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3D reconstruction and histopathological analyses on murine corporal body

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Abstract

Purpose: Erectile dysfunction (ED) is one of the increasing diseases with aging society. The basis of ED derived from local penile abnormality is poorly understood because of the complex three-dimensional (3D) distribution of sinusoids in corpus cavernosum (CC). Understanding the 3D histological structure of penis is thus necessary. Analyses on the status of regulatory signals for such abnormality are also performed.

Methods: To analyze the 3D structure of sinusoid, 3D reconstruction from serial sections of murine CC were performed. Histological analyses between young (2 months old) and aged (14 months old) CC were performed. As for chondrogenic signaling status of aged CC, SOX9 and RBPJK staining was examined.

Results: Sinusoids prominently developed in the outer regions of CC adjacent to tunica albuginea. Aged CC samples contained ectopic chondrocytes in such regions. Associating with the appearance of chondrocytes, the expression of SOX9, chondrogenic regulator, was upregulated. The expression of RBPJK, one of the Notch signal regulators, was downregulated in the aged CC.

Conclusions: Prominent sinusoids distribute in the outer region of CC which may possess important roles for erection. A possibility of ectopic chondrogenesis induced by alteration of SOX9/Notch signaling with aging is indicated.

KEYWORDS

aging, corpus cavernosum, erectile dysfunction, penis, sinusoid

1 | INTRODUCTION

The penis is required for erection during copulation that plays fundamental roles by regulating the inner blood flow. Male corporal tissue, corpus cavernosum (CC), develops in the upper (dorsal) part of penis. When CC is filled with bloods, the microvascular complex termed sinusoids expands during erection.[1] Because of fundamental function of CC for erection, various CC-related reproductive abnormalities have been known. Currently, 5%-20% of men in the world suffer from moderate-to-severe erectile dysfunction (ED).[2] ED is often characterized by pathological conditions of penis associated with diabetes mellitus, hypertension, and dyslipidemia.[3-6] The potential reasons for the above conditions have been suggested as correlated with the "aging" society and the worldwide increase of lifestyle diseases.[7,8,9] Searching

and development of drugs to treat ED are considered as one of the essential modern medical topics.[10,11] Vascular system including microvasculatures is generally affected by these diseases.[12] It has been suggested that cellular alterations and histological changes of penises are generally correlated with impaired erection in such pathological conditions.[13,14] As for histopathogenic conditions of CC, analyses have been performed for abnormal CC in case of diabetes for animal models and human patient erectile tissues.[15] Aged and diabetes patients' penises show various abnormalities related to smooth muscle cells, and the increased level of collagen production has been reported.[15,16] However, experimental models covering structural parameters of penises and sinusoidal structures are still poorly described.

Histological analysis of CC has been mainly performed on pathological conditions including diabetes and aged samples.[17] It has been suggested that accumulation of fibrosis is reported in such conditions.[18] Augmented expression of collagen and other ECM components have been reported in case of fibrosis of pathogenic CC.[19] Ectopic fibrosis and adipocyte accumulation are also reported in case of reproductive abnormalities.[20-22] Penile fibrosis or abnormal chondrogenesis is also indicated in human patients of Peyronie's disease.[23] Detailed histological studies utilizing electron microscopy have been also performed.[24] Disorganization of smooth muscle cells in castrated animal models by histological studies has been also reported.[19]

Sinusoids are widely distributed inside the CC. In the middle of CC, the deep artery delivers blood through helicine artery to the surrounding sinusoids. Such sinusoids are necessary for veno-occlusive functions to increase inner pressure of CC for erection.[25] However, three-dimensional location of sinusoids inside the entire CC structures has been poorly described due to the lack of suitable technologies covering such 3D CC structures. Thin sections by H&E staining can reveal a part of the sinusoidal structures. However, due to irregular shapes of each sinusoid unlike regular shaped hepatic sinusoids limited the usage of such thin sections by H&E staining to reveal the 3D structural images of sinusoids. In order to circumvent such difficulties, we performed 3D reconstruction of histological images covering sinusoidal structures.

In addition to the histopathological analyses, examination of chondrogenic regulator status was performed in order to get clues to understand the potential causes of ectopic chondrogenesis in the current study. Sox9 regulates chondrogenesis, and its ectopic expression was detected in the aged mouse CC. Notch signaling pathway is essential for bone and cartilage formation.[26] It is suggested that Sox9/Notch signaling also regulates vascular smooth muscle and mesenchymal differentiation during embryogenesis.[27,28] Disruption of such signaling results in ectopic bone and cartilage formation.[29] Prominently reduced extent of Notch signaling judged by reduced RBPJK expression was detected in the case of aged CC. The current work indicates potential involvement of Notch signals for the ectopic chondrogenesis of aged CC. Altogether, the current work discusses on the utility for the 3D distribution of sinusoids with such altered extent of regulators.

2 | MATERIALS AND METHODS

2.1 | Animals

Male ICR mice (2 months old and 14 months old) were purchased from CLEA Japan Inc Several criteria show that 14-month-old mice correspond to senescent staged mouse.[30,31] All procedures and protocols are approved by the committee for animal researches at Wakayama Medical University, Wakayama, Japan (approval number: 867).

2.2 | Corpus cavernosum isolation

The mouse penis consists of corpus cavernosum glandis (CCG), which includes baculum, and corpus cavernosum (CC). The CC region was isolated by microdissection removing the prepuce and CCG (Figure 1A).

2.3 | Histological analysis and 3D reconstruction

The mouse corpus cavernosum tissues were fixed overnight in 4% paraformaldehyde (PFA) in Phosphate-buffered saline (PBS). After the fixation, 6-μm thickness paraffin sections were prepared for hematoxylin and eosin (H&E) staining and Masson's trichrome staining was performed by standard procedures as previously described.[32,33] The numbers of chondrocytes were counted per 0.1 mm^2 region by imageJ software.

