Early Development of Mammary Gland is Associated with FGF8 Signaling in Mice

SOO-JIN SONG

Department of Medical Science

The Graduate School, Yonsei University

Early Development of Mammary Gland is Associated with FGF8 Signaling in Mice

Directed by Professor HAN-SUNG JUNG

The Master's Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of master of Medical Science

SOO-JIN SONG

June 2003

The certifies that Master's Thesis of

SOO-JIN SONG is approved.

Thesis Supervisor : HAN-SUNG JUNG

Thesis Committee Member #1 : HEE-JIN KIM

Thesis Committee Member #2 : HYE-YEON LEE

The Graduate School

Yonsei University

June 2003

Acknowledgments

I would like to thank Professor Han-Sung Jung for invaluable advice and guidance. My thanks must also go to Professor Cheryll Tickle at University of Dundee for her help and support, Dr. Keiichi Akita at Tokyo Medical and Dental University for help me on several experiments.

It is great pleasure to thank Jae-Young Kim, Eun-Jung Kim, Maxwell C. Eblaghie for letting me collaborate on several experiments.I am grateful to many people from the Department of Oral Biology, College of Dentistry, Yonsei University, past and present, who made my MSc course enjoyable.

Special thanks to Professor Hee-Jin Kim, Dr. Yamamoto Hitoshi for his encouragement.

Finally, special thanks go to my family, for supporting me always.

Soo-Jin Song

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT.....1

I. INTRODUCTION.....3

II. MATERIALS AND METHODS 10

1. Experimental Animals 10

2. Histology 10

3. Whole-mount *in situ* hybridization.....10

4. *In vitro* organ culture 11

5. Di.I. microinjection 11

6. FGF8 and SU5402 soaked beads 12

III. RESULTS.....13

1. Development of mammary gland is asynchronous 13

2. Early development of mammary gland is associated with BMPs signaling 15

3. Epithelial signaling in mammary gland is induced during early embryonic development.....21
4. Mammary glands form by cell migration.....21
5. FGF8 and SU5402 soaked beads 25

IV. DISCUSSION.....32
1. Asynchronous development of mammary gland.....32
2. The predicted position of the milk line in mice 34
3. Signal from epithelium or mesenchyme at initial stage for mammary gland development.....36
4. Relationship between FGFs signaling and BMPs signaling.....37

V. CONCLUSION.....40

REFERENCES.....42

ABSTRACT (In Korean) 48

LIST OF FIGURES

Figure 1. Diagram and histological observation during early development of mammary glands from E11.5 to E15.5 in mouse embryos. 4

Figure 2. Schematic ventral views of the transformation from milk line to the mammary ridge in human. 6

Figure 3. Summary of mammogenesis in mouse embryo from E11.5 to E15.5 7

Figure 4. Serial sections of each mammary bud at E12. 14

Figure 5. Expression of *Bmp2*, *Bmp4 Lef1*, *Msx1* and *Msx2* from E11 to E13.5. 18

Figure 6. The position of each mammary bud during the early development of mammary gland. 19

Figure 7. Sections after whole-mount *in situ* hybridization by *Bmp2, Bmp4, Lef1*, *Msx1* and *Msx2* at E12. 23

Figure 8. Tracing of the cell migration using Di.I. microinjection after 48 hours and 72 hours *in vitro* organ culture. 24

Figure 9. *In vitro* cultured mammary gland for 48 hours and 72 hours.....27

Figure 10. FGF8 soaked beads. 28

Figure 11. SU5402 soaked beads.....30

Figure 12. SU5402 and FGF8 soaked beads. 31

Figure 13. The model for the genetic pathway integrating FGF8, *Bmp2*, *Lef1*, *Msx1* and *Msx2* in epithelial-mesenchymal interactions that regulate mammary glands in mice.....39

LIST OF TABLES

Table. 1. The developmental order of each stage during early development of mammary glands. 20

Abstract

Early Development of Mammary Gland is Associated with FGF8 Signaling in Mice

SOO-JIN SONG

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor HAN-SUNG JUNG)

The patterned distribution of mammary glands suggests there are similarities between the development of the mammary glands and other epithelial appendages, including tooth, feather, hair and tongue papillae. The formation of skin appendages requires an interaction between the epithelium and mesenchyme.

In mice, there are five pairs of mammary buds. Three mammary buds are located in the thoracic region and two in are located in the inguinal region. The mammary glands are derived from the "milk line", which is formed by the migration of epidermal cells, rather than by cell proliferation.

A histological study on the early development of the mammary gland showed that each mammary bud has an asynchronous developmental process with the different

sizes and developmental stages. In addition, the *Bmp2, BMp4, Lef1, Msx1* and *Msx2* expression patterns are different in all five individual mammary buds during the early stages of mammary gland development. Moreover, all five individual mammary buds have their own identity. The development of individual mammary buds appear in the order of 3rd, 1st, 4th, 2nd, and 5th mammary bud, as indicated by the expression pattern of the above genes.

A study on the Di.I. labeled cell migration pattern showed the two lines of the thoracic and inguinal regions are the milk line. The migration pattern of the Di.I. labeled cells appeared to coincide with the changing gene expression pattern between E11.5 and E12.5.

This results of this study showed that FGF signaling is involved in the early development of mammary glands, and FGF8 induces the ectopic expression pattern of *Bmp2, Lef1*. FGF8 controls a genetic hierarchy, involving the *Bmp2* and *Lef1* signals, which regulates the early development of the mammary glands.

The mammary glands are derived from the milk line, which has a potent to develop from each mammary bud. The mammary glands can be considered to be an epithelial appendage, which are formed throughout the spacing pattern through epithelial - mesenchymal interactions. This also might be a good model system for investigating the pathway of the signaling molecules in induction, maintenance and morphogenesis.

Key words : epithelial-mesenchymal interaction, mammary bud, milk line, asynchronous, gene expression pattern, FGF8, signaling pathway

Early Development of Mammary Gland is Associated with FGF8 Signaling in Mice

SOO-JIN SONG

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor HAN-SUNG JUNG)

Ⅰ. INTRODUCTION

The mammary one type of skin appendage that develops under the influence of the sequential reciprocal interactions between its epithelium and adjacent mesenchyme.[1] In mice, the development of mammary glands is the one of the spacing pattern that five individual mammary glands form in the milk line running on the ventral side of body. The early development of mammary glands begins on the epithelium of both lateral flanks of the embryo around E10.5.[2] By E11.5-E12.5, five mammary placodes are detected as epithelial thickenings, which then develop epithelial down-growths into mesenchymal cells and form into bud-like structures. At E13.5, the mammary buds sink into the underlying dermis, and at E14.5, a fat pad precursor differentiates from the deeper mesenchyme. At E15.5, each mammary bud is elongates as a sprout (Fig. 1).[1,2] The mammary gland is located at three thoracic regions and two inguinal

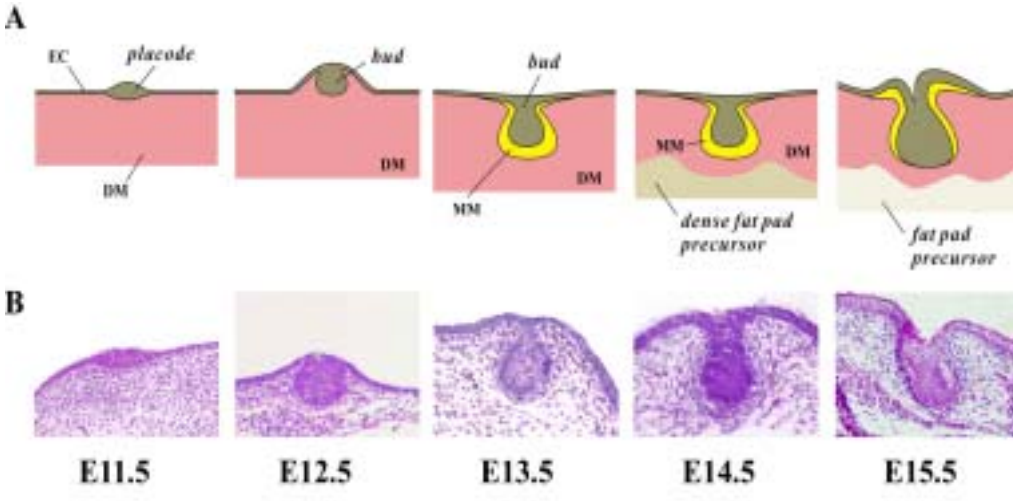

Fig. 1. Diagram and histological observation during early development of mammary glands from E11.5 to E15.5 in mouse embryos. The schematic diagram (A) of and histologic section (B) of the early development in mouse mammary glands. At E11.5, placode formation, at E12.5, each placode has transformed into a bulb of epithelial cells. At E13.5, the bud sinks into the underlying dermis. At E14.5, the mesenchyme differentiates into a fat pad precursor. Finally, at E15.5, each bud is elongated as a sprout (DM : dermal mesenchyme, EC : ectoderm, MM : mammary mesenchyme).

regions sharing these morphological developmental processes. The mammary gland is derived from the "milk line", which is formed by the migration of epidermal cells, rather than by cell proliferation (Fig. 2).[3,4]

Although many studies have examined the mammary glands after birth, little is known about the genes that regulate the initial phases of formation. *Lef-1* RNA is first found at the sites where the placodes are formed[5], and is followed by a shift to the mesenchyme[6]. The homeobox containing the transcription factor, *Msx1* and *Msx2*, are initially coexpressed in the developing placodes.[7] At a slightly later stage, *Msx1* gene expression is down regulated, whereas *Msx2* gene expression occurs in both the placode and mesenchyme.[6] Mammary gland development is not affected by the absence of *Msx1* alone. However, mice in which both *Msx1* and *Msx2* have been inactivated lack mammary buds.[6] The expression of the *Msx1* and *Msx2* homeobox genes has been shown to be coordinately regulated with the *Bmp2* and *Bmp4* ligands in variety of developing tissues such as the tooth, hair and whisker follicles. Transcripts from all four genes are developmentally regulated during both fetal and postnatal mammary gland development.[7] The T-box transcription factors, *Tbx2* and *Tbx3*, are expressed in the early mammary rudiment. *Tbx3* mutations in humans lead to severe mammary hypoplasia, or sometimes a complete lack of mammary glands (Fig. 3).[8,9] *Fgf8* plays a critical role in initiating the formation of epithelial-mesenchymal interaction organs by activating epithelial cells. As a representative example, *Fgf8* induced AER in the initial development of the limbs.[10] *Pyst1* is activity

