exon 7, expressed in spinous, tonsils and granular layers of the epidermis;

ECM1c has 559 aa, expressed in restricted to the basal layer of the epidermis (Mongiat et al,. 2003); ECM1d has 57aa (Horev et al., 2005). In the mouse ECM1 reported two alternativesplicing forms. ECM1a has 599aa and expresse in various tissues. ECM1b has 435aa and expresse in tail, front paws and skin (Smits et al., 1997; Bhalerao et al., 1995). ECM1 has been shown to play an important role in endochondral bone formation by inhibiting alkaline phosphatase activity and bone mineralization (Deckers et al., 2001). In the chickin chorioallantioic membrane assay, ECM1 promote blood vessel formation (Han et al., 2001). ECM1 is involved in cell proliferation (Smits et al., 2000). In breast tumor, it is angiogenic properties (Han et al., 2001). Lipoid proteinosis caused by ECM1 mutation is autosomal recessive disorder characterized by thickening of the skin, mucosae, and certain viscera (Hamada et al., 2002).

Though ECM1 has many function in tissue remodeling, it’s roles in implantations is not uncovered. In this study, mRNA and protein expression level of ECM1 were analyze using the PCR and western blot. Spatio-temporal expression of ECM1 was analyzed with the immunohistochemistry.

MATERIALS AND METHODS

Experimental animals

The animals used in this research were grown by the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and grown in the Sungshin University. CD-1 mice were maintained under the light-on at 06:00 a.m. and light-off at 08:00 p.m., and clean room system. Animals were fed a standard rodent diet and water ad libitum from weaning at 21 days after birth.

Uterus sampling

Conducted an experiment that female mouse was placed with male mouse and then checked the female early the following morning for a vaginal plus. The day of vaginal plug checked was designated as day 1 of gestation. The mouse uteri were sampled of day 1, 2, 3, 4, 5, 6, 7, 9 and 12 of gestation. The pregnancy was confirmed by the presence of embryos from the reproductive tracts. The uterus were frozen in -80℃ until used.

Total RNA extraction

Total RNAs were extracted by using TRIzol reagent (Invitrogen, Cat # : 15596026, San Diego, CA, USA). Uterus tissues were homogenized in TRIzol reagent (1 ml / 0.1 g) for 30 seconds and stored for 10min at room temperature (RT). Next chloroform of 0.2 ml / ml TRIzol reagent added in homogenized tube and vigorously shake at 15 sec and stored for 15min at RT and centrifuged 12,000 g for 15min at 4℃. Supernatant was transferred to new tube, and added 0.5ml of isopropanol in 1ml TRIzol, mixed gently and kept for 10min at RT. Then centrifuged 12,000 g for 8 min at 4℃. The supernatant was removed and RNA pellet were floated in 1ml 75% ethanol by inverting, and centrifuged 7,500 g for 5min at 4℃. The supernatant were removed, dried to remove ethanol for 4min at RT, and added 50 μl DEPC treated water. Total RNA quality and quantity were identified by Agilent bioanalyer™ 2100 analysis.

First strand cDNA synthesis and PCR analysis

In order to conduct reverse transcription polymerase chain reaction (RT-PCR), progressed cDNA synthesis. First cDNAs were synthesized using Accuscript first strand cDNA synthesis kit

(Stratagene, Cat # : 600559, CA, USA) according to the manufacturer's instruction. Reaction reagents were 5㎍ total RNA, 5㎕ Accuscript buffer (10X), 1㎕ oligo dT primer (0.5㎍/㎕), 1㎕ random primers (0.1㎍/㎕), 2㎕ dNTP mix (100mM) and added DEPC treated water to 20㎕.. Mixtures were spin down and reacted at 65℃ for 5min, and kept at RT for 10min. Thereafter added 4㎕ DTT (100mM), 2㎕ Rnase block ribonuclease inhibitor (40U/ml), and 1㎕ Accuscript multiple temperature RT. The mixture were incubated at 42℃ for 1hr after that 70℃ for 15min to terminate cDNA synthesis. Reverse transcription polymerase chain reaction (RT-PCR) was performed according to the manufacturer's instruction. Briefly, 1㎕ cDNAs transformed to each PCR tube and place the 10X buffer 2㎕, 2.5mM dNTP mix 0.4㎕, *taq* polymerase 0.1㎕, sense primer 1㎕, antisense primer 1㎕, and autoclaved 18.2mΩ H2O to 20 ㎕. Then cunducted RT-PCR using My Gene™ L Series peltier Thermal cycle (Long gene, Model : MGL 96+, Canada). RT-PCR cycles appeared in Table1.

