DOCTORAL THESIS

Efficacy of fibroblast growth factor (FGF) on epithelialization of the neovagina in patients with Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) who underwent vaginoplasty

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Efficacy of Fibroblast Growth Factor on Epithelialization of the Neovagina in Patients with Mayer-Rokitansky-Küster-Hauser Syndrome Who Underwent Vaginoplasty

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ABSTRACT

Study Objective: To evaluate the effect of fibroblast growth factor (FGF) on epithelialization of neovagina in patients with Mayer-Rokitansky-Küster-Hauser syndrome who underwent vaginoplasty.

Design: Observational study.

Setting: University hospital.

Participants: Seven patients with Mayer-Rokitansky-Küster-Hauser syndrome.

Interventions: Cytological examination was done on vaginal smear samples from the site of completed epithelialization, and tissue was collected from the epithelialized part for histological evaluation. Immunostaining for estrogen receptor α, and keratin 13 and 14, and reverse transcription polymerase chain reaction (RT-PCR) analysis of the FGF receptor (FGFR) 1-4 were performed in samples from case 2 three times (ie, during the surgery, during the period of vaginal creation, and at 3 months and 6 months after the surgery).

Main Outcome Measures: The primary outcome was the FGF effects on the epithelialization speed and FGFR expression in the neovagina. The second was the role of FGF in the mechanism of vaginal epithelial cell proliferation.

Results: The histological structure of the neovagina was consistent with that of normal vagina. RT-PCR analysis revealed that FGFR was expressed in the control vaginas and neovaginas. Among the FGFR subtypes, FGFR-4 was overexpressed during the process of epithelialization and its level decreased after completion of creation of the new vagina.

Conclusion: The epithelium of the neovagina was morphologically similar to that of normal vagina. It is suggested that FGF plays the role as a growth factor.

Key Words: Vaginoplasty, Mayer-Rokitansky-Küster-Hauser syndrome, Oxidized regenerated cellulose, Fibroblast growth factor

Introduction

Congenital vaginal agenesis, with a prevalence of 1 in 4,500 women, occurs mainly as a feature of Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS). The patients often present at adolescence with primary amenorrhea, normally developed sexual characteristics, and normal 46,XX karyotype. The cause of vaginal agenesis is often referred to as abnormal differentiation of the Mullerian ducts.1

In vaginoplasty for MRKHS, a variety of techniques have been used. Conventional techniques include the Frank technique using self-compression, the Ruge operation using intestine (such as sigmoid colon), Williams operation using a skin graft, and Davydov operation using peritoneum. The Vecchietti procedure is a method of vaginoplasty that is performed using laparotomy in which the vaginal antral membrane is pulled with a bead called an olive. For minimally invasive surgery, the laparoscopic Vecchietti method is performed. Recently, techniques using an artificial material (artificial dermis and synthetic fabrics), atelocollagen membrane (Terudermis; Olympus Terumo Biomaterials, Tokyo, Japan), or oxidized regenerated cellulose membrane (Intercell; Ethicon [part of Johnson & Johnson Family of Companies], Chiyoda-ku, Tokyo, Japan) have been used to completely create a vaginal lumen that had been created with a Wharton operation using vaginal antral epithelium.2-7

Jackson and Rosenblatt for the first time had reported a technique using oxidized regenerated cellulose membrane, and mentioned that there were no intraoperative or postoperative complications and that epithelialization of the newly created vaginal membrane was completed in 3-6 months.8 Meanwhile, Inagaki et al. histologically evaluated vaginal epithelium that had been completely created with this oxidized regenerated cellulose membrane and observed findings similar to those of normal vaginal epithelium.9

In our department, this oxidized regenerated cellulose membrane has been used in vaginoplasty for patients with MRKHS. The vaginal cavity created by this technique is
similar to that of the normal vagina in appearance, and
the operation is minimally invasive. However, it took
approximately 6 months for complete regeneration of
the new vaginal epithelium and shortening the period has been
an issue to solve.

Nakajima et al. reported that fibroblast growth factor
(FGF) was involved in differentiation and proliferation of
the uterus and vaginal epithelium in newborn mice. They
used 5-bromo-2'-deoxyuridine for detection of proliferative
cells in DNA replication, and it was added to organ cultured
mouse vaginal epithelial cells, and detection of proliferating
cells in organ-cultured vaginai was performed using
immunohistochemistry for 5-bromo-2'-deoxyuridine as
described.

In the mouse it was reported that basic FGF administra-
tion accelerated epithelialization of new vaginal epithe-
lum, but the precise mechanism remains to be clarified. In
this study, we histologically and biochemically analyzed the
effect of FGF on the creation of vaginal mucosa using this
technique.

