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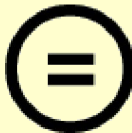

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석사학위 청구논문

Angiogenic role of Bradykinin During Decidualization

2016

성신여자대학교 대학원

생 물 학 과

백 정 원

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전용필 교수 지도

이 논문을 석사학위논문으로 제출함.

2016년 5월

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Angiogenic role of Bradykinin During Decidualization

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Submitted in partial fulfillment of the requirements for the degree of master.

May, 2016

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2016년 5월

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논 문 개 요

자궁내막에 포배가 착상하는 과정에서 관찰되는 뚜렷한 조직학적 특징은 착상부위를 중심으로 한 혈관의 형성과 내막의 탈락막화이다. 탈락막화는 태반형성 전단계로 정상적 개체 발생을 가능하게 한다. 착상 시작 단계의 포배는 저산소증을 겪게 되며 이를 극복하기 위해서 혈관형성이 빠른 시간 내에 진행된다고 알려져 있다. 이는 착상 초기에 실험적인 blue band의 형성을 통해 알 수 있다. 한편, 브래디키닌 (bradykinin)은 노나펩타이드 (nonapeptide)로, 키니노겐 (kininogen)이 칼리크레인 (kallikrein)의 효소 작용에 의해 분해됨으로 생성된다. 칼리크레인은 자궁수축을 유도하고 증식기의 자궁내막 성장과 관련이 있다. 브래디키닌은 염증반응, 혈관확장, 신생혈관형성과 같은 생리적 과정을 조절하는 것으로 알려져 있다. 브래디키닌은 내피세포의 증식을 유도하고 브래디키닌 수용체는 혈관성장인자로 알려진 VEGF의 수용체 KDR/Flk1의 전사를 촉진하여 내피세포의 신생혈관형성과 관련된 물질들을 분비하도록 한다. 착상 시기에 자궁의 착상 위치에서 칼리크레인이 발현함이 알려졌고, 이전 연구 결과는 칼리크레인, 키닌노겐, 브래디키닌 수용체가 mRNA 수준에서 임신시기 특이적으로 발현됨을 밝혔다. 그러나 칼리크레인-키닌 시스템이 착상시기에 어떻게 적용되는지는 잘 밝혀져 있지 않다. 따라서 본 연구에서는 탈락막화 과정에서 칼리크레인-키닌 시스템이 혈관 형성에 있어서의 역할과 에스트로겐에 의한 조절여부를 알아보고자 하였다. 생쥐 임신 초기 시기

자궁에서 칼리크레인5 mRNA는 임신 1일에 기저 수준을 보였고 임신 3일까지 증가하다 감소하여 임신 5일, 7일에 발현하는 특징을 보였다. 단백질의 양은 임신 1일 이후 일정량 유지되었다. 브래디키닌 수용체2 mRNA 수준은 칼리크레인에 비해 상대적으로 매우 높은 수준으로 발현하였고, 이를 반영하여 단백질의 양적 변이가 관찰되었다. 브래디키닌 수용체2는 면역형광법을 통하여 위치를 확인하였으며 혈관내상피와 일정부분에서 함께 표지 되었다. 착상지연모델에서 칼리크레인5 단백질은 에스트로겐 처리 전후 모두 높은 수준으로 발현하였다. 브래디키닌 수용체2 단백질은 에스트로겐 처리 1시간 이후부터 9시간까지 높은 수준으로 발현하였다. 에스트로겐 수용체 알파 낙아웃 생쥐에서 브래디키닌 수용체2의 발현이 감소함이 나타났고, 탈락막 조직에서 혈관의 분포가 상대적으로 적게 생겼다. 이러한 결과를 바탕으로 임신기간 동안 자궁에 존재하는 칼리크레인과 브래디키닌 수용체가 혈관형성과 관련되어 있고 이것이 에스트로겐에 의해 조절됨이 제안된다.

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INTRODUCTION

Implantation is a critical event for mammalian development that is initiated when embryo attaches to the uterine epithelium (Carson et al., 2001). After attachment of the embryo, the uterine stromal cells at implantation site start to proliferate and differentiate into glycogen and lipid rich decidual cells (Douglas et al., 2014). During this process, called decidualization, the stromal cells undergo dramatic transformation to form a specialized decidua tissue (Das et al., 2012). Decidualization is significant for pregnancy, because the decidua prepares placentation and supports embryo with nutrients (Zhu et al., 2014). If maternal uterus does not proper decidualizaiton, the embryo can not develop normally (Zhang et al., 2013). During implantation, the morphological and functional changes in the uterus are regulated by the actions of the steroid hormones 17β-estradiol (E_2) and progesterone (P_4) (Cyril et al., 2010).

Angiogenesis, the process of new blood vessels growth from existing blood vessels (Walter et al., 2005), is important feature of decidualization. By the attachment of embryo to endometrium, the endothelial cells proliferate and form vascular network (Kim et al., 2013).