Transformation and miniprep for sequencing

In order to sequencing the PCR product, first PCR product extracted from agarose gel. To extracted PCR product from agarose gel, we used

the QIAEXⅡ® Gel Extraction Kit (QIAGEN, Cat # : 20021, Hilden, Germany) according to the manufacturer's instruction. Agarose gel bands were cutted and transferred to 1.5ml tube and added QXI buffer in tube for three fold of agarose gel weight. Next added QIAEXⅡ buffer 30㎕ and vortexed 30sec. Thereafter incubated for 10min at 50℃. After melt the gel, centrifugation 13,000 rpm for 30sec at RT, remove the supernatant. Added QXI 0.5ml and vortexing for 30sec, centrifugation 13,000rpm for 30sec at RT and remove the supernatant. Next 05 ml PE buffer was added and vortexed for 30sec, centrifuged with 13,000 rpm for 30sec at RT, removed the supernatant. After repeated once more pellet was dried for 30min at RT. Extraction was performed with 20㎕ of DEPC treated water. Second, refined PCR product were ligation with pCR™2.1-TOPO® vector (Invitrogen, San Diego, CA, USA) according to the manufacturer's instruction. Mixed with purified PCR fragment 4㎕, salt solution 1㎕ and vector 1㎕ in 1.5ml tube, respectively. And mixture incubated for 30min at RT. Third, inserted vector were transformed with TOP 10 competent cell (Thermo Fisher, Cat # : C404006, Waltham, USA). And transfacted TOP 10 cells amplified in water at 37℃, and then slided in petri dish. Next colonys of petri dish were amplification in LB broth for overnight. And then prismid

extraction to use the Gene All exprep plasmid kit (Gene All, Cat # : 000001429, Seoul, Korea) according to the manufacturer's instruction. Purified plasmid were conformed by restriction enzyme cutting, and PCR product inserted vector was send to Bioneer(Daejeon, Korea) for sequencing.

Protein extraction and Western blotting analysis

Before protein extraction, tissue were washed using cold Y-PBS (0.7mM PMSF, 1mM Benzamidine-HCl, 4 μg/ml Leupeptin, 2 μg/ml Aprotinin, 2 mM EDTA). Uterine stroma cell were homogenized in cold homogenization buffer (50 mM Tris-Cl, 150 mM NaCl, 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1 mM PMSF, 1 μM Leupeptin, 1 μM Pepstatin, 0.5 mM EDTA, 15% Glycerol, and 0.1% NP-40). The homogenates were centrifuged to remove insoluble materials. The protein concentration was determined by Bradford assay using protein dye reagent (Bio-Rad Laboratories, Inc., Richmond, CA). 30 μg/ml of protein were boiled in SDS/β-mercaptoethanol sample buffer, and loaded onto each lane of 10% SDS-PAGE. The proteins were separated by electrophoresis and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories,

Inc., Richmond, CA) in transfer buffer (25 mM Tris base, 192 mM Glycine, 0.1% SDS, and 20% Methanol, pH 8.3). The membranes were blocked in 5% skimmed dry milk in TBST buffer (10 mM Tris-HCl, 150 mM NaCl and 0.05% Tween-20) for 1 hr at RT, and washed three times with TBST. The membranes were incubated during overnight with rabbit polyclonal ECM1 antibody (dilution 1:1000); rabbit monoclonal beta actin antibody (dilution 1:1000) at 4℃. After incubation, membranes were washed three times and incubated for 1 hr with horseradish peroxidase conjugated goat anti-rabbit IgG (dilution 1:5000); goat anti-mouse IgG-HRP (dilution 1:5000) for 1 hr. The bands were detected using ECL solution (GE Healthcare, Little Chalfont, UK) by Kodac Image station. And then development to use the X-ray film for 5sec, 10sec, 30sec, 1min and 5min.

