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ORIGINAL ARTICLE

Characterization of the Onychomatricodermis Containing Onychofibroblasts of the Nail Unit : Histology, Immunohistochemistry, and Electron Microscopic Study

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Background: We recently discovered the presence of specialized nail mesenchyme below the nail matrix and designated it as onychomatricodermis. **Objective:** We did further research to characterize the histologic, histochemical, immunohistochemical and ultrastructural features of the onychomatricodermis containing onychofibroblasts in the nail unit. **Methods:** Ten polydactyly nail unit specimens and 8 nail matrix biopsies were included. H&E-stained slides were reviewed. We did Alcian blue staining and Masson Trichrome staining, as well as immunohistochemical staining for type Ⅰ collagen, CD10, CD13 and CD34. In addition, polydactyly nail units were examined by transmission electron microscopy. **Results:** In H&E staining, the specialized mesenchyme called onychomatricodermis was observed to be slightly distant from the undersurface of the nail matrix and be less eosinophilic area. Onychomatricodermal onychofibroblasts showed light purple abundant cytoplasm. Masson Trichrome staining revealed fewer collagen fibers within the onychomatricodermis. In Alcian blue staining the onychomatricodermis showed mucin deposition within the onychofibroblasts and around them. Immunohistochemically, type Ⅰ collagen was expressed much less in the onychomatricodermis while it was strongly expressed elsewhere in the nail unit. In nail matrix biopsy specimens onychomatricodermal onychofibroblasts expressed CD10 and CD13 strongly, and expressed CD34 as well. Ultrastructurally, collagen fibrils were found sparsely within the onychomatricodermis, whereas collagen fibrils were densely distributed in the dermis of other parts of the nail unit. **Conclusion:** We demonstrated that there was less collagen expression in the onychomatricodermis containing onychofibroblasts. In addition, we found morphological and immunohistochemical features of onychomatricodermal onychofibroblasts (onychofibroblasts of Dongyoun). These findings support the presence of onychomatricodermis containing onychofibroblasts in the nail unit. **(Ann Dermatol 33(2) 108～115, 2021)**

-Keywords-
Collagen, Onychodermis, Onychofibroblast, Onychomatricodermis

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INTRODUCTION

The nail unit is a major skin appendage. It is composed of epithelial tissue such as nail matrix and nail plate, and mesenchymal tissue. The nail unit and hair follicle have some similarities including their origin[1], anatomical structures[2,3], and common involvement in various diseases[4].

Previously, based on the finding in the mesenchymal cells of hair follicle[5], we first discovered CD10 positive mesenchymal cells below the nail matrix and proximal nail bed, and proposed to name these specialized cells as onychofibroblasts[6]. We also demonstrated the existence of specialized mesenchyme containing onychofibroblasts beneath the nail matrix and nail bed and proposed to name this mesenchyme as onychodermis because it was distinguished by morphology and immunohistochemistry (IHC) from other areas of the nail unit[7]. In addition, another group verified the concept of onychodermis in the developing nail organ[8]. Moreover, we demonstrated that onychodermis exists in ectopic nails[9]. Very recently, by histology and elastin IHC we further characterized the onychodermis in the nail unit. We proved that the specialized nail mesenchyme below the nail matrix (nail matrix onychodermis) was distinguished from the mesenchyme below the nail bed (nail bed onychodermis) and designated it as onychomatricodermis[10]. Onychomatricodermis of the nail unit might be the nail counterpart of follicular dermal papilla of the hair follicle[10].

The dermis is primarily composed of the extracellular matrix (ECM) and cellular components. Fibroblasts are essential dermal cells that are responsible for generating ECM. Collagen is a major component of dermal ECM. Over 90% of the collagen in the human body is type I collagen. Other ECM components include elastic fibers, glycosaminoglycans, and proteoglycans. Previously, we demonstrated the presence and location of the specialized mesenchymes (onychodermis and onychomatricodermis) in the nail unit by expression of ECM components including versican[7], elastin[10] and fibrillin[11]. In addition, we verified the existence of specialized mesenchymal cells (onychofibroblasts) by expression of CD10[6] and CD13[12].

In this study we did further research to characterize the histologic, histochemical, immunohistochemical, and ultrastructural features of the onychomatricodermis containing onychofibroblasts in the nail unit. We investigated the onychomatricodermal expression of collagen, the most important ECM protein in the dermis, and the immunohistochemical features of onychomatricodermal onychofibroblasts.