Bradykinin is a nonapeptide generated from cleavage of kininogen by kallikrein (Bhoola et al., 1992). The kallikrein is a highly conserved multi-gene family of serine proteases that are expressed in a wide variety of tissues and act on a diverse range of substrates (Clements, 1997; Holland et al., 2001). The kininogen is protein substrates for kallikrein. Mouse has two kininogens, Kng1 and Kng2, and has tissue-specific expression between Kng1 and Kng2 (Cardoso et al., 2004; Shesely et al., 2006). The bradykinin exerts its action

through two known receptors, B1 and B2 receptor. The B2 receptor is constitutively expressed in various tissues and is responsible for most of the effects of bradykinin (Miura et al., 2003).

It is reported that kallikrein or B2 receptor expressed in uterus of human (Corthorn et al., 2006), rat (Valdés et al., 1998; Figueroa et al., 2001) and mouse (Chan et al., 1999) during pregnancy, but their role is not fully understand.

A number of studies suggest that E_2 induces endometrial endothelial cell rapid proliferation (Heryanto et al., 2002) and migration (Morales et al., 1995), which are required in vascular growth of endometrial tissue remodeling. Locally produced E_2 in uterus is critical for the progression of the stromal differentiation, which enables the synthesis of factors that are likely mediators of angiogenesis in the decidual uterus (Das et al., 2009). These observations suggest that E_2 is related to angiogenesis during decidualization however, network of angiogenesis by E_2 have not been defined.

Previous studies showed that is expression change of kallikrein, kininogen and bradykinin receptor in ovariectomized mouse uterus and in a delayed implantation uterus (Jeon, 2013). It suggests that steroid hormones regulate the kallikrein, kiniogen and bradykinin receptor. Previously, we explored the possibility of kallikrein-kinin system during decidualization for angiogenesis. In this study, protein profiling and their roles were examined.

MATERIALS AND METHODS

Experimental animals

Animals were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health. Animals were maintained under standard condition at Sungshin Women's University circadian rhythm kept under the 14L : 10D schedule with light-on at 0600 and clean room system. Animals were fed a standard rodent diet and water ad libitum from weaning at 21 days after birth.

Estrogen receptor-alpha knockout (ERαKO) female mice were generated by ERα heterozygote (ERα$^{+/-}$) breeding. ERα mice carries two loxP sites in the introns flanking exon3 of the ERα gene, was used as a target of ERα gene excision. The genotyping of the mice were determined by PCR analysis of genomic DNA from ear tissue. The following primers were used for genotyping the mice: ERα-P1 (5'-TTG CCC GAT AAC AAT AAC AT-3'), ERα-P2F (5'-GTG TCA GAA AGA GAC AAT-3') and ERα-P3 (5'-GGC ATT ACC ACT TCT CCT GGG AGT CT-3'). Primer combinations of ERα-P1 + ERα-P3 and ERα-P2F + ERα-P3 were used to determine the presence or absence exon3.

Uterus sampling

Female CD-1 mice were mated with fertile male mice of the same strain. The following morning, these mice were examined for the presence of vaginal plug,

and this was defined as day 1 of pregnancy. The mice were sacrificed to collect uteri on day 1, 2, 3, 4, 5, 6, 7, 9 and 12 of pregnancy. Pregnancy on day 1–12 was confirmed by recovering embryos from the reproductive tracts at from 10:00 to 11:00 AM. The embryos were removed from oviducts or uteri by flushing with DEPC-treated PBS. The uteri were frozen in liquid nitrogen and kept at -80℃ until RNA and protein extraction.

Delayed implantation model

Female CD-1 mice were injected with 2.5 IU of PMSG and after 48 hr, the mice were additionally injected with 2.5 IU of hCG. Female mice were mated with fertile male mice of the same strain. The following morning, these mice were examined for the presence of vaginal plug, and this was defined as day 1 of pregnancy. On day 4 of pregnancy, ovariectomized at the morning (0800-1000). Female mice were injected daily with progesterone (2 mg / 0.1ml cotton seed oil) from day 5 through 7 of pregnancy, at the morning (0830). On day 7 of pregnancy, the mice were additionally injected with 17β-estradiol (25 ng / 0.1ml cotton seed oil) (9000). Uterin were collected at Progesterone only, 0.5hr, 1hr, 1.5hr, 3hr, 6hr, 9hr, 12hr post 17β-estradiol, respectively. The embryos were removed from oviducts or uteri by flushing with Y-PBS (0.7 mM PMSF, 1 mM Benzamidine-HCl, 4 μg / ml Leupeptin, 2 μg / ml Aprotinin, 2 mM EDTA). The uteri were frozen in liquid nitrogen and kept at -80℃ until RNA and protein extraction.