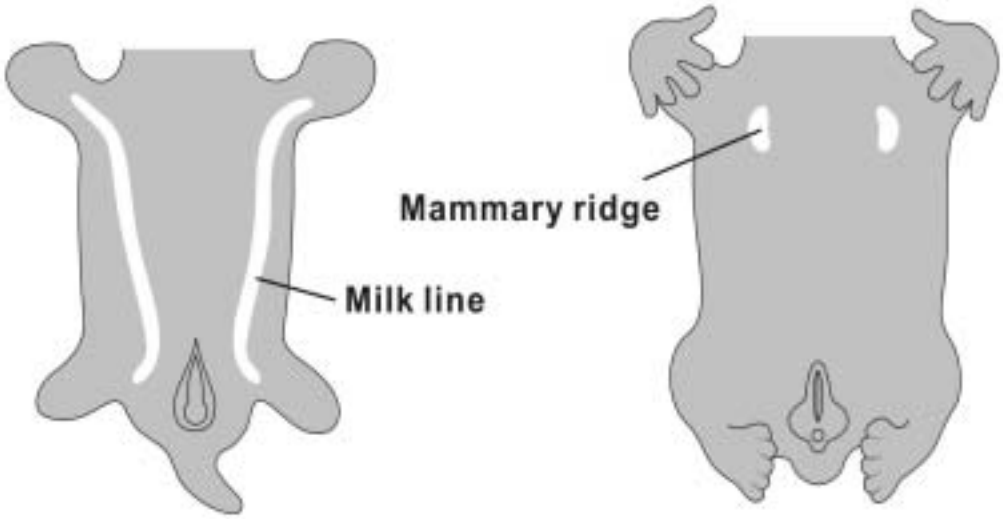

Fig. 2. Schematic ventral views of the transformation from the milk line to the mammary ridge in humans (Moore KL. et al., 1998).[12]

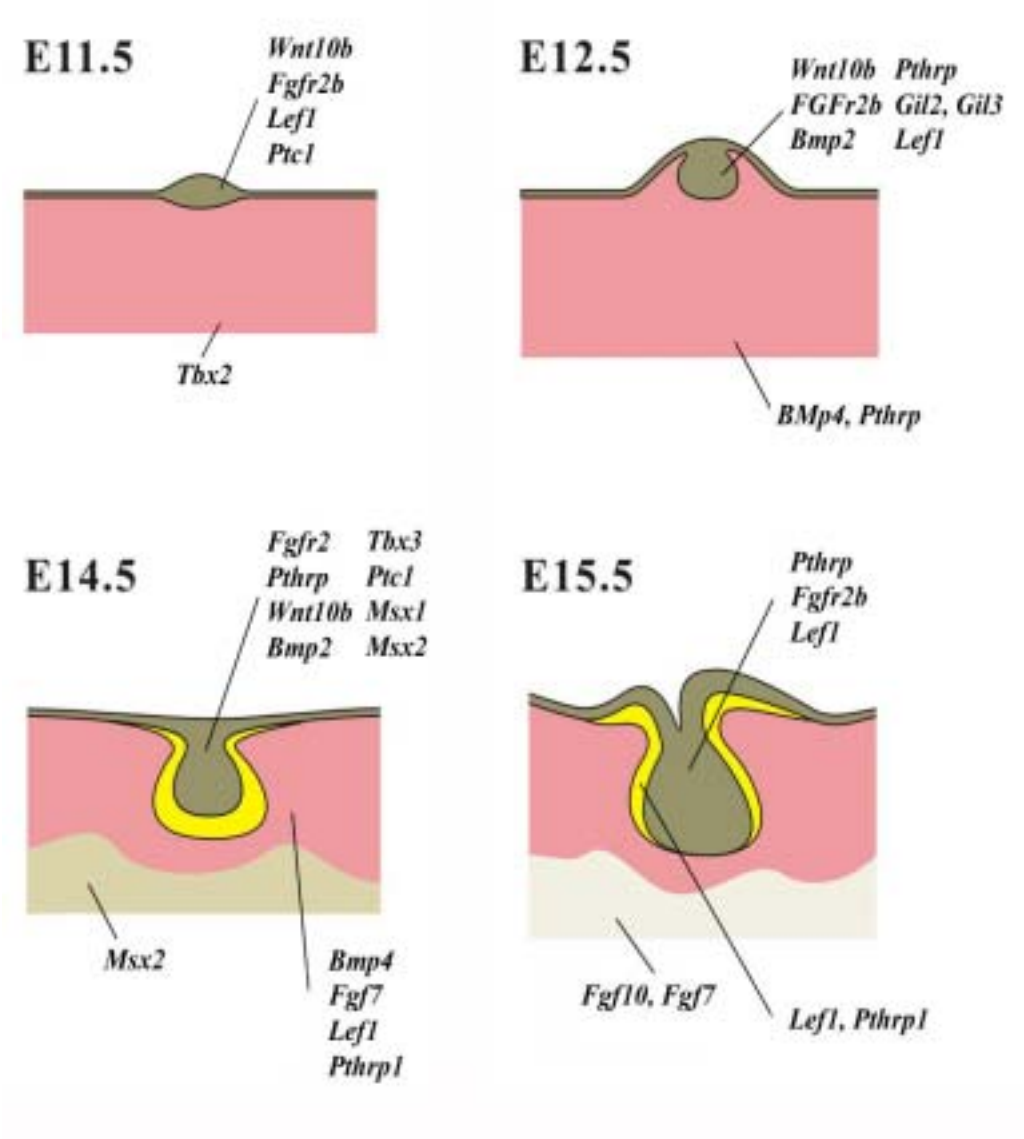

Fig. 3. Summary of mammogenesis in a mouse embryo from E11.5 to E15.5. The expression is known for some genes in embryonic development (Veltmaat JM. et al., 2003).[1]

involved in the developmental function of FGF/FGFR signaling, particularly with respect to the *Fgf8* function during embryogenesis.[11] SU5402 is known as an inhibitor of FGF pathway activity, and inhibits the tyrosine kinase activity of FGFR1 by interacting with the catalytic domain of FGFR1, and inhibiting the FGF- induced phosphorylation of ERK1 and ERK2.

Nevertheless, the histological and molecular processes during the early development of the mammary glands are still unclear. In order to understand the early development of the mammary glands, histological studies on mouse embryos between E11.5 to E15.5 were carried out using the Hematoxylin-Eosin staining method. It shares similar morphological changes during the early development of the mammary glands such as hair follicles and salivary glands. However, in case of mammary gland development, each mammary bud undergoes an asynchronous development process. The *Bmp2, Bmp4, Lef1, Msx1* and *Msx2* expression patterns during the initiation of mammary gland development from E11 to E13.5 were examined by whole-mount *in situ* hybridization. The expression levels of the five genes showed different patterns in all of five individual mammary buds respectively. In addition, the Di.I. micro-injection results were examined in order to understand the origins of the mammary glands in early development. Mouse embryos slightly younger than E11 were injected with Di.I. in the border between the body and the limb. As the injected cells of the forelimb moved ventral-cranially along the milk line, the formation of individual mammary buds is derived from the origins of the mammary gland by cell migration. Finally, in order to understand the implication of FGF signaling in the early

development of the mammary glands, FGF8 soaked beads were implanted in the flanks in mice slightly younger than E11. Cultured explants have the ectopic expression pattern around the FGF8 soaked beads. FGF8 is involved in the early development of mammary gland.

This study aimed to understand the roles of the tissue interactions and the molecules in induction, maintenance and morphogenesis during the early development of the mouse mammary glands.

Ⅱ. MATERIALS AND METHODS

1. Experimental Animals

Adult ICR mice were housed in a temperature-controlled room (22°C) under artificial illumination (lights on from 05:00 h to 17:00 h) and at 55% relative humidity, with access to food and water *ad libitum*. The mouse embryos were obtained from time-mated pregnant mice. The designated embryonic day 0.5 (E0.5) was determined the day that the presence of a vaginal plug was confirmed.

2. Histological observation

The mouse embryos were fixed in 4% paraformaldehyde in PBS (pH 7.4) overnight at 4°C. After dehydration, the mouse embryos were embedded in paraffin wax. Five-micrometer thick sections were cut from the paraffin blocks and dewaxed in xylene 4 times for 5 min each. After rehydration in a graded series of ethanol, the sections were stained with Hematoxylin and Eosin *ad libitum*. The tissue was then dehydrated in a graded series of ethanol and mounted using Canadian balsam in xylene.

3. Whole-mount *in situ* hybridization

The mouse embryos were fixed in 4% paraformaldehyde in DEPC-PBS overnight at

4°C. They were then dehydrated in graded series of methanol (25% to 100%) and stored at –20°C for up to one month. Anti-sense RNA probes were labeled with digoxygenin (BMS), and whole-mount *in situ* hybridization was performed, as described by Wilkinson (1992).[13]

4. *In vitro* organ culture

The E11 mouse embryos were quickly extracted from the uteri, driven out in BGJb with 0.5% penicillin streptomycin. Mouse embryos were dissected into two pieces along the midline in BGJb with 0.5% penicillin streptomycin and 0.2% ascorbic acid. Each side of the tissue was cultured in BGJb with 0.5% penicillin streptomycin and 0.2% ascorbic acid at 37°C in a humidified atmosphere of 92.5% air and 7.5% CO_2.
All media used contained 10mg/ml penicillin streptomycin and 100mg/ml ascorbic acid.