Immunohistochemistry

Dissected mouse uterine horns were cut into 0.5 segments, fixed in 4% formaldehyde for 24 hr and embedded in paraffin. 4 μm sections were mounted on glass slides and subjected to antigen retrieval in boiling 10mM citriate buffer(pH 6.0) for 10min. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide in H_2O for 5min. ECM1

immunoreactivity was detected according to the Vectastain ABC kit method (Vector Laboratories, Inc., Burlingame, CA). Briefly, tissues were incubated with 1% normal goat blocking serum in 0.1% BSA in PBS for 1hr. And then samples were incubate with rabbit polyclonal ECM1 antibody (dilution 1:1000) in 0.1% BSA in PBS during overnight at 4℃. Next day, washing in PBST; Tissues were incubated with biotinylated anti-rabbit IgG (dilution 1:250) in 0.1% BSA in PBS for 1hr; Tissues were washed with PBST and PBS; Incubated with avidin-biotin complex regeant containing horseradish peroxidase for 30min. Slides were washed with PBST and PBS. And color development was achieved using DAB substrate. And then the tissues were stained with hematoxylin.

Statistics

All experiments were conducted at least in triplicate. The Student's t-test was performed to evaluate the statistical significance between control and experiment group. Results were presented as mean ± SEM. Values of $P < 0.05$ were considered to be significantly different.

Table 1. PCR cycle schedule

step		Temperature (℃)	Time
Segment #1 cycle	Hold	94	5min
	Hold	59	2min
	Hold	72	1min
Segment #2 cycle	Hold	94	1 min
	Hold	59	2 min
	Hold	72	1 min
Segment #3 cycle	Hold	94	1 min
	Hold	59	2 min
	Hold	72	7 min
Segment #4 cycle	Hold	4	∞
Segment #5 cycle		END	

Table 2. Sequence of primers

Gene	symbol	NCBI gene reference	Premer sequence (5'−3')
Extracellular matrix protein 1	ECM1	NM_007899.2	S CCCTTCAAGTCTAGATCTGCCTGTGA AS GCTCAGGGTGACTCATTCTTCCTT
36B4	Rplp0	NM_007475	S CGACCTGGAAGTCCAACTACTTCCT AS GCACCTTATTGGCCAACAGCAT

Table 3. Antibodies information

Name		company
ECM1	-Rabbit polyclonal	ABBIOTEC
β-actin	-Mouse monoclonal	Sigma
Goat Anti-Rabbit IgG-HRP conjugate Goat anti-mouse IgG-HRP conjugate		Bio-Rad

RESULTS

Expression pattern of ECM1 in mouse uterus of early pragnancy

PCR analysis was performed as mentioned in Metereals and Methods (Fig 1). PCR band of the top was appeared to 1600bp and described as mECM1a. Second band had appeared to 1200bp and described as. mECM1b. 500bp was unseen pattern in previous reports. It was mentioned as mECM1s in here. mECM1s was expressed in all examined physiological status uterus. Based on the expression patterns, the sequences of bands were analysed (Fig 2, 3, 4).

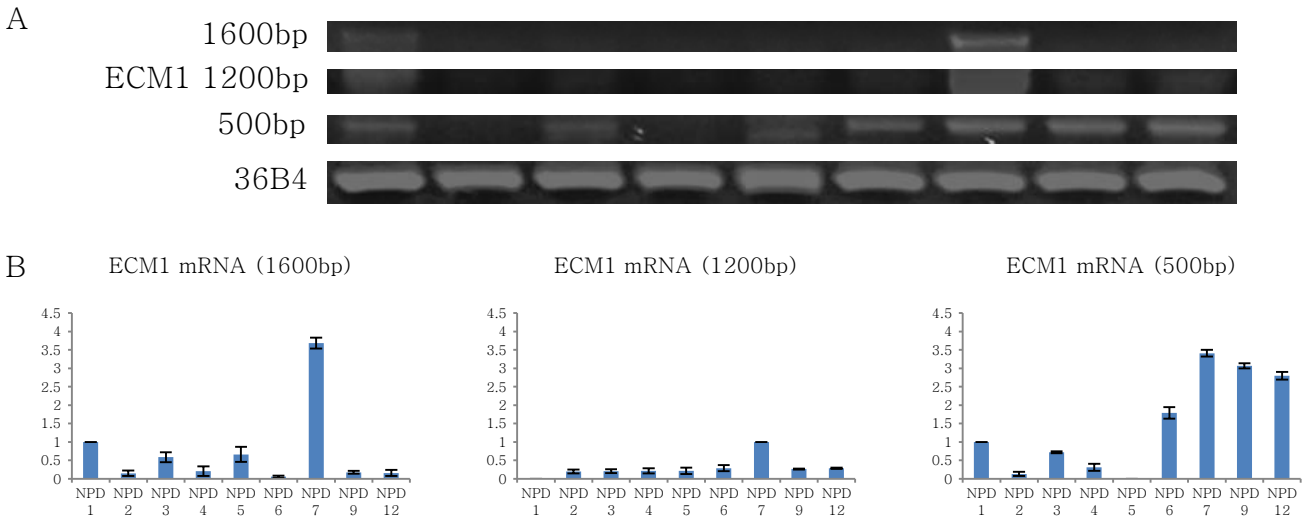

Fig 1. Expression pattern of ECM1, 36B4 mRNA was used as internal control.