Artificially stimulated decidualization

Female ERαKO and its wild type mice were ovariectomized and, day 7 later, were injected with 17β-estradiol (100 ng / 0.1ml cotton seed oil) for 3 consecutive days. Following two days, the mice kept free from hormonal administration. After then, mice were injected daily with 6.7 ng of 17β-estradiol and 1mg of progesterone until sacrificed. At third day, artificial decidualization was induced with mechanical trauma: insertion of a blunt needle into the uterine horn just proximal to the cervix and longitudinally scratching the entire length of the uterine horn along the anti-mesometrial side. Mice were received the trauma in one horn. Induced female mice were sacrificed 24 hr and 48 hr after trauma.

Total RNA extraction

Total RNAs of uteri were extracted using TRIzol reagent (Invitrogen, San Diego, CA, USA). Briefly, the uterine tissues in TRIzol reagent (100 mg / 1ml) were homogenized and stored for 10 min at room temperature (RT). The chloroform of 0.2 ml/1 ml TRIzol reagent were added to the homogenates and vigorously shaken for 15 sec. After then, the mixture kept for 15 min at RT and centrifuged 12,000g for 15 min at 4℃. The clear supernatant was transferred to new tube, added 0.5 ml isoprophanol per 1ml TRIzol reagent, mixed softly, kept for 10 min at RT, and centrifuged 12,000g for 8 min at 4℃. The supernatant was removed, added 1 ml DEPC-treated 75% ethanol to wash, mixed by inverting, and centrifuged 7,500g for 5 min at 4℃. The supernatant was removed, dried to

remove ethanol about 4 min and added 50 μl DEPC-treated water. Quantity of total RNAs was measured up using NanoDrop 2000/2000c Spectrophotometer (Thermo, Wilmington, DE, USA).

First strand cDNA synthesis and quantitative analysis

In order to reverse-transcription, 5 μg total RNAs were used. First strand cDNAs were synthesized using Accuscript first strand cDNA synthesis kit (Stratagene, CA, USA) according to the manual of manufacture. Briefly, reaction reagents were 5 μg total RNA, 5.0 μl Accuscript buffer (10x), 1.0 μl oligo dT primer (0.5 μg / μl), 1.0 μl random primers (0.1 μg / μl), 2 μl dNTP mix (100 mM), RNase-free water. Reaction mixture was incubated at 65℃ for 5 min, placed the tube at RT to allow the primers to anneal to the RNA for 10 min, after then added 4.0 μl DTT (100 mM), 2.0 μl RNase block ribonuclease inhibitor (40 U / ml), 1.0 μl Accuscript multiple temperature RT. The mixture was incubated at 42℃ for 1 hr and 70℃ for 15 min. Real-time PCR was performed to quantity the mRNA levels using SYBR Premix Ex Taq™ II and Thermal Cycler Dice Real Time System TP800 (TaKaRa, Tokyo, Japan). Each reaction was run in triplicate and consisted of 1.0 μl cDNA, 10 μl SYBR Premix Ex Taq™ and 10 pM of the primers listed in Table 1. The fold change in gene expression was calculated using the ΔΔCt method with the housekeeping gene, a ribosomal protein, 36B4, as the internal control.

Protein extraction and Western blotting analysis

Before protein extraction, tissue was washed using cold Y-PBS (0.7 mM PMSF, 1 mM Benzamidine-HCl, 4 μg / ml Leupeptin, 2 μg / ml Aprotinin, 2 mM EDTA). Uterine tissues were homogenized in cold homogenization buffer (50 mM Tris-Cl, 150 mM NaCl, 10 mM β-mercaptoethaol, 2 mM $CaCl_2$, 0.1 mM PMSF, 1 μM Leupeptin, 1 μM Pepstatin, 0.5 mM EDTA, 15% Glycerol, 0.1% NP-40). The homogenates were centrifuged to remove insoluble materials. The protein concentration was determined using protein dye reagent (Bio-Rad Laboratories, Inc., Richmond, CA) by Bradford assay. 1 mg/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, 20% Methanol, pH 8.3). The membranes were blocked in 5% skimmed dry milk in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20) for 1 hr at RT, and washed three times with TBST. The membranes were incubated for overnight at 4℃ with rabbit polyclonal BDKRB2 antibody (Biorbyt, UK, diluted 1:500); rabbit polyclonal kallikrein5 antibody (Abcam, UK, diluted 1:500); mouse monoclonal actin antibody (Sigma, USA, diluted 1:2000). After incubation, membranes were washed three times and incubated for 30 min with goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology, CA, USA, diluted 1:2,000); goat anti-mouse IgG (Santa Cruz Biotechnology, CA, USA, diluted 1:2,000), and washed

three times. The bands were detected using ECL solution (GE Healthcare, Little Chalfont, UK) by Kodac Image Station 4000MM PRO.