Material and Methods

This study was approved by the ethical committee of our
institution. Written informed consent was obtained from all
patients. Vaginoplasty was carried out using the following
technique in the 7 patients. In these 7 patients, the chief
complaint was primary amenorrhea, and we confirmed the
loss of the uterus and the vagina with magnetic resonance
imaging, and MRKHS was diagnosed in all patients.

Surgical Techniques and Postoperative Management

Surgical techniques and postoperative management
were performed according to the method described by
Imagaki et al. The outline is as follows.

1. A cross incision was made in the closed vaginal antral
mucosa and a new vaginal cavity was created by blunt
maneuvers. The incision was continued until an S-size
Cusco vaginal speculum could be fully inserted.

2. Laparoscopy was used in combination with the vaginal
approach to observe the intraperitoneal cavity and
confirm the direction from the vagina to the pelvic
peritoneum.

3. Oxidized cellulose membrane was wrapped around
the prosthesis to which lubricant gel had been applied, and
the prosthesis was inserted into the new vaginal cavity
for completion of creation.

4. On postoperative day 2 (48 hours later), the prosthesis
was removed, and insertion and removal of the prosthesis
by the patient herself were started on postoperative day
4. Before insertion, lidocaine hydrochloride (xylocaine
gel) and gentamycin sulfate (gentamycin salve) were
applied to the prosthesis for alleviation of pain and pre-
vention of infection. When the patient could comfortably
insert and remove the prosthesis by herself, the patient
was discharged from the hospital and followed up at the
outpatient clinic once a month.

5. The prosthesis was continuously inserted during the
day and night for 1 month after the procedure, and the
insertion time was gradually shortened according to the
condition of the vaginal epithelium.

Collection of Samples

Samples were collected from the vaginal mucosa created
by this procedure 3 times (ie, during the procedure, during
the period of vaginal creation after the procedure [approximately 3 months], and after completion of vaginal
creation [approximately 6 months]). Control samples were
collected from the normal vaginal wall and skin tissue
(abdominal wall skin) from other patients who underwent
total hysterectomy during their surgery. Collection of the
samples was performed using punch biopsy, and the size of
the samples were 5 mm in diameter.

Evaluation of the New Vaginal Epithelium

Cytological examination was done on vaginal smear
samples obtained every month from the site of completed
epithelialization, and tissue was collected from the ep-
thelialized part 3 months after the procedure for histolog-
ical evaluation.

In addition, immunostaining for estrogen receptor
(ER) α, keratin 13 (K13), and keratin 14 (K14) was done to
study if the newly created vagina was similar to the
normal vagina anatomically. Normal vaginal mucosa is
positive for all of ERα, K13, and K14, and skin tissue
consisting of stratified squamous epithelium is positive
only for K14.

For immunostaining, the ENVISION method (Dako,
Kyoto, Japan) was used with K13 monoclonal anticyto-
keratin peptide 13 (Sigma-Aldrich, St Louis, MO), K14 mouse
monoclonal antibody (Thermo, Fremont, CA), and ER
monoclonal mouse antihuman ERα (Dako).

Problems of FGF on Epithelialization of New Vaginal Epithelium

To investigate the involvement of FGF, the levels of FGF
receptor (FGFR) subtype FGFR-1, FGFR-2, FGFR-3, and FGFR-
4 were quantified using real-time RT-PCR at each step of
formation of the vaginal mucosa. Peptidylprolyl isomerase
A was chosen as an internal standard.

Results

Outcome of the Surgery

Vaginoplasty was performed in 7 patients with MRKHS.
The mean surgical time was 119.6 minutes (range, 76-
161 minutes) with mean blood loss of 89.2 g (5-170 g), and
the mean length of hospital stay was 8.9 days (5-14 days).
The surgical time was relatively long because laparoscopy
was used in combination with the vaginal approach, but no
remarkable complications were observed in any case. Three
cases (cases 2-4) exhibited postoperative stenosis and
underwent repeated surgery. They steadily exhibited epithe-
lialization of vaginal epithelium, and creation of the vaginal
cavity was completed with a cavity length of 7-8 cm. Its length was measured using a ruler.

**Histological Evaluation of the New Vaginal Epithelium**

Cytological examination revealed an accumulation of squamous cells 3 months after the procedure, and histological examination at the same time point revealed findings of stratified squamous epithelium similar to those in normal vaginal mucosa (Fig. 1A-C). Immunostaining showed that the control skin tissue was positive for only K14, whereas normal vaginal epithelium and the newly created vaginal epithelium in all cases were positive for ERα, K13, and K14 (Fig. 2).