5. Di.I. Microinjection

Di.I.(1,19-dioctadecyl-3,3,39,39-tetramethyl indocar bocyanine perchloride; Molecular Probes, Eugene, OR) is a vital dye and a member of the carbocyanine dye family. Di.I. is a highly fluorescent lipophilic dye, which labels the cell membrane and has been widely used for investigate the cell fate. It is passed on to the progeny of labeled cells but does not leak to the neighboring cells and is non toxic.[14,15]

0.3% w/v Di.I. in DMSO was used for the microinjection. The Di.I. injection was performed using 10 cm borosilicate capillary pipettes(Sutter Instruments, BF 120-94-10), pulled using a Sutter Instruments Flaming Brown micropipette puller, and filled by capillary action. Using a electric instrument of electroporation, the lipophilic carbocyanine dye inserts into the cell membrane of the cells adjacent to the injection site. The exact position of the dye can be determined using a fluorescent microscope.

Di.I. was injected in the border between the bodies and limbs at E11. After culturing for 72 hours using an *in vitro* culture method, the Di.I. movement was observed in the border each 24 hours.

6. FGF8 and SU5402 soaked beads

For FGF8, heparin acrylic beads (sigma) were washed several time in PBS and incubated for 1 hour at room temperature in 1mg/ml FGF8b (R&D system, cat# 423-F8). The FGF8-soaked beads were then implanted to the *in vitro* cultured explants.
For the pharmacological inhibition of the FGFR activity, SU5402 (calbiochem, cat# 572630)-soaked beads were implanted into the *in vitro* cultured explants. The SU5402 was diluted in DMSO to a concentration of 1mg/ml, and is incubated at room temperature in the dark for 1 hour with the beads.

All protein-soaked beads were stored at 4°C for up to 1week prior to use.

Ⅲ. RESULT

1. Development of Mammary Gland is Asynchronous

The mouse embryos at from E11.5 to E15.5 were processed using the H-E staining method to demonstrate the asynchronous development of the mammary buds. Each mammary bud was numbered from the neck to the genital regions as the 1st, 2nd, 3rd, 4th and 5th mammary bud. The result shows H-E stain at E12 (Fig.4). The mouse embryos at E12 have only four pairs of mammary buds, except for the 5th mammary bud. Four pairs of mammary buds have a different size in the order of the 1st, 3rd, 4th and 2nd. In addition, they had different developmental stages, that is, initially, the 2nd mammary bud occurred at the stage of placode formation (Fig. 4-B), and the 3rd and 4th mammary buds were detected in the next stage, which is epithelial downgrowth into mesenchymal cells (Fig. 4-C, D). The 1st mammary bud developed later than the 3rd and 4th buds (Fig. 4-A). This result shows that when the milk line is transformed into a bud-form of epithelial cells, all five pairs of the mammary buds have their own developmental time course. This means that the development of each mammary bud in mice is not synchronous.

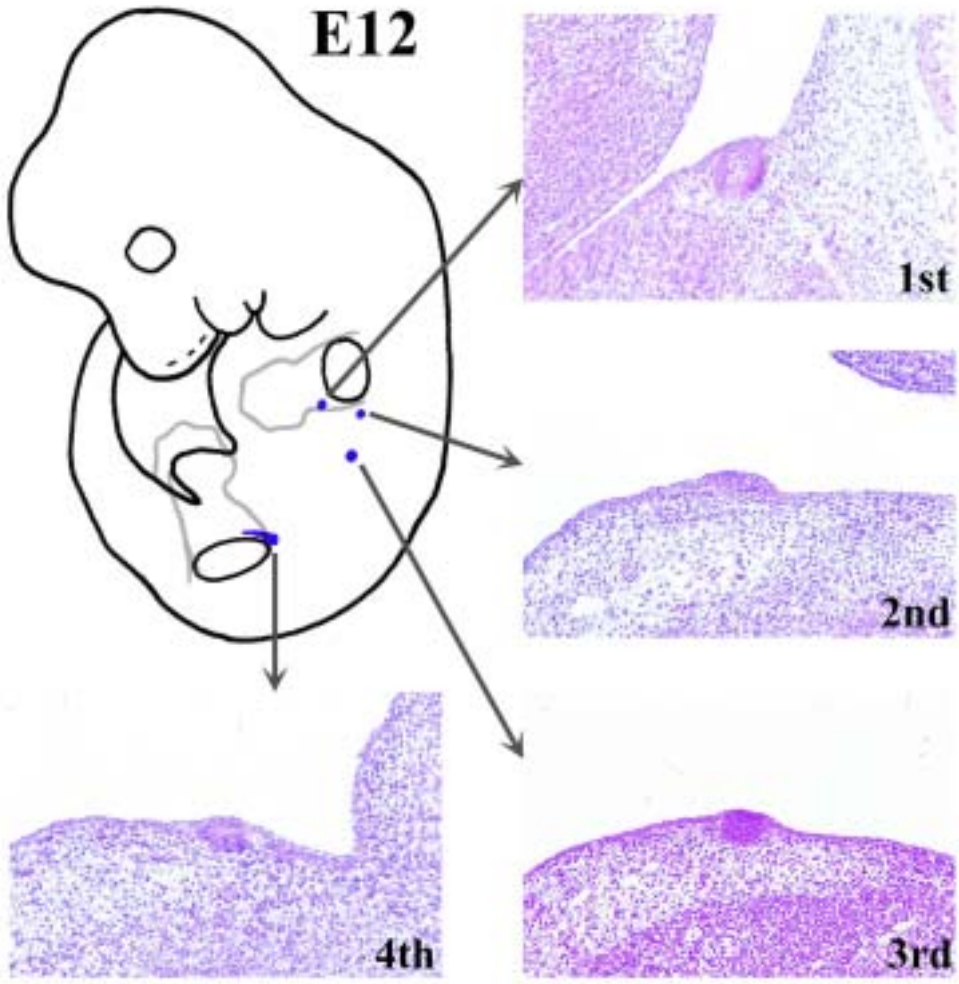

Fig. 4. Serial sections of each mammary bud at E12. Each mammary bud from the neck to the genital region is referred to as the 1st, 2nd, 3rd, 4th and 5th mammary bud. The mouse embryos at E12 have only four pairs of mammary buds (1st : 1st mammary bud, 2nd : 2nd mammary bud 3rd : 3rd mammary bud, 4th : 4th mammary bud).

2. Early Development of Mammary Gland is Associated with BMPs Signaling

Mouse embryos taken from E11 to E13.5 were used in whole-mount *in situ* hybridization (Fig. 5) in order to demonstrate the developmentally regulated *Bmp2, Bmp4, Lef1*, *Msx1* and *Msx2* expression pattern,. *Bmp2* expression was the line-form on the border between the limbs and bodies at E11 (Fig. 5-A). At E11.5, the 1st, 2^{nd,} 3rd and 4th mammary buds expressed *Bmp2.* In particular, the 3rd mammary bud was strongly expressed, whereas the 2nd mammary bud was weakly expressed (Fig. 5-B). At E12, four pairs of mammary buds were expressed, but the 4th mammary bud is peculiarly shaped in that was oval shaped and had a tail (Fig. 5-C). All mammary buds began appearing at E12.5, whereas the 3rd mammary bud was less than the other expression patterns (Fig. 5-D). At E13.5, the *Bmp2* expression of the 3rd mammary bud disappeared (Fig. 5-E).

The mammary buds at E11 expressed the line-form of *Bmp4* expression on the border between the limbs and the bodies (Fig. 5-F), and at E11.5, the 1st, 3rd, and 4th mammary buds were detected, but the 3rd mammary bud was weakly detected. Remarkably, the 1st and 4th mammary buds were detected as the shape of a bud on the line (Fig. 5-G). *Bmp4* was clearly expressed in the 1st, 2nd and 4th mammary buds, whereas, *Bmp4* was weakly expressed in the 3rd mammary bud at E12 (Fig. 5-H). At E12.5, four pairs of mammary bud expressed *Bmp4* except for the 3rd mammary bud (Fig. 5-I), and the *Bmp4* expression at E13.5 was similar to that observed E12.5 (Fig. 5-J).

Lef1 is expressed on the border between the limbs and bodies at E11 (Fig. 5-K). At E11.5, *Lef1* expression was detected in the 2nd and 4th mammary buds and *Lef1* expression was weak in the 3rd mammary bud but it was different from *Bmp2* expression (Fig. 5-L). *Lef1* was expressed 1st, 2nd, 3rd and 4th mammary buds at E12 (Fig. 5-M). At E12.5, five pairs of mammary buds were detected (Fig. 5-N). At E13.5 the *Lef1* expression pattern was almost the same as that at E12.5 (Fig. 5-O).

Msx1 expression was detected in the 3rd mammary bud because this embryo is in the slightly developed stage than E11 (Fig. 5-P). At E11.5, *Msx1* expression was detected in the 1st, 2nd, 3rd and 4th mammary buds (Fig. 5-Q). The *Msx1* expression pattern at E12 was similar to that observed at E11.5 but expression level was higher except for the 3rd mammary bud (Fig. 5-R). At E12.5, five pairs of mammary buds were expressed, but still *Msx1* expression in the 3rd mammary bud was expressed weakly (Fig. 5-S), and at E13.5 was a similar with E12.5 (Fig. 5-T).

Finally, at E11, *Msx2* was expressed on the border between the limbs and the bodies (Fig. 5-U). As well as in the 1st, 2nd, 3rd and 4th mammary bud at E11.5, *Msx2* expression was weakly expressed in the 3rd mammary bud (Fig. 5-V). *Msx2* expression was detected in the 1st, 2nd, 3rd and 4th mammary buds at E12 (Fig. 5-W). At E12.5, *Msx2* was expressed in five pairs of mammary buds, but was weakly expressed in the 3rd mammary bud (Fig. 5-X), and at E13.5, the result was similar to that observed at E12.5 (Fig. 5-Y).