(A) ECM1 mRNAs expressed in uterus for three patterns. Top band was relevant to ECM1a, middle band was ECM1b, below band was not reported size band. To make sure, we progress sequencing.

(B) Normalization of the ECM1 mRAN in each of alternative splicing form.

Alrternative splicing forms in mouse uterus

In mouse ECM1 has 11exons consist of 559 amino acids. And human ECM1 have 10 exons consist of 540 amino acids (Fig 6). In human, ECM1 has four alternative splicing forms; ECM1a, ECM1b, ECM1c, ECM1d. In the experience, the alternative splicing forms were identified which were designated as mECM1a, mECM1b, and mECM1s. mECM1a was full length ECM1 mRNA. It was corresponded to ECM1a. mECM1b was defect the exon 8. It was corresponded to ECM1b. mECM1s had half part of exon 9 and exon 10, 11. It is new splicing variant (Fig 6).

1600bp PCR product blast with ECM1 / accuracy : 1752/1764(99%)

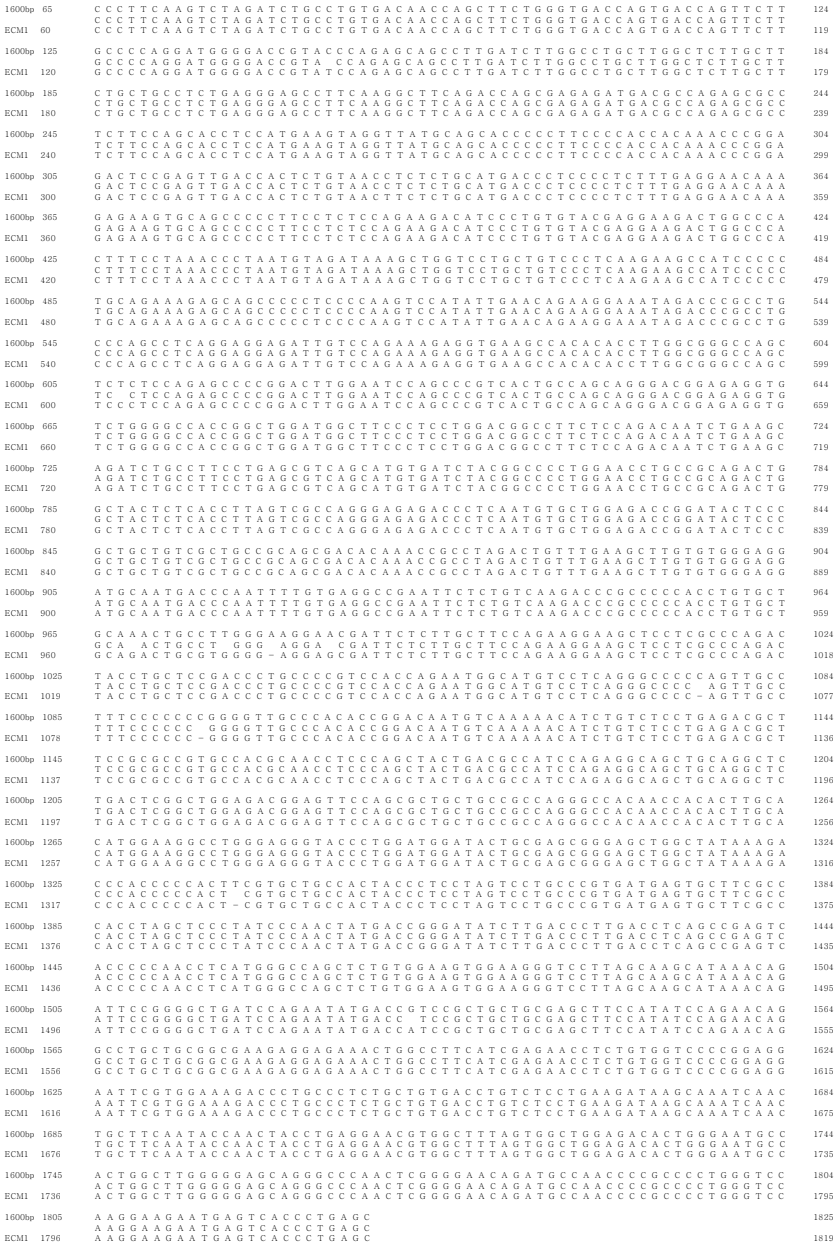

Fig 2. Blast of ECM1 product of 1600bp

PCR product sequence was blast with mouse ECM1 sequecne with BLAST wep tool of NCBI. It is 99% (1752/1764) math with ECM1a.