Whole mount immunohistochemistry

Small pieces (~2 mm X 3 mm X 1 mm) of the uteri were excised and placed in 15 ml tubes. The uterine tissues were blocked using Fc block (BD biosciences, USA) in PBA (phosphate-buffered saline + 1% bovine serum albumin + 0.1% sodium azide) with shaking at 4℃ for 20 min. The primary antibody, Cy5-conjugated anti-BDKRB2 (Antibodies-online, Germany, diluted 1:50) and FITC-conjugated anti-CD31 (Novusbio, USA, diluted 1:200), were added directly to the tubes and incubated with shaking at 4℃ for 2 hr. The sample was washed twice by adding 4 ml of PBA to tube and rotating at 4℃ for 1 hr. After the final wash, samples were removed from the tubes, and placed on slides with two drops of PBA. A coverslip was placed on top of the piece of the sample, gently pressed down and excess PBA. This fragment was then analyzed by Zeiss LSM 700 laser scanning microscope with ZEN software.

Statistics

The t-test was used to evaluate the difference between control and experiment group. Results were presented as mean ± SEM. Values of $P < 0.05$ were considered significant.

Table 1. Sequence of primers

Gene	NCBI gene reference	Primer sequence (5’-3’)	Amplified length (bp)
Klk5	NM_026806.2	TACCCTGATCACAACCCTGGTTCT GATTCTGAACACTGGCTTTCTGC	313
Kng1	NM_001102411.1	GCTCAGAGTGGCCTCGCAT GATATGGCATGCACACAACCAAT	194
Kng2	NM_201375.2	GCTCAGAGAGGCCTGGCGT GATATGGGATGCACACAACTCGC	194
B2 receptor	NM_009747.2	TTTCGAAGGACAACTGCCCA ACAACACCTCTCCAAACACCCA	264
36b4	NM_007475	CGACCTGGAAGTCCAACTACTTCCT ATGCTGTTGGCCAATAAGGTGC	303

Table 2. Thermal cycler schedule

Step	Temperature (℃)	Time	Cycles
Initial cycle	94	5min	1
	59	30sec	
	72	1min	
Denaturation	94	1min	37
Annealing	59	30sec	
Extension	72	1min	
Final cycle	94	1min	1
	59	30sec	
	72	7min	
Hold	4	Indefinitely	

RESULTS

Expression of *Klk, Kng* and *B2 receptor* mRNA and protein in mouse uteri during pregnancy

To investigate the mRNA expression of *Klk, Kng* and *B2 receptor*, during pregnancy, real-time PCR analysis was performed. *Klk5* mRNA levels were increased until day3 of pregnancy and showed peak again on day 5 and 7. *Kng 1* and *Kng2* were expressed highly from on day 6 to 12 of pregnancy. *B2 receptor* was increase on day 3 of pregnancy and very row until on day 6 of pregnancy. It peaked on day 7 of pregnancy and decreased until on day 12 of pregnancy (Fig. 1).

The profiles of Klk5 and B2 receptor protein was analyzed by Western blot. Protein of Klk 5 and B2 receptor were detected during pregnancy (Fig. 2).

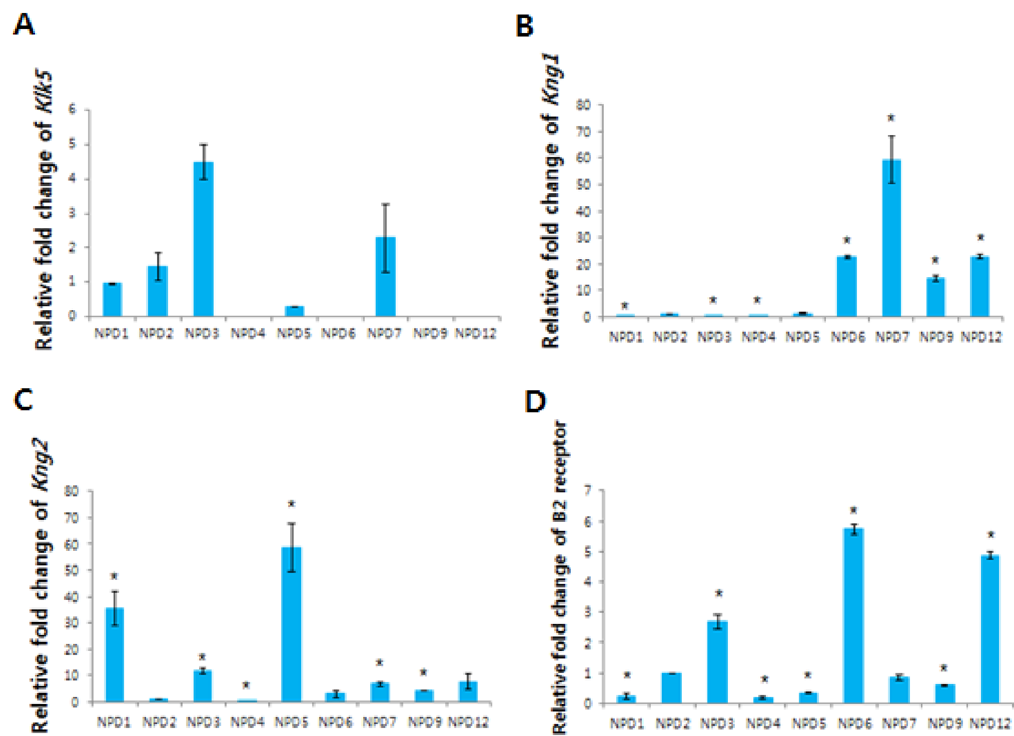
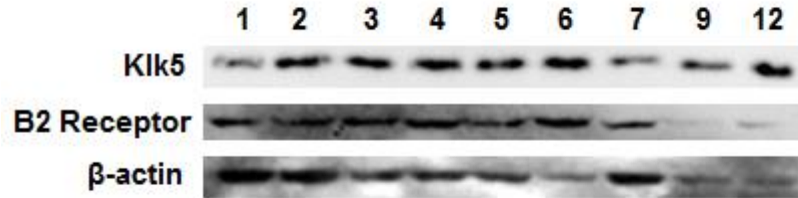