The gene expression pattern in the mammary glands by whole-mount *in situ* hybridization in mouse embryos from E11 to E13.5 about the position of each

mammary bud are shown as a diagram of each stage (Fig. 6), and the developmental order of each mammary bud is shown as a table of each stage (Table. 1). At E11, mammary glands were detected in line-form on the border between the limb and the body. At a slightly older than E11, the beginning of placode formation was indicated by the formation of the 3rd mammary bud. At E11.5, the 1st, 2nd, 3rd and 4th mammary bud appeared. Characteristically, the 1st mammary bud was shaped like a bud on the line, and the 4th mammary bud was oval shaped with a tail. However, the gene expression pattern at the 2nd mammary bud was observed weakly or not at all. With the exception of the 5th mammary bud, all mammary buds were detected at E12. At E13, all five pairs of mammary buds were expressed, but the expression level in the 3rd mammary bud was the lowest.

According to these five genes expression patterns, the development of each mammary bud occurred in order of the 3rd then 1st, 4th followed by 2nd and finally 5th mammary bud.

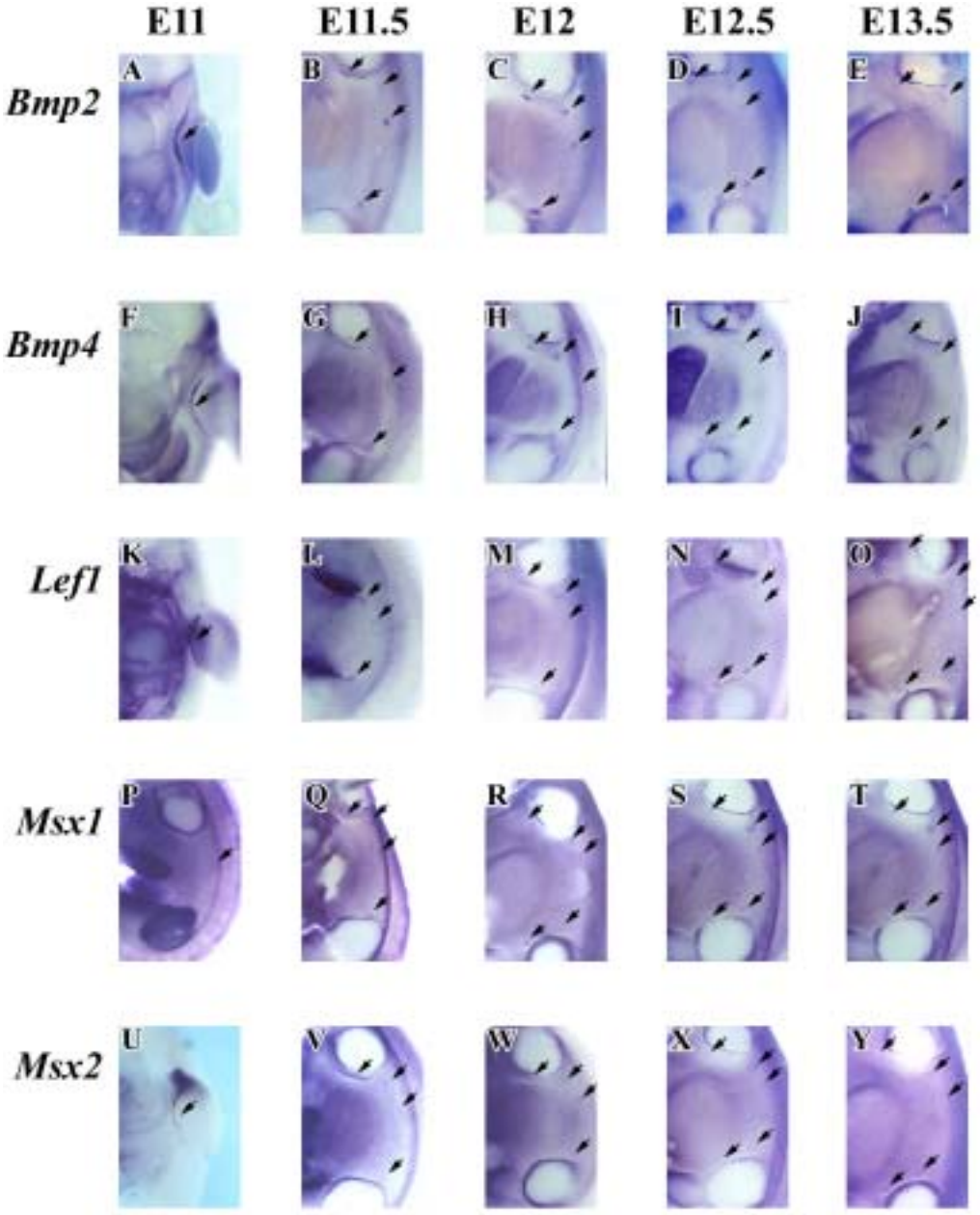

Fig. 5. Expression of *Bmp2, Bmp4 Lef1, Msx1* and *Msx2* from E11 to E13.5.

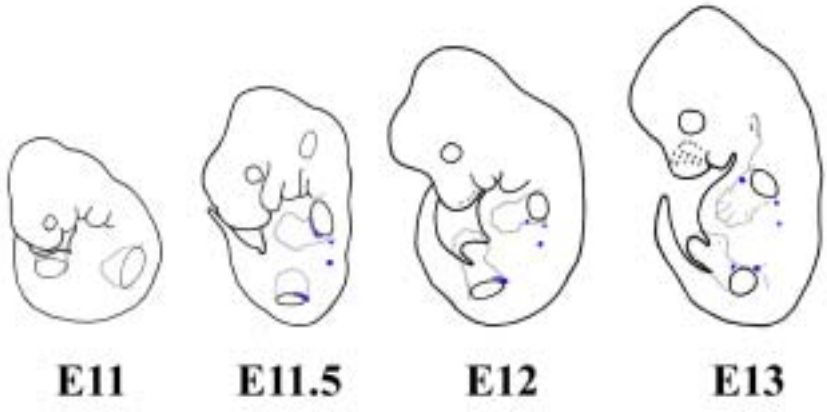

Fig. 6. The position of each mammary bud during the early development of the mammary gland. From E11 to E13, the location of the mammary bud changes during mouse embryo development.

Table. 1. The developmental order of each stage during the early development of the mammary glands. At a slightly older than E11, the 3rd mammary bud formed. At E11.5 the 1st and 4th mammary buds appeared with indications of the 2nd and 5th mammary bud. At E12, the 2nd mammary bud appeared. After E12.5, all five pairs of mammary buds appeared (1st : 1st mammary bud, 2nd : 2nd mammary bud 3rd : 3rd mammary bud, 4th : 4th mammary bud, 5th : 5th mammary bud, # of M.B. : number of mammary bud , * : too weak or non-expression).

Stage / # of M.B.	E11	E11.5	E12	E12.5	E13.5
1st		○	○	○	○
2nd		*	○	○	○
3rd	○	○	○	○	○
4th		○	○	○	○
5th				○	○

3. Epithelial Signaling in mammary gland is induced during Early Embryonic Development

The embryos were sectioned after the whole-mount *in situ* hybridization in order to identify the expression of *Bmp2, Bmp4, Lef1, Msx1 and Msx2* whether the epithelium or mesenchyme (Fig. 7). *Bmp2* expression at E12.5 was detected at the center of the mammary epithelial cells of the 1st, 2nd, 3rd, 4th and 5th mammary bud. Similarly, *Bmp4, Lef1, Msx1 and Msx2* at E12.5 were expressed in the center. The mammary glands from E11 to E13.5 showed similar results. This suggests that epithelial signaling is important for the early development of mammary glands.

4. Mammary Glands form by Cell Migration

A Di.I. microinjection was used in embryos younger than E11 in the *in vitro* tissue culture To detect the origins of the mammary glands. Between E11 and E11.5, the form of the mammary bud was changed from the milk line into an individual bud. When the gene expression pattern in the mammary buds of E11.5 was compared with those at E12, the 1st and 4th mammary bud have potential ability to 2nd and 5th mammary buds respectively**.**

Di.I. was injected in the ventral-lateral flank of the fore limb in the embryos younger than E11. After culturing for 48 hours (Fig. 8-B) and 72 hours (Fig. 8-C) using the *in vitro* tissue culture, Di.I. migration was observed. The injected cells

around the flank of the forelimb moved ventral-cranially following the border between the body and the limb. This suggests that the Di.I. migration pattern in the flank of the fore limb compares with the changing gene expression pattern between E11.5 and E12.5

It was concluded that the mammary glands were formed as a result from the migration of epidermal cells, which expressed in the 1st and 4th mammary bud at E11.5. In addition, the 1st and 4th mammary buds have the potential ability to form the 2nd and 5th mammary buds, respectively.