1200bp PCR product blast with ECM1 / accuracy : 1379/1385 (99%)

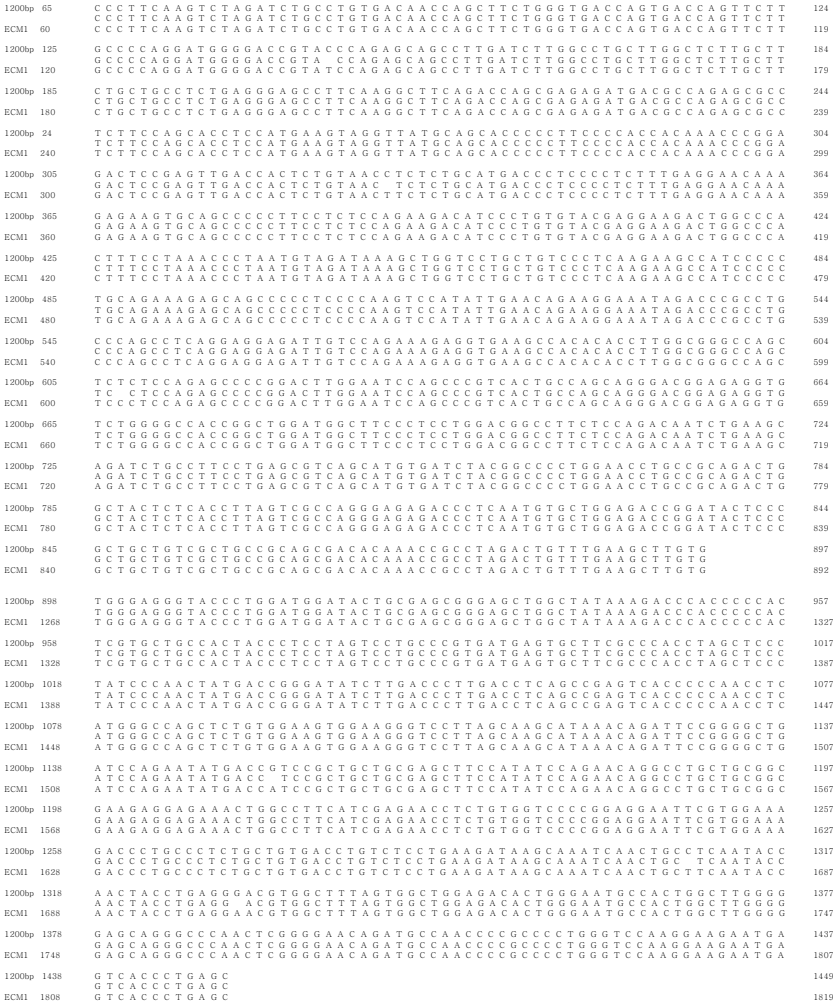

Fig 3. Blast of ECM1 product of 1200bp

PCR product sequence was blast with mouse ECM1 sequecne with BLAST wep tool of NCBI. It is 99% (1379/1385) math with ECM1b.