MATERIALS AND METHODS

Ten cases of polydactyly were evaluated in this study. Use of all specimens was approved from the institutional review board at Samsung Medical Center (IRB no. 2017-10-137). The specimens were sectioned longitudinally along the long axis of the nail plate, transversely across the nail matrix or nail bed, or horizontally parallel to the surface of the nail plate. Formalin-fixed paraffin-embedded blocks of 8 punch or incisional biopsy specimens were retrieved from the archive materials of the Pathology department. Hematoxylin and eosin (H&E)-stained slides of the speci-

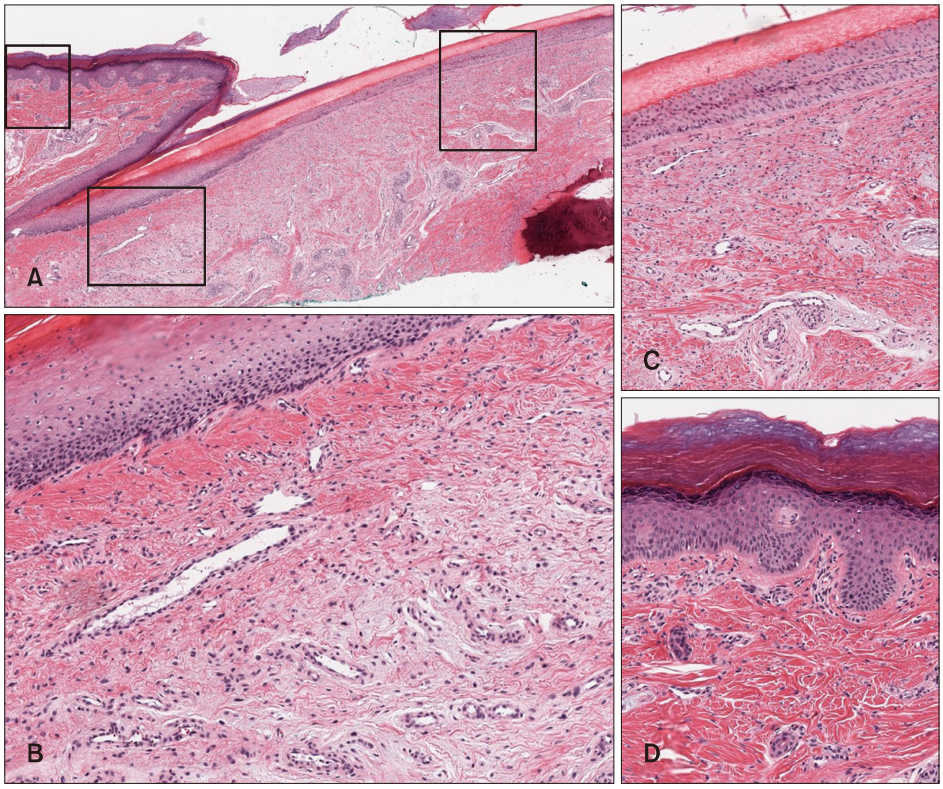

Fig. 1. Hematoxylin and eosin (H&E) staining of longitudinal section of polydactyly nail unit. (A) Low-power view (40×); (B) high-power view of nail matrix epithelium and subjacent onychodermis (400×); (C) high-power view of nail bed epithelium and subjacent onychodermis (200×); (D) high-power view of proximal nail fold (400×).

mens were reviewed. We did Alcian blue (pH 2.5) staining and Masson trichrome staining to demonstrate acid mucopolysaccharides and collagen respectively. We did immunohistochemical staining on the nail units of polydactyly and nail matrix biopsy specimens using a monoclonal antibody against type Ⅰ collagen (Abcam, Cambridge, UK). In addition, we used monoclonal antibodies against CD10 (Novocastra, Newcastle, UK), CD13 (Novocastra), and CD34 (DAKO, Santa Clara, CA, USA) for IHC on nail matrix biopsy specimens. For IHC, tissue sections were deparaffinized with xylene and rehydrated with ethanol. Tissue sections were immersed in boiled sodium citrate for antigen retrieval. To block endogenous peroxidase in tissue sections, we treated slides with H_2O_2 in methanol. Sections were treated with a blocking solution (Protein block, DAKO) to prevent nonspecific antibody binding. The sections were then incubated with each antibody for a few hours at room temperature. The slides were washed in phosphate buffered saline, followed by antigen detection kit (DAKO EnVision System; Dako) and diaminobenzidine for visualization. They were counterstained with hematoxylin solution.