Figure 1. Expression profiles of *Klk, Kng and B2 receptor* mRNA expression level in mouse uteri during pregnancy.

(A) Klk5, (B) Kng1, (C) Kng2, (D) B2 receptor. NPD; Natural pregnancy day. Values represent the mean ± SEM. * indicated p-value < 0.05.

A



B

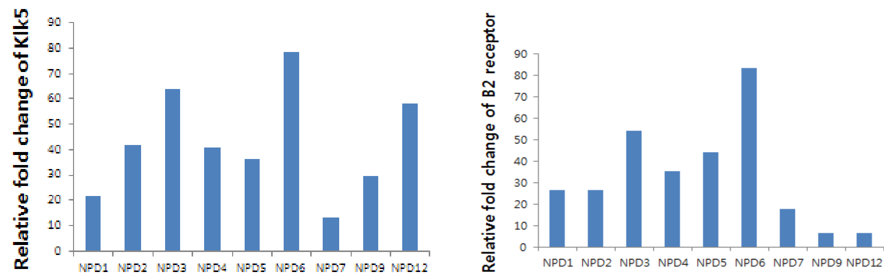


Figure 2. Profiles of Klk5 and B2 receptor protein expression in mouse uteri during pregnancy.

(A) The Klk5 and B2 receptor protein produced in the mouse uterus during pregnancy were detected by Western blot. (B) Normalization of Klk5 and B2 receptor proteins with β-actin.

Localization of B2 receptor protein in decidua

To evaluate the cell specific expression whole mount immunohistochemistry was performed with B2 receptor and endothelial cell specific antibodies respectively. B2 receptor was localized around the neogenic blood vessel in uterus of embryonic day 5.5 (Fig. 3 A-C).

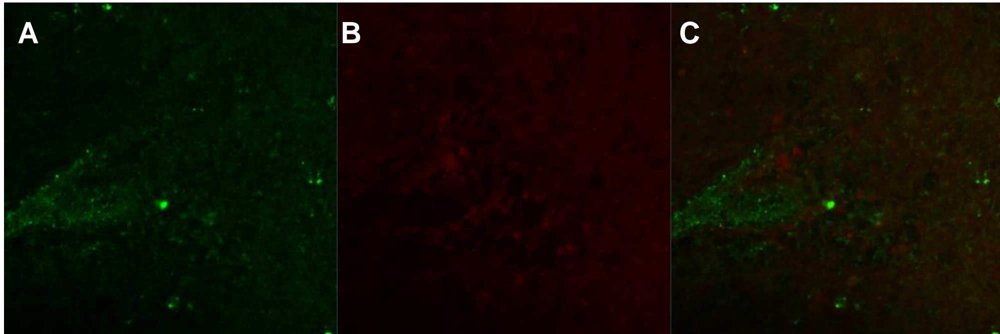


Figure 3. Localization of B2 receptor protein in decidua

(A), (B), (C) embryonic day 5.5; (A) endothelial cell; (B) B2 receptor; (C) (A) and (B) merge. Magnification; A-C, 200.

Expression of *Klk, Kng* and *B2 receptor* mRNA and protein in mouse uteri of delayed implantation

The mRNA expression profiles of *Klk, Kng* and *B2 receptor* in the delayed implantation model were performed with real-time PCR analysis methodology. The relative quantity of *Klk5* was dramatically increased after administration of E_2. *Kng1* was peaked at 12hr after administration of E_2. *Kng2* was detected more higher than *Kng1*. B2 receptor was increased after administration of E_2 (Fig. 4).

The profiles of Klk5 and B2 receptor protein were analyzed by Western blot. Proteins of Klk5 and B2 receptor were detected (Fig. 5). Klk5 was highly expressed. B2 receptor was increased E_2 post 1.5hr until 9hr.