500bp PCR product blast with ECM1 / accuracy : 468/473(99%)

```
500bp   76      CTAGATCTGCCTGTGATGAGTGCTTCGCCCACCTAGCTCCCTATCCCAACTATGACCGGG   135
                CTAG  CTGCC GTGATGAGTGCTTCGCCCACCTAGCTCCCTATCCCAACTATGACCGGG
ECM1    1347    CTAGTCCTGCCCGTGATGAGTGCTTCGCCCACCTAGCTCCCTATCCCAACTATGACCGGG   1406

500bp   136     ATATCTTGACCCTTGACCTCAGCCGAGTCACCCCCAACCTCATGGGCCAGCTCTGTGGAA   195
                ATATCTTGACCCTTGACCTCAGCCGAGTCACCCCCAACCTCATGGGCCAGCTCTGTGGAA
ECM1    1407    ATATCTTGACCCTTGACCTCAGCCGAGTCACCCCCAACCTCATGGGCCAGCTCTGTGGAA   1466

500bp   196     GTGGAAGGGTCCTTAGCAAGCATAAACAGATTCCGGGGCTGATCCAGAATATGACCGTCC   255
                GTGGAAGGGTCCTTAGCAAGCATAAACAGATTCCGGGGCTGATCCAGAATATGACC TCC
ECM1    1467    GTGGAAGGGTCCTTAGCAAGCATAAACAGATTCCGGGGCTGATCCAGAATATGACCATCC   1526

500bp   256     GCTGCTGCGAGCTTCCGTATCCAGAACAGGCCTGCTGCGGCGAAGAGGAGAAACTGGCCT   315
                GCTGCTGCGAGCTTCC TATCCAGAACAGGCCTGCTGCGGCGAAGAGGAGAAACTGGCCT
ECM1    1527    GCTGCTGCGAGCTTCCATATCCAGAACAGGCCTGCTGCGGCGAAGAGGAGAAACTGGCCT   1586

500bp   316     TCATCGAGAACCTCTGTGGTCCCCGGAGGAATTCGTGGAAAGACCCTGCCCTCTGCTGTG   375
                TCATCGAGAACCTCTGTGGTCCCCGGAGGAATTCGTGGAAAGACCCTGCCCTCTGCTGTG
ECM1    1587    TCATCGAGAACCTCTGTGGTCCCCGGAGGAATTCGTGGAAAGACCCTGCCCTCTGCTGTG   1646

500bp   376     ACCTGTCTCCTGAAGATAAGCAAATCAACTGCTTCAATACCAACTACCTGAGGAACGTGG   435
                ACCTGTCTCCTGAAGATAAGCAAATCAACTGCTTCAATACCAACTACCTGAGGAACGTGG
ECM1    1647    ACCTGTCTCCTGAAGATAAGCAAATCAACTGCTTCAATACCAACTACCTGAGGAACGTGG   1706

500bp   436     CTTTAGTGGCTGGAGACACTGGGAATGCCACTGGCTTGGGGGAGCAGGGCCCAACTCGGG   496
                CTTTAGTGGCTGGAGACACTGGGAATGCCACTGGCTTGGGGGAGCAGGGCCCAACTCGGG
ECM1    1707    CTTTAGTGGCTGGAGACACTGGGAATGCCACTGGCTTGGGGGAGCAGGGCCCAACTCGGG   1766

500bp   496     GAACAGATGCCAACCCCGCCCCTGGGTCCAAGGAAGAATGAGTCACCCTGAGC   548
                GAACAGATGCCAACCCCGCCCCTGGGTCCAAGGAAGAATGAGTCACCCTGAGC
ECM1    1767    GAACAGATGCCAACCCCGCCCCTGGGTCCAAGGAAGAATGAGTCACCCTGAGC   1819
```

Fig 4. Blast of ECM1 product of 500bp

PCR product sequence was blast with mouse ECM1 sequence with BLAST wep tool of NCBI. It is included ECM1 RNA but unknown splice variant in previous report.