**Expression of FGFR During the Process of Epithelialization of New Vagina**

The results of real-time RT-PCR in case 3 are shown. The level of FGFR in the control skin sample was defined as 1, and the level of FGFR was expressed relative to that in the control sample. Among the FGFR subtypes, FGFR-4 was overexpressed during the process of epithelialization and its level decreased after completion of creation of the new vagina. There were no significant changes in the levels of other subtypes of FGFR during the process of vaginal epithelialization (Fig. 3). These results suggested that FGF was involved in the process of vaginal epithelialization.

**Discussion**

**The following 2 Findings Were Made in This study**

The first is that vaginal membrane created according to the method of Motoyama et al. not only exhibited squamous epithelium histologically, but also had characteristics comparable with those of normal vaginal mucosa in the expression pattern of keratin and ERα. The second finding is that FGFR was expressed in the newly created vaginal mucosa.

In vaginoplasty for MRKHS, biografts using tissue from the intestine, peritoneum, and skin have been conventionally used, but problems with this procedure include the surgical invasiveness of graft transplantation, cosmetic aspects, and postoperative quality of life. Recently, vaginoplasty using oxidized regenerated cellulose membrane (Intercell), instead of biografts, has been performed. Oxidized regenerated cellulose membrane plays a role as a protective membrane that reduces physical irritation by the vaginal prosthesis and prevents intravaginal inflammation and closure due to adhesion of the vaginal epithelium during proliferation of the vaginal antrum. This technique is minimally invasive because no graft is collected, and it is considered that the technique leads to satisfactory results with regard to cosmetic aspects and naturalness.

In this study, a procedure using oxidized regenerated cellulose membrane was performed in the patients. In case 3, cytological examination was done in vaginal smear samples obtained every month after the surgery, and histological examination was carried out 3 months after the procedure, and findings of squamous epithelium similar to those in normal vagina were observed. As reported by Inagaki et al., the vaginal epithelium created using this technique was histologically the same as normal vaginal mucosa.

Cytokeratin, which is present in all epithelia, has been widely used as an epithelial marker. Cytokeratin has been categorized into approximately 20 subtypes to date and a variety of characteristics of epithelial cells and tumor histological types can be estimated on the basis of their features. It has been reported that K13 is not expressed in

![Fig. 1. Smears taken from the neovagina (case 2) at (A) 1 month and (B) 4 months after surgery. (C) Punch biopsy specimen taken from the neovagina 3 months after surgery is shown. Squamous epithelialization of the neovagina was noted. Magnification in (A), ×200; (B), ×200; and (C), ×200.](image-url)
normal skin squamous epithelium, but K14 is expressed in normal skin squamous epithelium and vagina. It is known that ERα is distributed predominantly in the female organs indispensable for reproduction such as the mammary glands and uterus. It is possible to differentiate normal skin from vagina by the presence or absence of ERα expression.

The results of immunostaining in this study showed that skin was positive only for K14 but normal vaginal walls and newly created vaginal epithelium were positive for all of ERα, K13, and K14, and it was proven that the vaginal epithelium created using this technique had characteristics comparable with those of normal vaginal epithelium.

FGF is one of the growth factors involved in neo-vascularization, wound healing, and embryogenesis, and all FGF subtypes from FGF-1 to FGF-10 bind FGFR. The FGFR family in mammals comprises 4 kinds of FGFR: FGFR1, FGFR2, FGFR3, and FGFR4. The distribution of their expression in a variety of tissues and organs in vivo has been elucidated. FGF expression in human normal skin and skin during wound healing has been investigated: all 4 FGFRs were expressed in normal skin and all FGFRs were expressed in the skin during wound healing. In particular, with strong expression of FGFR1 and FGFR4. In addition, all 4 FGFRs were expressed during neo-vascularization of capillaries in granulation tissue, and FGFR1 and FGFR3 were expressed in fibroblasts and myofibroblasts. Taken together, it was shown that FGF exerted its activity via FGFR in the process of tissue wound healing.

With regard to acceleration of epithelialization by FGF administration, Fu et al reported that FGF administration shortened the healing time and accelerated epithelium regeneration in the process of wound healing for grade 2 burns.

The results of real-time RT-PCR in this study showed the presence of FGFR in the newly created vaginal epithelium, which suggested the involvement of FGF in the proliferation of vaginal epithelium.

Raya-Rivera et al reported a technique in which epithelial tissue of the vaginal antrum was collected for culture and
used for vaginoplasty using autotransplantation for vaginal epithelium,\(^{39}\) and the utility of vaginal antral epithelium was elucidated. Our surgical technique is similar in terms of using vaginal antral epithelium, but the additional use of FGF enabled creation of vagina similar to normal epithelium more quickly.

Because only 7 patients were examined in this study, we plan to further accumulate similar cases and investigate the utility of this technique, the satisfaction of patients in terms of sexual function,\(^{31}\) and the role of FGF in the epithelial proliferation of human neovagina.

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