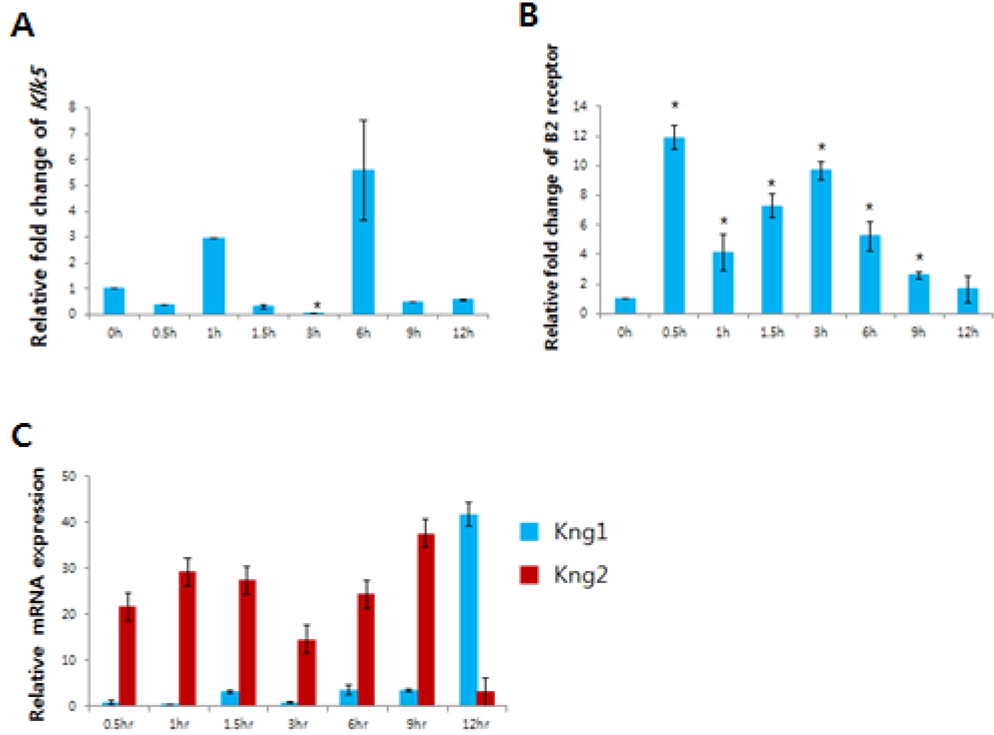
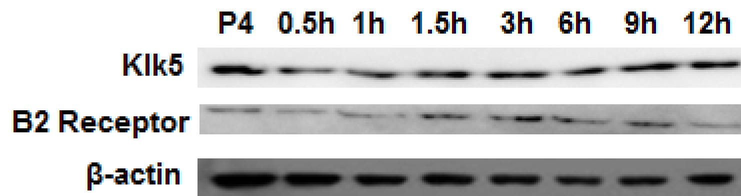


Figure 4. Expression profiles of *Klk, Kng and B2 receptor* mRNA level in mouse uteri of delayed implantation.

(A) Klk5, (B) B2 receptor, (C) Kng1 and Kng2. 0h: P_4 only, E_2 post 0.5h-12h. Values represent the mean ± SEM. * indicated p-value < 0.05.

A



B

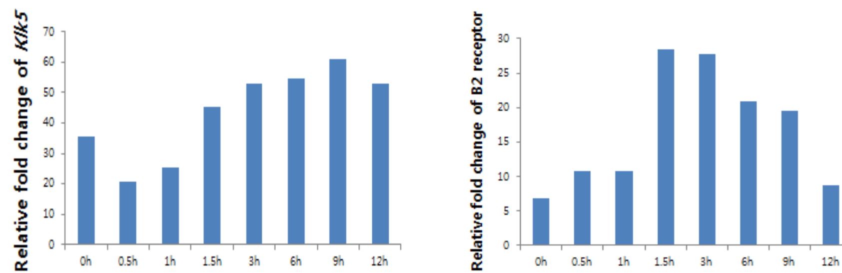
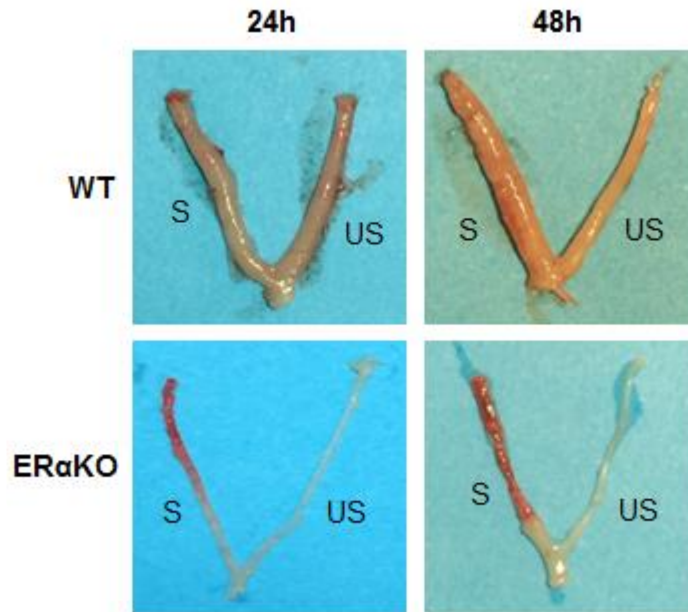


Figure 5. Profiles of Klk5 and B2 receptor protein expression in mouse uteri of delayed implantation.