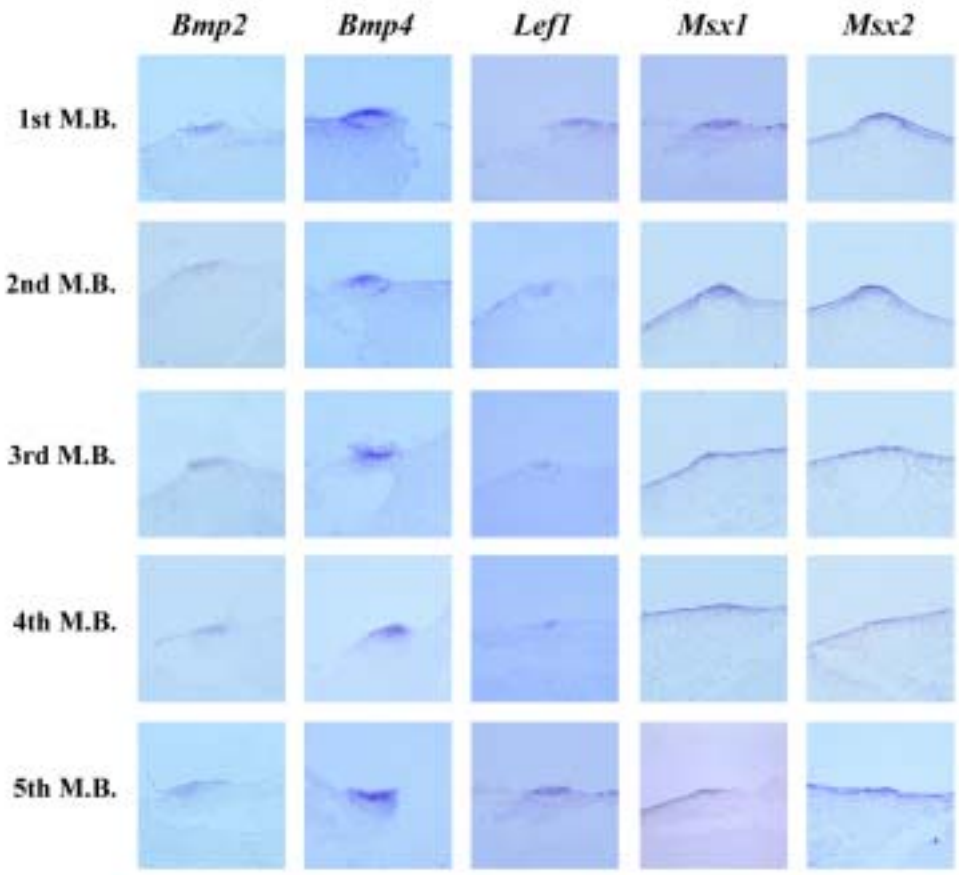

Fig. 7. Sections after whole-mount *in situ* hybridization by *Bmp2*, *Bmp4*, *Lef1*, *Msx1* and *Msx2* at E12.5. The signal derives from the mammary epithelium during the early development of the mammary glands.

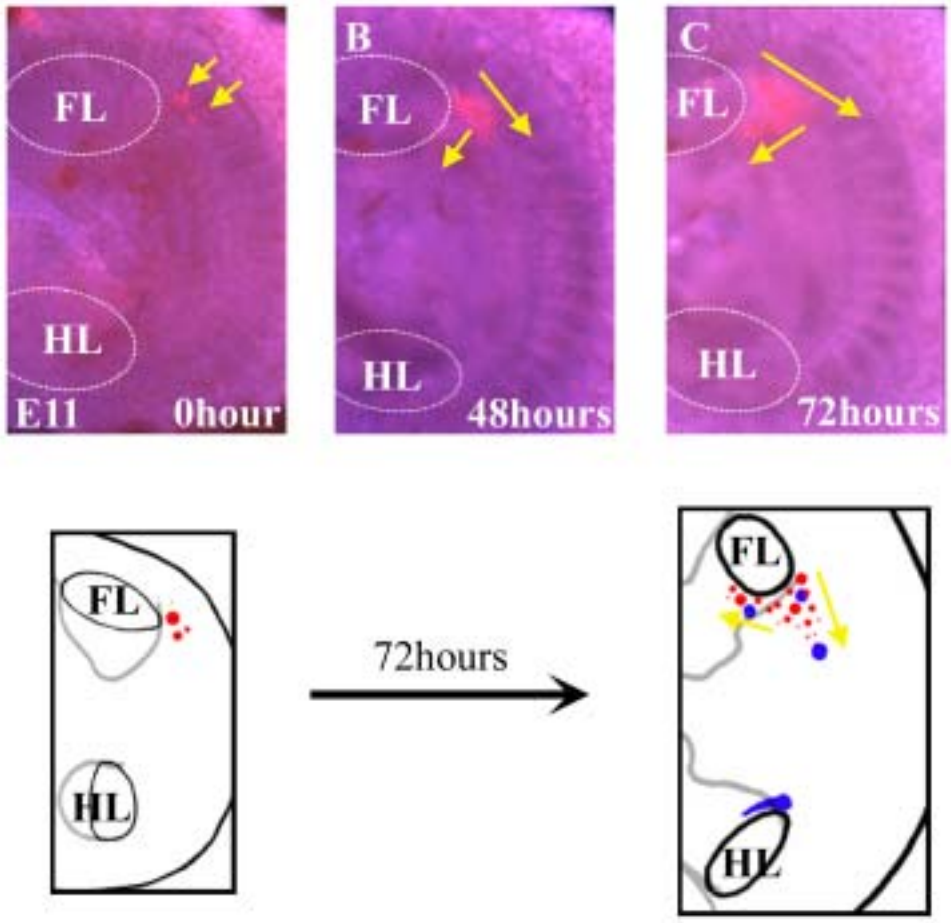

Fig. 8. Tracing of cell migration using a Di.I. microinjection after 48 hours and 72 hours *in vitro* organ culture. Di.I. injected cells around the flank of the forelimb moved ventral-cranially (FL : Forelimb, HL : hind limb, Red point : Di.I., Blue point : mammary bud, Yellow arrow : Di.I. migration).

.

5. FGF8 and SU5402 soaked Beads

A study was carried out using mouse embryos younger than E11 in order to establish the *in vitro* tissue culture system. After the mouse embryos were dissected into two pieces along the midline, one half side was fixed immediately as control, and the other half side was cultured for 48 hours or 72 hours. The cultured explants were then fixed and processed for the whole-mount *in situ* hybridization. The control was not detected in any mammary bud (Fig. 9-A, C, E, G, I). After the other half was cultured for 48hours, *Bmp2* was expressed at the 3rd and 4th mammary buds (Fig. 9-B), and *Tbx3* was expressed at the 2nd, 3rd, 4th and 5th mammary buds (Fig. 9-D). After culturing for 72 hours, *Bmp2* expression was observed at the 1st, 2nd, 3rd and 4th mammary buds (Fig. 9-F), *Lef1* was expressed at the 1st, 3rd and 4th mammary buds (Fig. 9-H), and *Tbx3* expression was observed in all five mammary buds (Fig. 9-J). This result shows that the *in vitro* tissue culture of the mammary glands was similar to the *in vivo* results.

In order to demonstrate the potential ability of the mammary gland on the line where gene expression pattern is present around the 1st and 4th mammary buds at E11.5, several protein-soaked beads were implanted on that line.

After the FGF8 soaked beads were implanted on that line in embryos slightly younger than E11, the dissected mammary glands tissue was used for *in vitro* tissue culture system and then by whole-mount *in situ* hybridization in order to demonstrate of the effect of the FGF8 protein.

After culturing for 48 hours, ectopic expression of *Lef1* and *Tbx3* were observed around the FGF8 soaked beads, which *Lef1* and *Tbx3* were expressed at the 3rd and 4th mammary buds (Fig.10-A, C). Ectopic expression of *Pyst1* was observed around the FGF8 soaked beads and *Pyst1* was expressed at the 4th mammary bud (Fig.10-B). After culturing for 72 hours, the cultured explants exhibited ectopic expression of *Bmp2, Pyst1* and *Tbx3* around the FGF8 soaked beads. However, no bud was detected**.** In the case of *Tbx3* expression, the FGF8 soaked beads were implanted in both the forelimb and hind limb regions (Fig.10- D, E, F).

In order to identify whether or not ectopic expression around the FGF8 soaked beads were affected by the FGF8 protein, FGFR inhibitor, SU5402 soaked beads were implanted in the same place where the FGF8 soaked beads were implanted.

After culturing for 48 hours, no *Bmp2* and *Lef1* expression was detected around the SU5402 soaked beads. Whereas, *Bmp2* was expressed on the upper side of the SU5402 soaked beads (Fig. 11-A), *Lef1* was expressed around the lower side of the SU5402 soaked beads as the line forms (Fig. 11-B). After culturing for 72 hours, *Lef1* was expressed beside the SU5402 soaked beads as the line formed (Fig. 11-C).

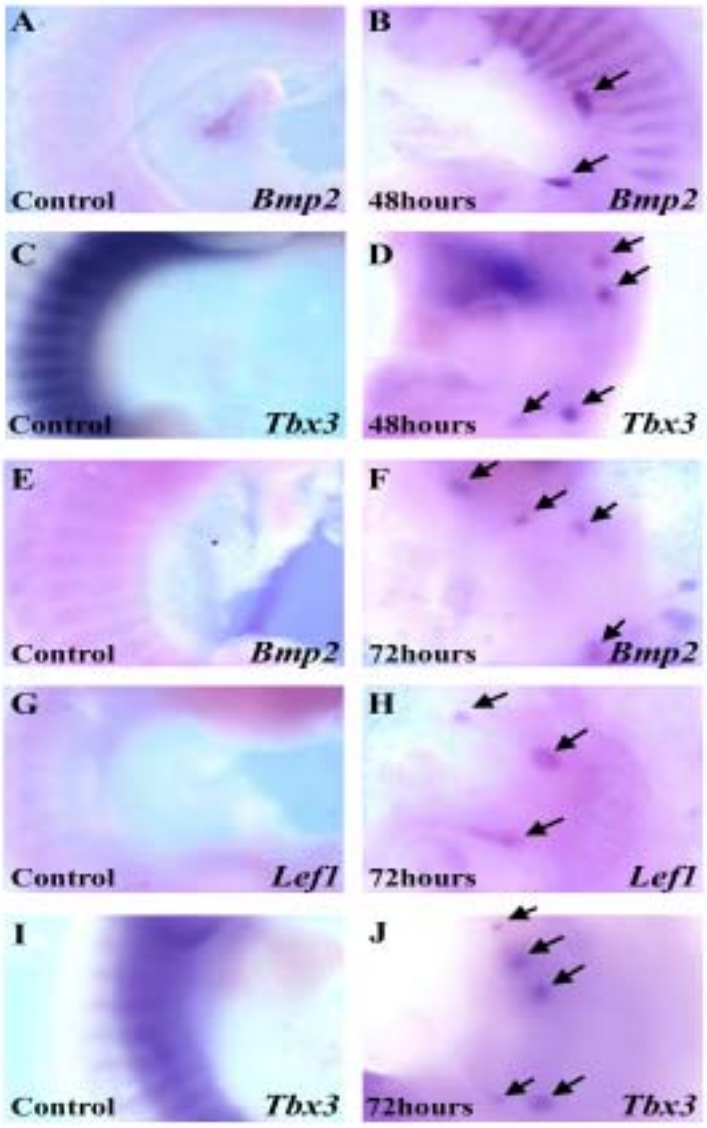

Fig. 9. *In vitro* cultured mammary glands for 48 hours and 72 hours.