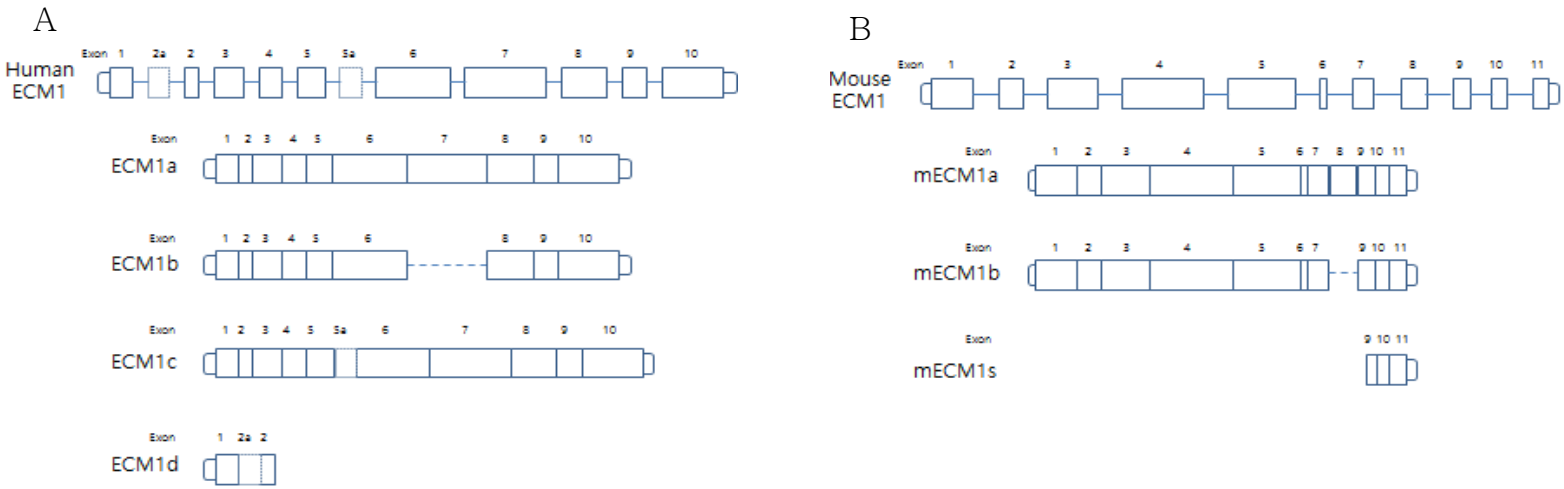

Fig 5. Comparison of alterlative spliced ECM1 mRNAs between human and mouse

(A) Four different splicing formsof human ECM1 (ECM1a, ECM1b, ECM1c, and ECM1d). (B) Three forms of mouse ECM1 (mECM1a, mECM1b, and mECM1s).

Profiles of ECM1 protein in early pregnant uteri

The used ECM1antiboy is specific to the C-terminal sequence of ECM1 proteins. Therefore it can bind to all three alternative splice mRNA products (Fig 6).

As expected from the PCR analysis, mECM1a, mECM1b, and mECM1s were detected in the pregnant uterus (Fig6). It expression levels were dramatically increased after implantation (Fig. 6).

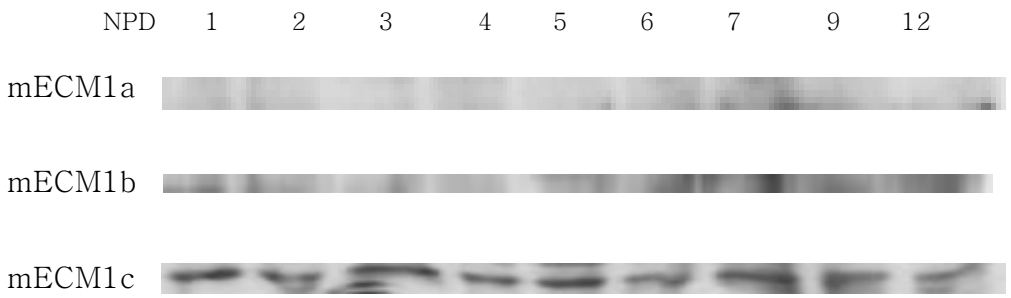

Fig 6. Identify ECM1 protein expression in mouse uterus of NPD 1-12

ECM1 protein was detected in mouse uterus at the time to experiment.

Tissue specific localization of ECM1

ECM1 was localized in uterine luminal epithelial cells and glandular epithelial cells (Fig 7). It was stronly localized in primary and secondary decidualized zones after implantation (Fig 7). Its intensity in tissues were similar with the results of western blot.