(A) The Klk5 and B2 receptor protein produced in the mouse uteri of delayed implantation were detected by Western blot. (B) Normalization of Klk5 and B2 receptor proteins with β-actin.

E_2-ER mediated regulation of angiogenesis through bradykinin-B2 receptor

To evaluate the role of E_2 and ER on angiogenesis artificial decidualization models were employed. Decidual responses were detected both in wild type and ERαKO mice (Fig. 6A). In ERαKO mice the development of blood vessels were decreased compared with wild mice in the given areas. The number of cell were not different (Fig. 6B, C). B2 receptor specific signals were also decreased in ERαKO mice (Fig. 6B, C).


A
24h
48h
WT
S
US
S
US
ERαKO
S
US
S
US

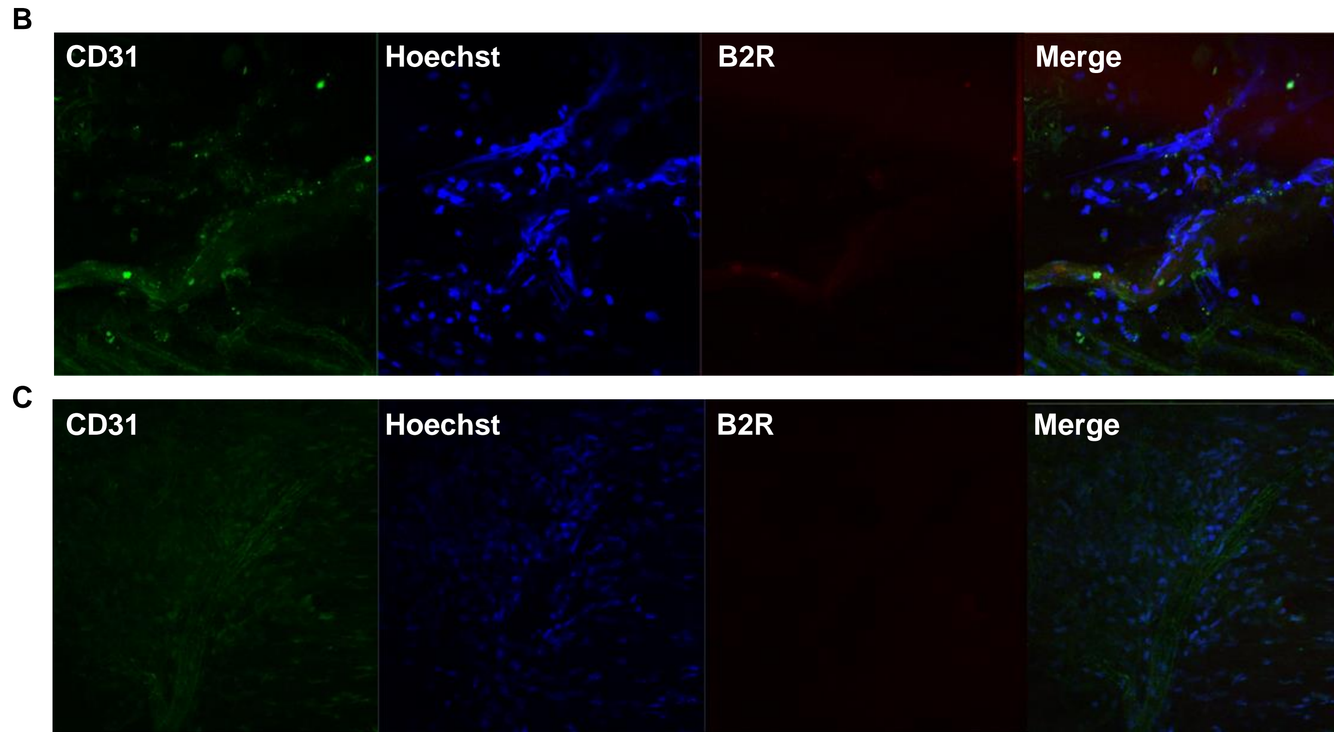


Figure 6. Whole mount immunohistochemistry of B2R and CD31 in deciduoma of estrogen receptor α knockout mice.

(A) Gross morphology of artificially stimulated decidualization model. (B), (C) Artificial decidualization was induced and sacrificed after 24h. (B) Wild type mice. (C) ERαKO mice. US: unstimulated, S: stimulated. Magnification; 200X

DISCUSSION

In the present study, we observed a change in the uterine expression of Klk5, Kng1, Kng2 and B2 receptor under conditions between normal pregnancy and delayed implantation model. During normal pregnancy, the expression levels both mRNA and protein of Klk5 was changed after implantation. It's substrates increased after implantation. Furthermore, B2 receptor also detected in the near implantation sites. It means that kallikrein-kinin system exist in the implantation site.