After dissecting the mouse embryos into two pieces along the midline, one half side was fixed immediately as the control (A, C, E, G, I) and the other side was cultured for 48 hours (B, D) and 72 hours (F, H, J). (Black arrow : mammary bud)

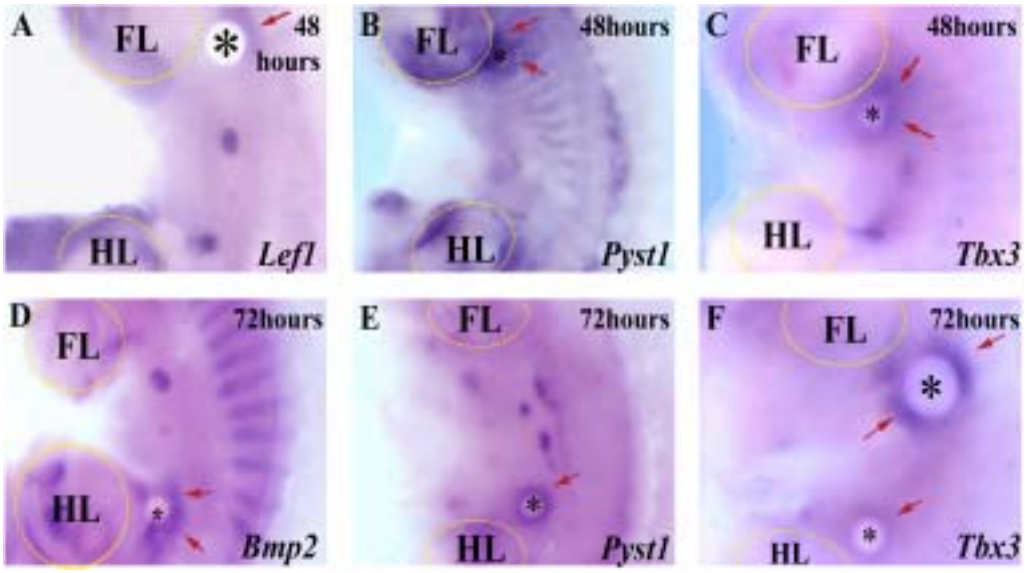

Fig. 10. FGF8 soaked beads. After implanting the FGF8 soaked beads, the explants was cultured for 48 hours (A, B, C) and 72 hours (D, E, F) using *in vitro* organ culture. (FL : forelimb, HL : hind limb, Black * : FGF8 soaked beads, Red arrow : ectopic expression)

Finally, the SU5402 and FGF8 soaked beads were implanted together on the line. The FGF8 soaked beads were implanted beside the SU5402 soaked beads along body axis where the dorsal and ventral region met. The reason for this is that the development of a mammary bud is inhibited by SU5402 but it is induced by FGF8.

After culturing for 48 hours, *Bmp2* expression was observed at the 4th mammary bud and around the FGF8 soaked beads. However, no *Bmp2* expression was detected around the SU5402 soaked beads (Fig. 12-A). After culturing for 72 hours, *Lef1* expression was observed at the 4th mammary bud and around the FGF8 soaked beads, but no *Lef1* expression was detected around the SU5402 soaked beads (Fig. 12-B). We cannot be sure whether or not the FGF8 protein induced this ectopic expression pattern. However, it can be conclude that FGF8 is involved in the initiation of mammary gland formation.

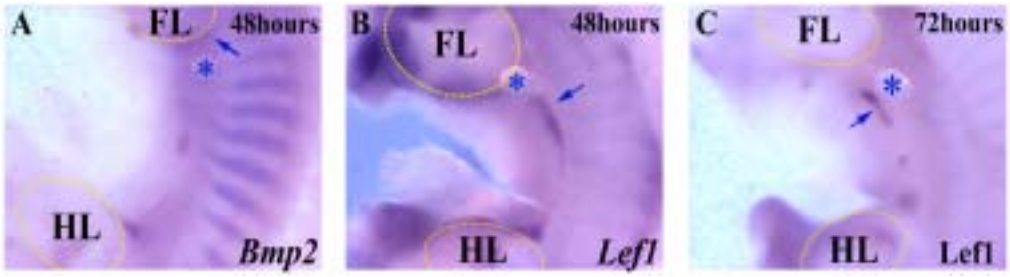

Fig. 11. SU5402 soaked beads. After implanting the SU5402 soaked beads, the explants was cultured for 48 hours (A, B) and 72 hours (C) using *in vitro* organ culture (FL : forelimb, HL : hind limb, Blue * : SU5402 soaked beads, Blue arrow : inhibited expression pattern of mammary bud) .

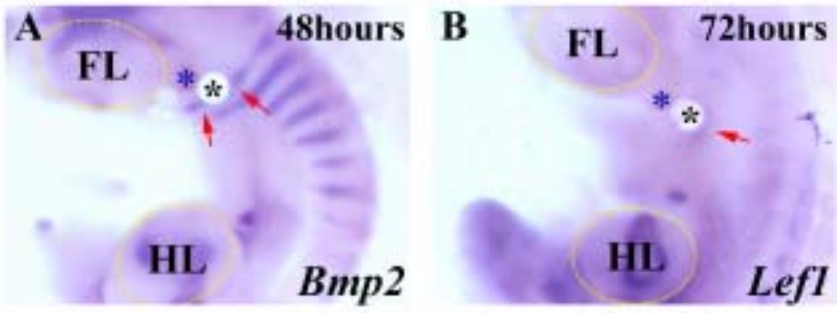

Fig. 12. SU5402 and FGF8 soaked beads. After implanting the SU5402 and FGF8 soaked beads, the explants were cultured for 48 hours (A) and 72 hours (B) using *in vitro* organ culture (FL : forelimb, HL : hind limb, Blue * : FGF8 soaked beads, Blue arrow : inhibits the expression pattern of the mammary bud. Black * : FGF8 soaked beads, Blue * : SU5402 soaked beads, Red arrow : ectopic expression) .

Ⅳ. DISCUSSION

1. Asynchronous development of mammary gland

The histological observations and *in situ* hybridization experiments showed that each mammary bud has its own developmental course and molecular mechanism. At E12, the H-E stains shows only four pairs of mammary buds, and each mammary bud has a different size and developmental stage. The *Bmp2, Bmp4, Lef1, Msx1* and *Msx2* expression pattern was different in the five individual mammary buds, having different developmental time course. Hence, during each mammary bud was derived from the milk line, it developed asynchronously having its own identity.

In addition, the mammary gland is one of the spacing patterns developed by epithelial-mesenchymal interactions, and the early development of the mammary gland resembles the development other epithelial appendages morphologically, such as tooth, and feather buds.[1] Tooth bud formation is one of the best examples for asynchronous development. From the dental lamina to the individual tooth, each tooth has its own identity during development in its proper place in both the maxilla and mandible.[16] Furthermore, signaling molecules are key factors for regulating development. As FGFs induce *Msx1, Dlx1* and *Dlx2* expression, FGFs may activate tooth development. In addition, BMPs plays a role as an inhibitor. Since antagonistic signaling between the FGF and BMP regulates tooth development, the diastema, which is located in the region between the incisors and molars, lack the tooth after the

dental lamina stage in mouse.[17] On the other hand, feather buds in chick skin are initiated from the ectoderm, and they form single row known as the primary row. This primary row appears to resemble other embryonic regions such as the milk line along which the mammary bud develops and the dental lamina from which tooth are initiated. [17] During feather bud formation, the primary row begins as a continuous stripe that is positive for *Fgf4, Shh* and *Ptc*. This stripe then breaks into periodic feather primordia with increased *Fgf4* and *Shh* expression in the primordia and disappearance in the interbud regions. At this time, the periodic primordia became positive for *Bmp2* and *Bmp4*.[18,19]

Feather bud formation has a definite interval and it own developmental time course as a result of an interaction between the signaling molecules-*Fgf4, Shh, Bmp2* and *Bmp4*.[20]

The order of individual mammary bud formation was summarized according to the expression pattern of the five genes. According to the *Msx1* expression pattern in mouse embryos slightly older than E11, placode formation occurred first in the 3rd mammary bud (Fig. 5-P). The 1st and 4th mammary buds then appeared in the shape of a bud on the line. This was followed by the 2nd mammary bud, and finally by the 5th mammary bud. However, a previous study showed that the placodes temporally appeared in the following order of the 3rd, then 4th, followed by 5th and 1st, and finally 2nd using electron microscopy.[21] The order of mammary bud formation observed by electron microscopy is different from the order of mammary bud formation in terms of gene expression pattern. Nevertheless, during mammary bud transformation from

the milk line, the formation of each mammary bud is followed by a developmental order.

2. The predicted position of the milk line in mice

The mammary gland is derived from the milk line. The mammary gland is formed by the migration of epithelial cells.[3,4] At E10.5, *Fgf10* expression is restricted to the milk line between the forelimb and hind limb.[21]

An *in situ* hybridization at E11 showed that the two lines of the thoracic and inguinal region was the milk line (Fig. 5). Hence, the 3rd mammary bud has the characteristic development. The milk line can be the same positions of the 1st and 4th mammary buds, which is expressed as the shape of the bud on the line at E11.5. In addition, the 1st and 4th mammary buds were accompanied the potential ability for the production of the 2nd and 5th mammary buds through morphological observations and the genes expression patterns. That is, the developmental mechanism of the 3rd mammary bud is different from that of the 1st and 4th mammary buds.