For transmission electron microscopy we dissected polydactyly nail units under microscopy. Small specimens including nail matrix, nail bed and proximal nail fold were respectively fixed in 2% paraformaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer. Postfixation was carried out with 1% (w/v) osmium tetroxide, and this was followed by dehydration in up to 70% ethanol and then the specimens were embedded in EPON812 resin. Ultrathin sections were collected on copper grids and stained with uranyl acetate and lead hydroxide. Microscopy and photography were performed with a Hitachi 7100 (Hitachi Corporation, Tokyo, Japan).

RESULTS

H&E staining of the longitudinal sections of polydactyly nail units showed findings consistent with our previous observations of the onychomatricodermis containing onychofibroblasts[10]. The onychomatricodermis was slightly distant from the undersurface of the nail matrix epithelium and was observed to be less eosinophilic area containing blood vessels compared to the dermis of other areas of the nail unit (Fig. 1). The mesenchymal area beneath the nail bed was also less eosinophilic; however, the onychomatricodermis looked different from the mesenchymal area beneath the nail bed in that the onychomatricodermal onychofibroblasts showed light purple abundant cytoplasm with round to oval nuclei. These findings were also observed in transverse and horizontal sections of the nail unit (Supplementary Fig. 1).

The biopsy specimens including nail matrix showed onychomatricodermis with histologic features similar to those seen in the onychomatricodermis of polydactyly nail units (Fig. 2). The onychomatricodermis was slightly distant from

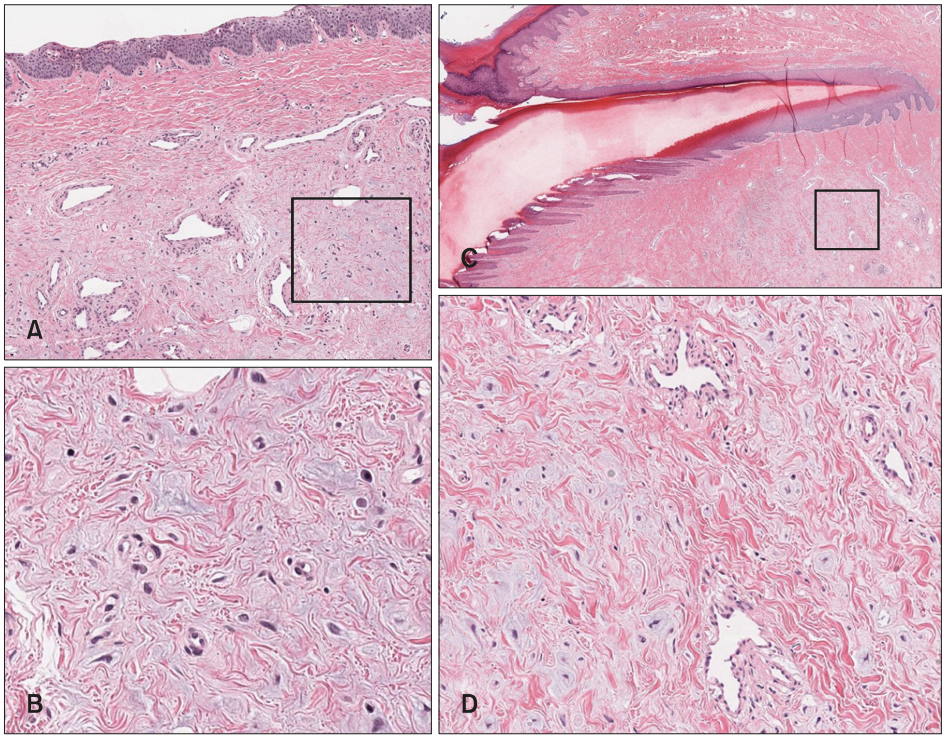

Fig. 2. Hematoxylin and eosin (H&E) staining of biopsy specimens including nail matrix and its surrounding areas. (A) Punch biopsy cases (100×); (B) high-power view of (A) (400×). (C) Incisional biopsy case (40×); (D) high-power view of (C) (200×).

the undersurface of the nail matrix epithelium by a zone of connective tissue and was observed to be less eosinophilic area containing blood vessels compared to the dermis of other areas of the nail unit. Onychomatricodermal onychofibroblasts showed abundant cytoplasm with variously shaped nuclei, mostly round to oval nuclei. These were fewer yet bigger than those seen in the polydactyly nail units.