Angiogenesis is a key factor for mammalian development during implantation. The study for angiogenesis is at the frontier of developmental biology and chronic interesting. Angiogenesis is a multistep process that can be regulated by a variety of factors. A few of angiogenic factors such as nitric oxide, VEGF, BK are known and their role were examined in various tissues but they relationship is not clear. Previous study, vessel specific site was decreased by B2 receptor antagonist during early pregnancy (Jeon, 2013). It is suggested that angiogenic role of bradykinin during decidualization.

The expression of kallikrein or B2 receptor were detected in various organs as mentioned previously. Rajapakse and colleagues analyzed the expression of Klk1 in mouse uterus. The authors reported that the Klk1 mRNA level was significantly higher at estrus than at diestrus and administration of estrogen resulted in an increase in the uterine expression of Klk1 in a dose-dependent manner in the ovariectomized mice (2007). Similarly, the kallikrein gene expression was elevated following the estrogen surge at proestrus (Clements et

al., 1997) and myometrial B2 receptor protein and mRNA levels were highest with estrogen level during late proestrus in rat uterus (Murone et al., 1999).

In delayed implantation model, expression of B2 receptor was increased after administration of estrogen in wild type mice, whereas expression of B2 receptor was decreased in estrogen receptor-α knockout mouse. It means that B2 receptor expression is regulated by estrogen via estrogen receptor-α. Previously, Johns et al (2001) and Losorde et al (2001) suggested that one of the ERs mediate the uterine angiogenesis. Present results are further suggested that E_2 −ER mediated kallirkein-kinin system is a mediator of E_2 regulated angiogenesis in uterine.

Recently it is reported that B2 receptor increases the expression of VEGF and VEGFR2 via the PI3 kinase/AKt/GSK3beta signaling pathway (Bader et al., 2009). Kim and his colleagues (2013) reported that P_4 - PR regulated VEGF-A-VEGFR2 signaling. Another study, showed that P_4 blockade, prevented implantation at all gestational time points examined, whereas inhibition of VEGFR2 only impacted embryo viability when administered at post-but not pre-implantation time (Goddard et al., 2014).

Put together these observations led to us suggested that estradiol induce the expression of kallikrein-kinin system components in a implantation stage specific manners. Through these regulation, angiogenesis occurs successfully to support embryonic developmental systems in uterus.

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// ABSTRACT

Angiogenic role of bradykinin during decidualization

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Implantation is a critical event in early pregnancy that is initiated when embryo attaches to the uterine epithelium. After attachment of the embryo, the uterine stromal cells undergo decidualization. Decidualization is significant for pregnancy, if maternal uterus does not undergo decidaulizaiton, the embryo will not develop normally. Angiogenesis, the process of new blood vessels growth from existing blood vessels, is important feature of decidualization. Bradykinin is a nonapeptide generated from cleavage of kininogen by kallikrein. The bradykinin regulates physiological process, such as inflammation, vasodilation, and angiogenesis. It is reported that kallikrein, bradykinin receptor expressed in uterus during pregnancy, but their role is not fully understand. Previous study showed that the numbers of blood vessel specific site were decreased by the B2 receptor antagonist. It is suggested that kalikrein-kinin system related to the uterine angiogenesis. Previous study showed that expression change of kallikrein,

kininogen, and bradykinin receptor in ovariectomized mouse uterus and in a delayed implantation uterus. These data suggest that steroid hormone estrogen regulates the kallikrein, kiniogen, and bradykinin receptor during periimplantation period. In addition, estrogen and kallikrein-kinin system is important in implantation maintaining. Interestingly, Klk5 mRNA was expression from day1 of pregnancy until day3 and peaked again at day 5 and 7. Kininogen1 and 2 showed different expression patterns after implantation. B2R mRNA expression pattern were similar Klk5 but the expression levels were relatively high compared with Klk5. B2R protein levels were maintained basal level and increased after implantation. In ERαKO mice, the angiogenic effects of estrogen was dramatically decreased.

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마지막으로, 사랑하는 아빠! 아빠에게 드디어 막내딸이 졸업했다고 말할 수 있게 되었네요. 이 논문을 받고 기뻐하실 아빠 모습을 생각하니 이 순간이 진심으로 기쁘고, 행복합니다. 감사하다고 사랑한다고 보고 싶다고 수없이 말해도 부족하네요. 무뚝뚝한 엄마를 대신해 무한애정을 표현하셨던 우리집의 사랑꾼 아빠를 만나서, 그런 아빠의 사랑과 보살핌을 받아서, 저는 지금도 아주 행복한 기억들을 가지고 살아갑니다. 아빠 딸로 태어나서 행복하고 또 감사합니다.

이 논문을 저의 첫사랑이자, 제 삶의 든든한 버팀목, 그리고 영원한 내편 우리아빠에게 드립니다.