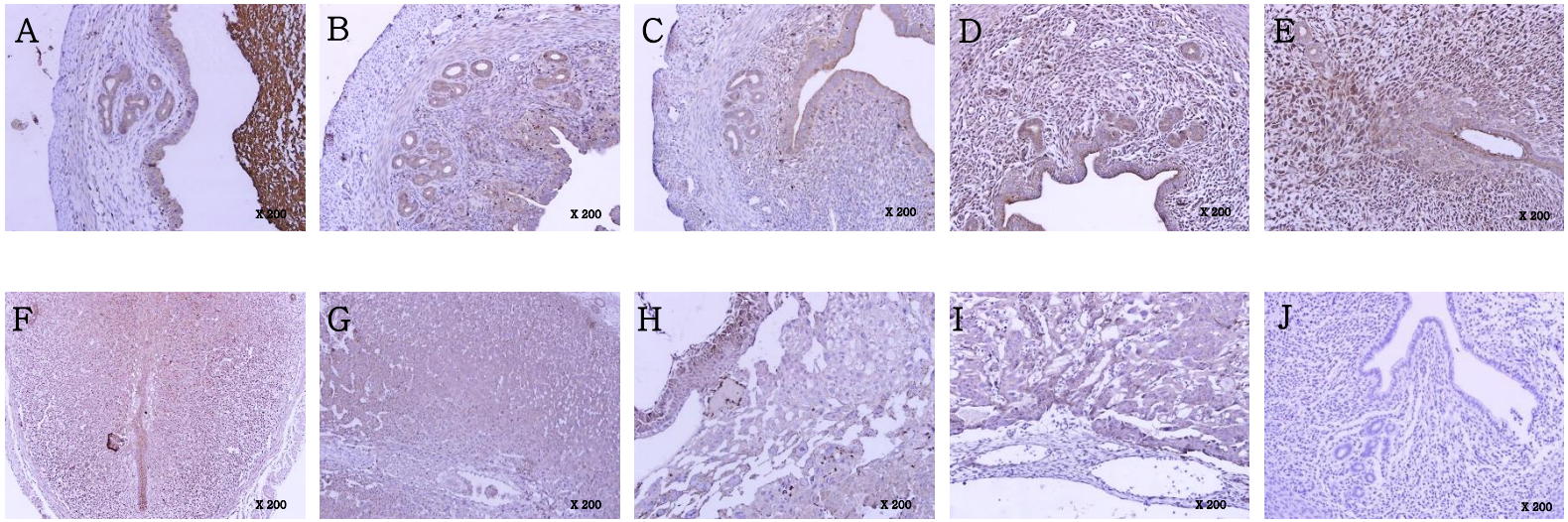

Fig 7. Photograph of Immunohistochemistry of ECM1 in pregnant uterus.

(A)－(I) : Immunohistochemistry was performed with ECM1 C terminal specific antibody in pregnant mouse uterus. A : day 1of pregnancy, B :day 2 of pregnancy, C day 3 of pregnancy, D : day 4 of pregnancy, E : day 5 of pregnancy, F : day 6 of pregnancy, G : day 7 of pregnancy. H : dya 9 of pregnacy, I : day 12 of pregnancy. (J) : Negative control.

DISCUSSION

It is known that ECM1 has four alternative splicing forms in human and two in mouse. It is evaluated that these alternative splicing forms have different expression regions for each. In here, we investigated the expression of ECM1 mRNAs in uterus during early pregnancy. Interestingly, three alternative splicing forms were detected in pregnant mouse uterus. They were designated as mECM1a, mECM1b, and mECM1s. mECM1a and mECM1b are matched with the previously known forms, ECM1 and ECM2. mECM1s is new alternative splicing form, it is identified in this report.

ECM1 can promote angiogenesis and proliferation. It is possible to bind other extracellular matrix protein and release or suppress of the binding proteins functions: ECM1 regulate the angiogenesis and cell proliferation, directly or indirectly.

mECM1 was localized in mouse uterus. From day 1 to day 4, mECM1 was localized across the endometrium especially luminal epithelium and gland cells. In day 5 to 7, mECM1 expressed in

primary and secondary decidualized zones. It suggests that mECM1 is seems to be relevant for uterine stromal cell proliferation and decidualization.

Consequently, in mouse uterus, ECM1 expresses in three alternative splicing forms and they are secreted from gland cells. Based on them, it is suggested that mECM1 may be have a role in endometrial proliferation and decidualization.

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ABSTRACT

Characterization of Extracellular matirx protein 1 (ECM1) expression during mouse preimplantation stages in uterus

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Based on previous our studies extracellular matrix protein 1 is suggested as a candidate for an important factors in embryo implantation. In this study, ECM expression was characterized in pregnant mouse uterus. ECM1 mRNA and protein expression were analyzed with PCR and western blot methodology. Its tissue specific expression was analyzed with immunohistochemistry method. In pregnant mouse uterus, there was three splicing forms were expressed and designated in here mECM1a, mECM1b, and mECM1s. mECM1a and mECM1b were

same with the ECM1a and ECM1b. Their expression patterns were stage specific.mECM1a and mECM1b were detected in day 1 and day 7. mECM1s was detected in all experimental groups. The profiles of protein levels were similar with that of mRNA. IHC results revealed that it was localized in the decasualized cells after implantation. It was localized in luminal epithelial cell and glandular epithelia cells from day 1 to day 4 of gestation. Though further studies are needed, these results suggested that ECM1 has a role in implantation process with the alternative splicing forms.

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