Masson Trichrome staining of the longitudinal sections of polydactyly nail units revealed much less and thinner collagen fibers around the onychofibroblasts within the onychomatricodermis than in the dermis of other parts of the nail unit. These findings were also observed in transverse and horizontal sections of the nail unit (Supplementary Fig. 2A～D). The biopsy specimens including the nail matrix also showed histochemical features similar to those seen in polydactyly nail units (Supplementary Fig. 2E～H).

In Alcian blue staining of the longitudinal sections of polydactyly nail units the onychomatricodermis showed abundant mucin deposition in the onychofibroblasts and around them. These findings were also observed in transverse and horizontal sections of the nail unit (Supplementary Fig. 3A～D). The biopsy specimens including nail matrix also showed histochemical features similar to those seen in polydactyly nail units (Supplementary Fig. 3E, F).

Immunohistochemically, type Ⅰ collagen was expressed much less in the onychomatricodermis while it was strongly expressed in the dermis of other areas of the nail unit. These findings were observed in longitudinal (Fig. 3), transverse (Supplementary Fig. 4A～C) and horizontal sections (Supplementary Fig. 4D～F) of the nail unit. The biopsy specimens including the nail matrix also showed IHC features similar to those seen in polydactyly nail units (Supplementary Fig. 5).

In nail matrix biopsy specimens CD10 was strongly expressed in onychomatricodermal onychofibroblasts (Fig. 4). CD13 was also positive in onychomatricodermal onychofibroblasts (Supplementary Fig. 6). CD10 and CD13 were expressed in onychofibroblasts below the nail bed, however, they were not observed in dermal fibroblasts. In addition, CD34 was weakly expressed in onychomatricodermal onychofibroblasts; however, it was negative in mesenchymal area below the nail bed except for blood vessels (Fig. 5).

Transmission electron microscopic examination of the onychomatricodermis from the polydactyly specimens showed electron lucent areas containing sparsely distributed collagen fibrils (Supplementary Fig. 7A). However, in the dermis of other parts of the nail unit including proximal nail fold (Supplementary Fig. 7B) and nail bed collagen fibrils were densely distributed around fibroblasts.

DISCUSSION

In the past, we first discovered the presence of specialized mesenchymal cells below the nail matrix and proximal nail bed by CD10 IHC, and proposed to name these cells onychofibroblasts[6]. We found weak expression of fibrillin, an ECM protein, in the mesenchymal area below the nail matrix, but it was strongly expressed in the dermis of the lateral nail fold[9]. In addition, based on histology and IHC for the expression of CD10 and versican, we demon-