As *ska*(scaramanga) mutations are involved in determining the pattern formation of the mammary gland in mice, *ska* mammary gland pattern abnormalities include absent 3rd mammary bud and supernumerary nipples connected to a functional milk producing ductal system.[22] This study supports the hypothesis that the 3rd mammary bud has a different developmental mechanism.

In a previous study, the milk line lies between the forelimb and the hind limb at

E10.5 and the 4th mammary bud processes the other signaling pathway by FGF10/FGFR2b.[21] However, the mammary gland is derived from the milk line, and each mammary bud develops with its own identity.

As it is possible that that the milk line could be in the axillary region, Di.I. was injected into the cells in the axillary region where the signaling molecules are expressed as the line. As expected, the Di.I. labeled cells moved ventral-cranially following the axillary region. Hence, the migration pattern of the labeled cells might be compared with the changing gene expression pattern between E11.5 and E12.5. In compliance with the result of cell migration, the two lines of the thoracic and inguinal region are the milk line.

If that milk line is the functional area, regulating the signaling molecular can result in a change in the spacing pattern of the mammary gland as making ectopic mammary buds. The implantation of protein-soaked beads was performed to determine if the axillary region is the milk line. *Lef1* is first found at the sites where the placodes are formed.[5] *Tbx3* is implicated in mammary hypoplasia and lack of mammary glands.[8,9] In addition, *Pyst1* is involved in developmental function of FGF/FGFR signaling, particularly *Fgf8.* As FGF8 induces the initial development of the epithelial appendages, the FGF8 soaked bead is implanted in the flanks of the mammary glands.

FGF8 induced the ectopic expression pattern of *Lef1, Pyst1* and *Tbx3.* From these results, the presumptive milk line has the potential to develop mammary glands, and FGF8 is involved in the early development of mammary glands.

3. Signal from epithelium or mesenchyme at initial stage for mammary gland development

The mammary gland is one of the skin appendages that develop under the influence of interactions between the epithelium and mesenchyme.[1] Sections after whole-mount *in situ* hybridization were analyzed to define the epithelial-mesenchymal interaction. The first signal localized in the epithelium as *Bmps, Bmp4, Lef1, Msx1*and *Msx2*.

This might be the key step for mammary gland development in mice. Although *Bmp4* is expressed in the mesenchyme at E13.5, *Bmp4* is indeed as initial marker for mammary gland development.[23] Another study showed that the mesenchyme may at least have a permissive function for inducing mammogenesis.[24] These mechanisms can often be observed during the development of other epithelial appendages as the tooth and feathers. In tooth development, the tooth forming potential shifts from the tooth epithelium to the mesenchyme after the bud stage. [25,26]

Lef1 and *Bmp2* then serve as the epithelial signals whereas *Bmp4* is localized in the dental mesenchyme. Moreover these signals are valuable for further development. *Lef-1* and *Bmp-4* knockout mice arrest their tooth development at bud stage and skeletal system is shown severe phenotype.

During limb development, *Fgf10* is expressed in the limb mesenchyme. *Fgf10* then induces *Fgf8* expression in the overlying ectoderm. FGF8 and Wnts are involved in maintaining *Fgf10* expression in the mesenchyme. Hence, *FGF10* induced *Wnt3a* expression in the ectoderm followed by *Wnt3a* via β-catenin activates *Fgf8*

expression, which then maintains *Fgf10* expression in a feedback loop.[27] However, it still unclear as to whether information for placode formation is intrinsic to the epithelium of the mammary region.

4. Relationship between FGFs signaling and BMPs signaling

The developing mammary gland forms via a series of reciprocal inductive tissue interactions where the signals were exchanged between the epithelium and mesenchyme, resulting in a progressive specification of organ fate same as the tooth, feathers.[28]

From a signal molecular point of view, the coexpression of the FGFs and BMPs observed in many developing vertebrate organs suggests a close relationship between FGFs and BMPs.

FGFs, in particular FGF8, play central roles as early an epithelial signal patterning the branchial arch mesenchyme and regulating the initiation of tooth formation.[17] In addition, the FGFs signaling cascade, which initiates limb formation and control, establish the apical ectodermal ridge. [27]

BMPs, including BMP2, BMP4, BMP7, have been shown to function as mitogens[29,30], although an opposing role for BMP4, that of repressing cell proliferation, has been suggested in lung development. [31,32]

In addition, during tooth development, FGF8 stimulated and BMP2 and BMP4 inhibited the *Pax9* required for tooth morphogenesis to proceed beyond the bud

stage.[17]

In the spacing of feathers, *Fgf*4 and *Shh* might be activators, while *Bmp4* is an inhibitor. Furthermore, both FGF4 and SHH proteins induced *Bmp4* expression, while the BMP4 protein inhibited the expression of the two former genes.[18]

In investigation into the relationship between FGFs and BMPs in mammary gland development using protein-soaked beads implantation showed that FGF8 induces the ectopic expression pattern of *Bmp2, Lef1,* and so on. On the other hand, SU5402, an inhibitor of the FGF receptor, inhibits the ectopic expression pattern of *Bmp2* and *Lef1*. The results showed that FGF8 is essential for maintaining *Bmp2* and *Lef1* expression in the mammary epithelium. Hence, FGF8 controls a genetic hierarchy, involving the *Bmp2* and *Lef1* signals, which regulate the early development of the mammary glands (Fig. 13).[33] These growth factors expressed in the different tissue layers appear to act as signals mediating such epithelial-mesenchymal interactions leading to the mammary glands.

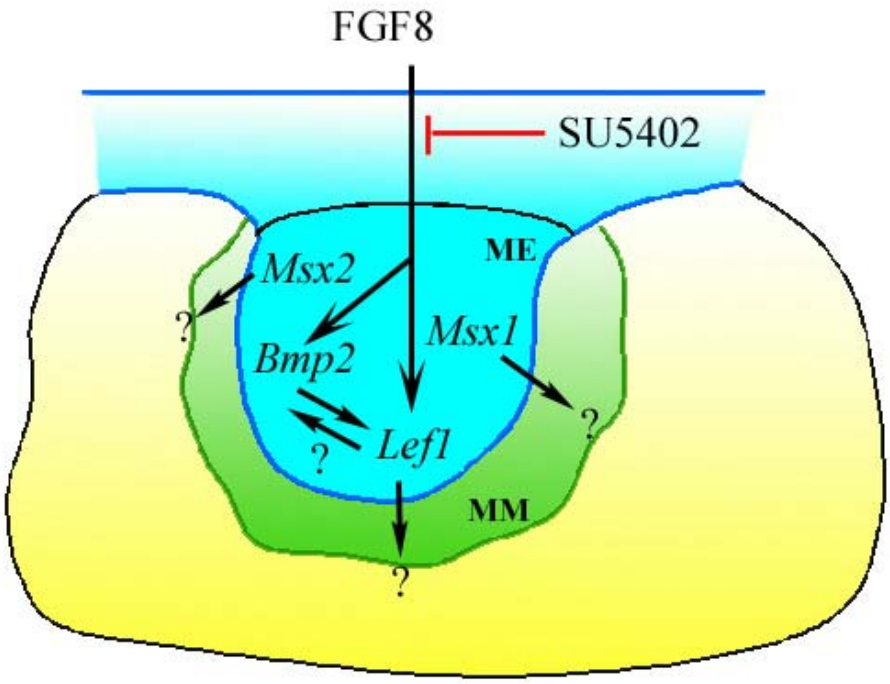

Fig. 13. The model for the genetic pathway integrating FGF8, *Bmp2, Lef1, Msx1* and *Msx2* in the epithelial-mesenchymal interactions that regulate the mammary glands in mice. (ME : mammary epithelium, MM : mammary mesenchyme)

Ⅴ. CONCLUSION

The mammary glands are one of the skin appendages that develop through continuous communication between its epithelium and adjacent mesenchyme.

During the initial development of the mammary glands, the mammary bud is derived from the milk line such as the dental lamina from which the tooth is initiated.

At the time of the transformation from the milk line to the mammary buds, the mammary buds develop independently each other at these positions. In addition, each pair of mammary buds responds differently to altered levels of gene expression. In particular the development of individual mammary buds appear in the order of the 3rd, then 1st and 4th, followed by the 2nd, and finally the 5th mammary bud, as summarized by the expression pattern of the genes.

All of the five individual mammary buds exhibited asynchronous development with their own developmental identity, different developmental time course and mechanism.

An investigation of the *Bmp2, Bmp4, Lef1, Msx1* and *Msx2* expression pattern and morphological observations showed that the two lines of the thoracic and inguinal regions are the milk line. Therefore, the 3rd mammary bud has a characteristic development pattern. According to the migration pattern of the Di.I. labeled cells in the axillary region, , the two lines of the thoracic and inguinal region are the milk line. In addition, as it was not confirmed whether or not the axillary region is the functional milk line, the presumptive milk line has the potential to develop mammary

glands, and FGF8 is involved in the early development of the mammary glands.

As mentioned above, the mammary gland developed by epithelial-mesenchymal interaction. Observations of the section after whole-mount *in situ* hybridization showed that the first signal is localized in the epithelium as *Bmps, Bmp4, Lef1, Msx1*and *Msx2* during the early development of the mammary glands. That is, the information for placode formation is intrinsic to the epithelium of the mammary region.

FGF8 plays an important role in the development of mammary glands. As FGF8 induces the ectopic expression of *Bmp2* and *Lef1*, FGF8 controls the genetic pathway, involving the *Bmp2* and *Lef1* signals in the mammary epithelium.