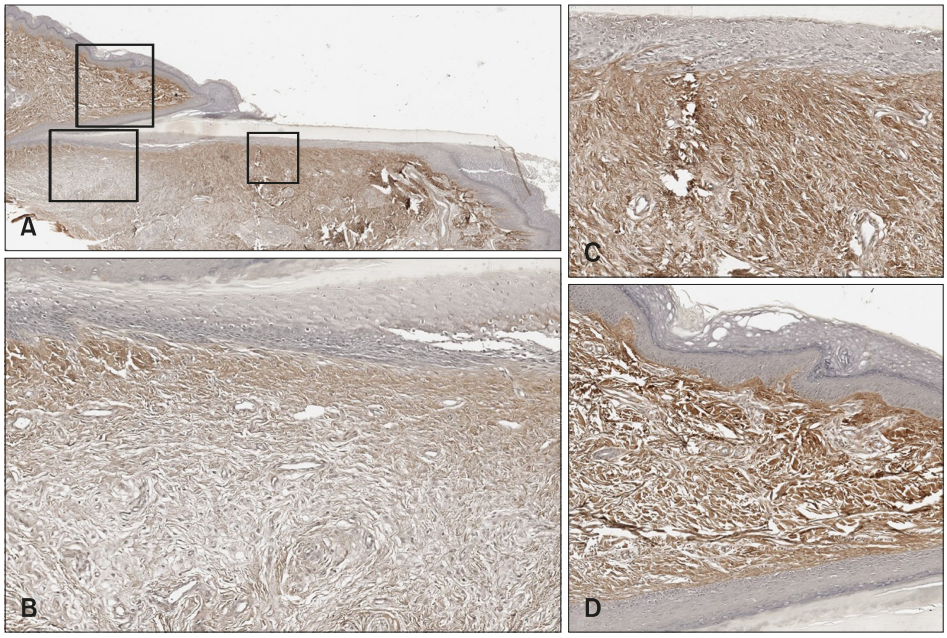

Fig. 3. Immunohistochemical staining for type Ⅰ collagen of longitudinal section of polydactyly nail unit. (A) Low-power view (40×); (B) high-power view of lower portion of nail matrix epithelium and subjacent onychodermis (400×); (C) high-power view of nail bed epithelium and subjacent onychodermis (200×); (D) high-power view of proximal nail fold (200×).

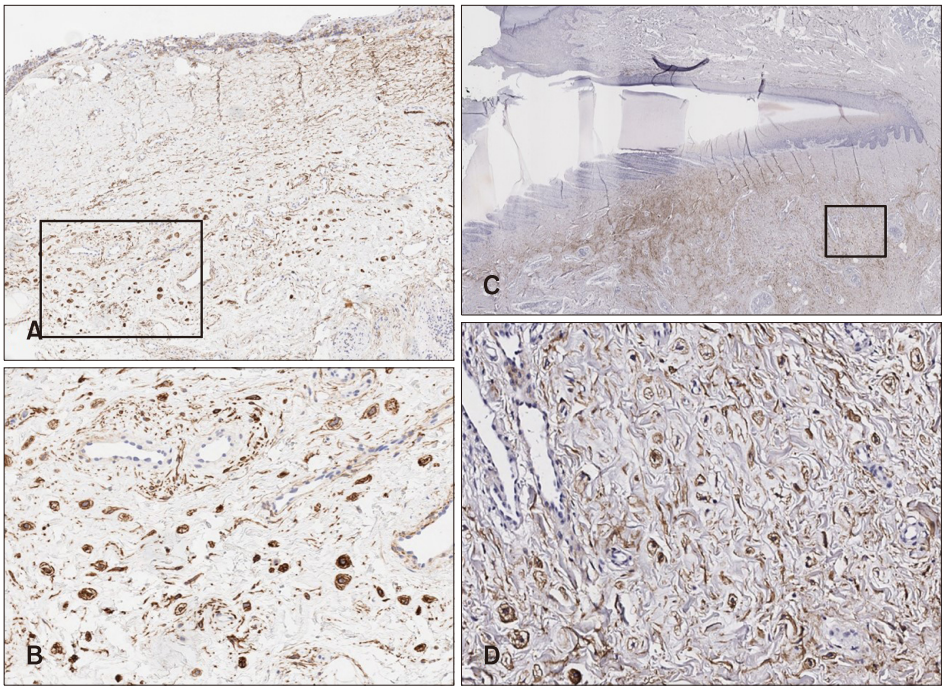

Fig. 4. Immunohistochemical staining for CD10 of biopsy specimens including nail matrix and its surrounding areas. (A) Punch biopsy case (100×); (B) high-power view of (A) (400×); (C) incisional biopsy case (40×); (D) high-power view of (C) (400×).