A line to form dotted spots as mammary buds is thought to be ideal for embryonic patterning according to previous studies in mammalian. The results in this study indeed demonstrate for the first time the role of FGF8, *Bmp2* and *Lef1* as well as the functional milk line in the development of mammary glands. This might be associated with large numbers of other signaling molecules for mammogenesis. Overall, this study provided information on the basic and functional mechanisms of the spacing pattern during the formation of mammary glands in mice.

REFERENCE

1. Veltmaat JM, Mailleux AA, Thiery JP, Bellusci S. Mouse embryonic mammogenesis as a model for the molecular regulation of pattern formation. Differentiation 2003 ; 71 : 1-17

2. Turner CW, Gomez ET. The normal development of the mammary gland of the male and female albino mouse. I. Intrauterine. Mo.Agric.Exp.Stn.Res.Bull. 1933 ; 182 : 3-20

3. Balinsky BI. On the pre-natal growth of the mammary gland rudiment in the mouse. J.Anat. 1950 : 84 : 227-235

4. Proper, A.Y. Wandering epithelial cells in the rabbit embryo milk line. Dev.Biol. 1978 ; 67 : 225-231

5. van Genderen C, Okamura RM, Farinas I, Quo R, Parslow TG, Bruhn L, Grosscheld R. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1 deficient mice. Genes Dev. 1994 ; 8 : 2691-2703

6. Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, et al. Msx2 deficiency

in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Natue genetics. 2000 ; 24 : 391-395

7. Deborah JP, Stephen JW, Paul TS, M. Stuart N, H. Jayatalake, Richard M, et al. Regulation of *Msx-1*, *Msx-2*, *Bmp-2*, and *Bmp-4* during foetal and postnatal mammary gland development. Dev. 1996 ; 122 : 2729–2737

8. Chapman DL, Garvey N, Hancock S, Alexiou M, Agulnik SI, Gibson-Brown JJ, et al. Expression of the T-box family genes, Tbx1-Tbx5, during early mouse development. Dev Dyn. 1996 ; 206(4) : 379-390

9. Agulnik SI, Papaioannou VE, Silver LM. Cloning, mapping, and expression analysis of TBX15, a new member of the T-Box gene family. Genomics 1998 ; 51(1) : 68-75

10. Yonei-Tamura S, Endo T, Yajima H, Ohuchi H, Ide H, Tamura K. FGF7 and FGF10 directly induce the apical ectodermal ridge in chick embryos. Dev. Bio. 1999 ; 211 : 133-143

11. Dickinson RJ, Eblaghie MC, Keyse SM, Morriss-Kay GM. Expression of the ERK-specific MAP Kinase phosphatase *PYST1/MKP3* in mouse embryos during morphogenesis and early organogenesis. Mech. Dev. 2002 ; 113 : 93-196

12. Moore KL, Persaud TVN. The developing human. 6th ed. Philadelphia (U.S.A.): W.B.SAUNDERS COMPANY ; 1998

13. Wilkinson DG. *In situ* hybridization. 1st ed. London (UK): IRL PRESS; 1992

14. Honig MG, Hume RI. Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures. J. Cell Biol. 1986 ; 103 : 171-187

15. Honig MG, Hume RI. Di.I. and DiO : versatile fluorescent dyes for neuronal labeling and pathway tracing. Trends Neuosci. 1989 ; 12(9) : 333-341

16. Kratochwil K, Dull M, Farinas I, Galceran J, Grosschedl R. *Lef1* expression is activated by BMP-4 and regulates inductive tissue interactions in tooth and hair development. Genes Dev 1996 ; 10 : 1382–1394

17. Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech. Dev. 2000 ; 92 : 19-29

18. Jung HS, Francis-West PH, Widelitz RB, Jiang TX, Ting-Berreth S, Tickle C, and Chuong CM. Local inhibitory action of BMPs and their relationshipa with activators in feather formation:Implications for periodic patterning. Dev. Bio.

1998 ; 196 : 11-23

19. Wolpert L. Pattern formation in epithelial development :the vertebrate limb and feather bud spacing. Phil. Trans. R. Soc. Lond. B 1998 ; 353 :871-875

20. Jernvall J, Jung HS. Genotype, penotype, and developmental biology of molar tooth characters. Yearbook of Physical Anthropology. 2000 ; 43 : 171-190

21. Mailleux AA, Spencer-Dene B, Dillon C, Ndiaye D, Savona-Baron C, Itoh N, et al. Role of FGF10/FGFR2b signaling during mammary gland development in the mouse embryo. Dev. 2002 ; 129 : 53-60

22. Howard BA, Gusterson BA. Mammary gland patterning in the AXB/BXA recombinant inbred strains of mouse. Mech. Dev. 2000 ; 91 : 305-309

23. Thesleff I, Vaahtokari A, Partanen AM. Regulation of organogenesis. Commom molecular mechanisms regulating the development of teeth and other organs. Int. j. Dev. Biol. 1995 ; 39 : 35-50

24. Sakahura T. (1987) Mammary embryogenesis. In : Neville, M.C. & Daniel, C.W.(eds) The Mammary Gland : Development, Regulation and Function. Plenum Press, New York, pp 37–66

25. Hardcastle Z, Mo R, Hui CC, Sharpe PT. The Shh signaling pathway in tooth development : defect in Gli2 and Gli3 mutants. Dev. 1998 ; 125 : 2803-2811

26. Zhang Y, Zhang Z, Zhao X, Yu X, Hu Y, et al. A new function of BMP4 : dual for BMP4 in regulation of Sonic hedghog expression in mouse tooth germ. Dev. 2000 ; 127 : 1431-1443

27. Tickle C, Munsterberg A. Vertebrate limb development-the early stages in chick and mouse. Curr Opin Genet Dev. 2001 ; 11 : 476-481

28. Chen Y, Zhang Y, Jiang TX, Barlow AJ, St Amand TR, Hu Y, et al. Conservation of early odontogenic signaling pathways in Aves. Proc. Natl. Acad. Sci. 2000 ; 97(18) : 10044-10049

29. Barlow AJ, Francis-West PH. Ectopic application of BMP-2 and BMP-4 can change patterning of developing of developing chick facial primordial. Dev. 1997 ; 124 : 391-398

30. Wang YH, Rutherford B, Uphot W, Mina M. Effects of BMP-7 on mouse tooth mesenchyme and chick mandibular mesenchyme. Dev. Dyn. 1999 ; 216 : 320-335

31. Bei M, Maas R. FGFs and BMP4 induce both *Msx1*-independent and *Msx1*-

dependent signaling pathways in early tooth development. Dev. 1998 ; 125 : 4325–4333

32. Basilico C, Moscatelli D. The FGF family of growth-factor and oncogenes. Advances in Cancer Research 1999 ; 59 : 115-165

33. Hennighausen L, Robinson GW. Signaling pathway in mammary gland development. Dev. Cell 2002 ; Vol.1 : 467-475

Abstract (in Korean)

흰쥐의 젖샘 초기 발생과 FGF8 신호 전달 물질과의 관계

(지도 교수 : 정 한 성)

연세대학교 대학원

의과학 사업단

송 수 진

젖샘의 발생 양상은 치아, 깃털, 머리털 그리고 혀의 돌기 등을 포함한 다른 상피성 부속 기관의 발생 양상에서 유사성을 제시할 수 있다. 피부 부속기관의 발생은 상피조직과 간엽조직의 상호 작용이 요구된다.

흰쥐 배아의 경우 다섯 쌍의 젖샘싹을 가지고 있으며, 그 젖샘싹들은 가슴부위에 세 쌍, 샅굴부위에 두 쌍으로 위치하고 있다. 또한 젖샘의 발생은 상피세포의 세포증식보다는 세포이동에 의해 milk line 으로부터 유래되어진다고 알려져 있다. 젖샘의 초기 발생 과정을 확인한 결과, 각각의 젖샘싹은 다른 크기와 다른 발생적 단계를 거침으로서 비동시적인 발생 과정을 가진다. 또한 젖샘의 초기 발생 과정 동안 *Bmp2, Bmp4, Lef1, Msx1* 그리고 *Msx2* 의 발현 양상은 각각 다섯 쌍의 젖샘싹에서 각기 다른 양상으로 나타남으로써 각각의 젖샘싹마다 각각의 고유성을 가진다. 이러한 유전자들의 발현양상에는 각각의 젖샘싹마다 발생학적 순서를 나타내며 발생한다. 발생적 순서는 3 번째, 그리고 4 번째와 1 번째 다음으로 2 번째 마지막으로 5 번째 젖샘싹으로 나타냈다.

E11 의 유전자 발현 양상이 선으로 나타나는 가슴부위에 Di.I.로 세포를 염색하여 확인한 세포의 이동양상 결과, 가슴부위와 샅굴부위의 두 개의 선이 milk line 이라는 확인을 할 수 있었다. 그리고 Di.I.로 표시된 세포의 이동양상은 E11.5 와 E12.5 사이에서 보여지는 유전자 발현 양상의 변화와 일치한다.

마지막으로 상피조직과 간엽조직의 상호작용에 의해 발생하는 상피성 부속기관의 초기 발생을 유도하는 FGF 신호전달물질의 영향을 젖샘에서 알아본 결과, FGF8 은 젖샘 초기 발생에 나타나는 *Bmp2* 와 *Lef1* 의 발현을 유지하도록 유도한다. 이러한 결과로 FGF8 는 젖샘 발생을 조절하며, *Bmp2* 와 *Lef1* 의 신호전달체계를 포함하는 유전적 경로를 조절함을 확인하였다.

젖샘의 발생은 각각의 젖샘싹을 발생시키는 잠재력을 가지는 milk line 에서 유래되며, 상피세포와 간엽세포의 상호작용 및 spacing pattern 을 거쳐 형성되는 상피성 부속기관 이다. 따라서 젖샘은 발생학적 유도, 보존 그리고 형태발생에서 신호전달물질 경로를 이해하는 좋은 model system 으로 생각한다.

핵심되는 말 : 상피조직와 간엽조직의 상호작용, 젖샘싹, milk line, 비동시성, 유전자 발현 양상, FGF8, 신호전달경로