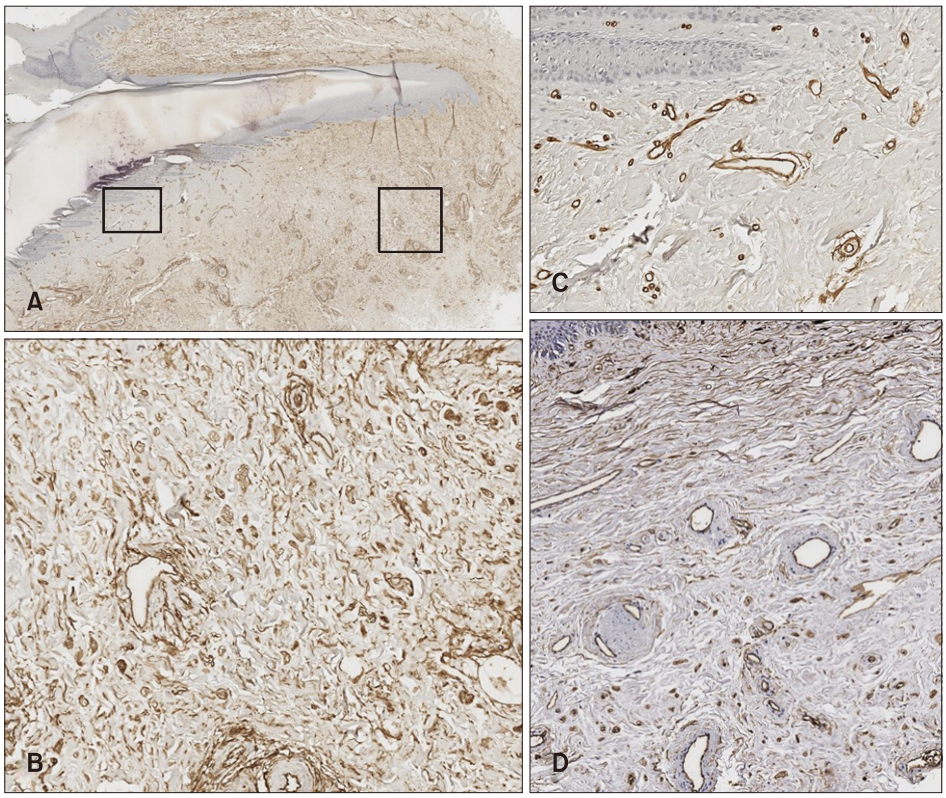

Fig. 5. Immunohistochemical staining for CD34 of biopsy specimens including nail matrix and its surrounding areas. (A) Incisional biopsy case (40×); (B) high-power view of nail matrix onychodermis (400×); (C) high-power view of nail bed epithelium and subjacent onychodermis (200×); (D) punch biopsy case (200×).

strated the presence and location of the specialized mesenchyme containing onychofibroblasts below the nail matrix and nail bed and proposed to call it onychodermis[7]. We recently found stronger CD13 expression in the mesenchyme below the nail matrix than in the mesenchyme below the nail bed[12]. Because the nail plate is produced by the nail matrix epithelium, we presumed that onychodermis below the nail matrix could be different from the

onychodermis beneath the nail bed. Very recently, we characterized histologic features that distinguish the nail matrix onychodermis from the dermis of other parts of the nail unit[10]. We also demonstrated much less elastin expression in nail matrix onychodermis in contrast to its strong expression elsewhere in the nail unit[8]. Based on these findings, we proposed the terminology onychomatricodermis for this mesenchymal area containing onychofibroblasts beneath the nail matrix[8]. In this study, we demonstrated that there is less collagen expression in the onychomatricodermis containing onychofibroblasts compared to the dermis of other parts of the nail unit by histology, histochemistry, IHC, and electron microscopy. These findings confirm the presence of specialized nail mesenchyme (onychomatricodermis) containing onychofibroblasts below the nail matrix in the nail unit (Fig. 6). Putting our previous findings[7,10,11] and present results together, onychomatricodermis was characterized by having less collagen, elastin and fibrillin, and more abundant mucin and proteoglycan such as versican.

In our previous study[10] as well as in our present study we found that onychomatricodermal onychofibroblasts had characteristic histologic features such as slight purple ample cytoplasm. In addition, onychomatricodermal onychofibroblasts had abundant mucin. Nail matrix biopsy specimens of our present study showed that onychomatricodermal onychofibroblasts expressed CD10 and CD13 strongly, consistent with the findings of the previous study[10]. CD34 was also expressed in onychomatricodermal onychofibroblasts; however, it was negative in the mesenchymal area below the nail bed except for the blood vessels. Based on the findings of our previous[10,12] and present studies, onychomatricodermal onychofibroblasts were unique, specialized mesenchymal cells with distinguishing histologic and IHC features below the nail matrix. Onychomatricodermal onychofibroblasts are part of the onychofibroblasts that express CD10[6]. However, onychofibroblasts below the nail bed are CD10 (+), CD13 (+), and CD34 (−). Thus, among CD10 positive onychofibroblasts we propose to call onychomatricodermal onychofibroblasts onychofibroblasts of Dongyoun (Fig. 6).

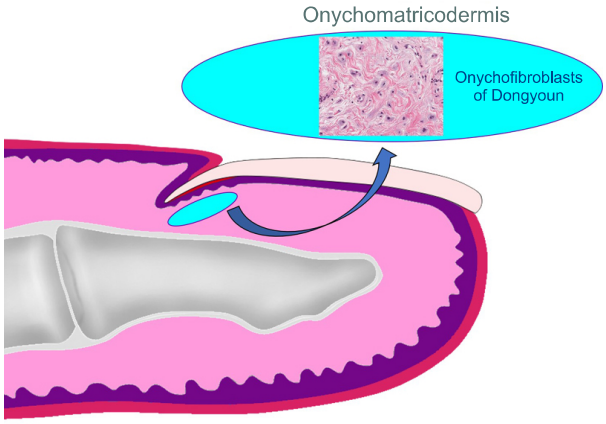

Fig. 6. Simplified picture of the onychomatricodermis containing onychofibroblasts of Dongyoun below the nail matrix in the nail unit. Modified from the article of Lee et al. (J Cutan Pathol 2019;46:490-497).[10]

Previously, based on IHC for CD13[12] and elastin[10], we suggested that onychomatricodermis may be the nail counterpart of follicular dermal papilla. In the present study, type Ⅰ collagen in the onychomatricodermis was expressed much less than was the dermis of other parts of the nail unit. Although we did not show the expression of type Ⅰ collagen in hair follicles, it was expressed much less in follicular dermal papilla than in the follicular dermal sheath and scalp dermis (data not shown). Decreased expression of type Ⅰ collagen in both the onychomatricodermis and follicular dermal papilla supports that the onychomatricodermis may be nail counterpart of follicular dermal papilla and also provides another evidence of resemblance between nail and hair. Onychomatricodermal onychofibroblasts (onychofibroblasts of Dongyoun) may be the nail counterpart of the follicular dermal papilla cells within the follicular dermal papilla.

Very recently, Perrin[13] pubished an interesting paper about nail anatomy. According to his schematic diagrams he divided the mesenchyme of the nail unit into several parts including matrical dermis, matrical hypoderm and nail bed dermis. The matrical hypodermis was located below the matrical dermis. However, he might have overlooked the concept of specialized nail mesenchyme called onychodermis containing onychofibroblasts[7]. In the past, although another group also demonstrated the presence of onychodermis in the fetal nail unit[8], he denied the presence of onychodermis in the adult nail unit[14]. However, we demonstrated that onychodermis is present in the adult nail unit as well[12,15]. Recently, we proposed the concept of nail matrix onychodermis (onychomatricodermis) in the nail unit[10,12]. In this study we also confirmed the presence of onychomatricodermis in the nail unit. Onychomatricodermis seems to correspond to the matrical hypodermis in Perrin's paper.

Onychomatricoma is a rare, benign tumor that is located at or around the nail matrix[16]. Histopathologically, it is a fibroepithelial tumor that is composed of epithelial and mesenchymal components and the concept of epithelial onychogenic tumor with onychogenic mesenchyme was proposed[17]. Previously, we reported the relationship of onychomatricoma to onychodermis by its location and expression of CD10[18] and CD13[19]. In the present study we

found CD34 expression in onychomatricodermal onychofibroblasts. Because CD34 is known to be expressed in the dermal portion of onychomatricoma[20], onychomatricodermal onychofibroblasts might participate in the development of onychomatricoma. Nail matrix-like epithelium in the onychomatricoma might be induced by neoplastic proliferation of onychomatricodermal onychofibroblasts.

The limitation of this study was the use of polydactyly samples instead of normal nail unit, but to overcome this limitation to some extent we also used nail matrix biopsy specimens.

In this study we further characterized the histologic, histochemical, immunohistochemical and ultrastructural features of the onychomatricodermis containing onychofibroblasts. Additional studies including the function of onychomatricodermis containing onychofibroblasts are necessary to find out whether onychomatricodermal onychofibroblasts play an important role in the formation of the nail plate through the epithelial-mesenchymal interactions.

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SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-33-108-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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