For 3D reconstruction, serial sections of CC were prepared and stained by H&E staining. Three-dimensional reconstruction using H&E images was performed by Amira 3D software (Amira5).[34] Video file (MPEG) was constructed from serial section image files (JPEG).

2.4 | Immunofluorescence staining

Harvested corpus cavernosum tissues were embedded in OCT compound (Sakura Tissue-Tek) after 4% PFA fixation. 20-μm thickness cryosections were prepared. Paraffin sections were also prepared for staining. The detailed protocols were previously described.[35] For primary antibody staining, the following antibodies were utilized: anti-CD31 (1/200, AF3628, AB_2161028, R&D systems), anti-ACTA2 (1/1000, U 7033, DAKO), anti-NG2 (1/100, AB5320, AB_11213678, Millipore), anti-SOX9 (1/1000, AB5535, Millipore), and anti-RBPJK (1/300, 2ZRBP2, Cosmo Bio). For secondary antibody reaction, the following antibodies were utilized: Invitrogen goat anti-rabbit IgG Alexa Fluor 488, Invitrogen donkey anti-goat IgG Alexa Fluor 488, Invitrogen goat anti-mouse IgG Alexa Fluor 488, and Invitrogen goat anti-rat IgG Alexa Fluor 546 (1/200, Thermo Fisher Scientific).

2.5 | Statistical analysis

For the number of chondrocytes in young and aged mice CC, Student's *t* test followed by the F test was performed (values of $P < .05$ were considered to be significant).

3 | RESULTS

3.1 | The location and structure of the mouse corpus cavernosum

To reveal the corpus cavernosum (CC) structure with its cellular composition, histological analyses with hematoxylin and eosin (H&E) and immunofluorescence staining for cellular markers were performed. A schematic illustration is shown for the location and structure of the mouse penis of adult mice (Figure 1A). In flaccid conditions, the majority of the mouse external penile region corresponds to the glans.[34] The location of major CC is indicated by red lines (Figure 1A).

The images of H&E Staining of CC showed prominently developed sinusoids, dorsal vein, and dorsal artery (Figure 1B). To reveal the distribution of extracellular matrix (ECM) with collagens inside CC, Masson's trichrome staining was performed. Collagen-rich ECM region was shown by the blue staining (Figure 1C). Several types of cells locating in the CC were demonstrated as CD31-positive endothelial cells, ACTA2- (alpha-smooth muscle actin) positive smooth muscle cells, and NG2-positive pericytes locating adjacent to tunica albuginea. (Figure 1D-F).

3.2 | Three-dimensional (3D) reconstruction of histological images for mouse CC

In order to understand 3D structure of CC with sinusoids, 3D reconstruction with approximately 400 H&E serial thin section images was performed with Amira5 software (Figure 2A). The obtained cross and sagittal images from 3D video showed prominently developed sinusoids locating adjacent to the outer regions of CC (adjacent to tunica albuginea). Figure 2B shows representative of cross and sagittal images. Entire 3D video is attached in the Video S1. Such images include the pink color regions for sinusoidal spaces, red color regions; dorsal artery, blue color regions; dorsal vein, green color regions; nerve bundle and yellow color regions; urethra.

3.3 | Detection of ectopic chondrocytes in the aged mouse CC

In order to analyze the histological status of aged mouse CC, the H&E staining for young and aged mouse CC was performed. Prominent numbers of chondrocytes were observed in the aged CC specimens in the region adjacent to tunica albuginea in contrast to controls (Figure 3A-C; white dotted line region). In order to further analyze chondrogenic status, Masson's trichrome staining for ECM detection was performed for young and aged mouse CC specimens. Prominently stained ECM regions were shown in aged CC samples (Figure 3D and E; white dotted line region).

3.4 | Detection of augmented SOX9 expression in the aged mouse CC

Sox9 is a key gene regulating chondrocyte differentiation and cartilage formation.[37,38] In order to analyze the status of regulator expression for chondrocytes, Sox9 expression was examined in the young and aged mouse CC specimens. Low level of SOX9 expression was detected in the control young (2 months old) mouse CC (Figure 4A). In contrast, augmented SOX9 expression was detected in aged (14 months old) mouse CC (Figure 4B; white arrows).

SOX9 was colocalized with chondrocytes in the aged mice CC (Figure 4C and D).

3.5 | Possibility of reduced Notch signals in the aged mouse CC

In order to examine the possible involvement of regulatory signaling for the ectopic chondrogenesis, immunofluorescence staining of Notch signaling was performed. Notch signal is one of the essential signals suppressing the chondrogenesis.[27,28] Among such Notch signal regulatory components, Rbpjk is one of the major coregulatory genes for Notch signal.[39,40] The reduced level of RBPJK expression was detected in the aged CC sample in contrast to control CC sample (Figure 5A and B; white dotted line). Implications of the reduced level of RBPJK expression with aging are discussed below (Figure 5C).

4 | DISCUSSION

Increased number of ED patients is reported recently. To understand the basis of such ED related to penile abnormality, analyses are necessary to understand the structure of corporal body (corpus cavernosum; CC) and sinusoids in experimental animal models. In order to also analyze the status and regulatory molecules of abnormal mouse CC, the current work analyzed 3D distribution of sinusoids in CC and performed expression analysis of key regulators for CC mesenchyme, Sox9, and Notch signal.

4.1 | 3D structure of CC/sinusoids and implications for erectile physiology and aging

In the current study, 3D structure of CC with sinusoids is revealed for the first time. Significant number of sinusoids was observed as locating adjacent to the outer regions of CC which are close to

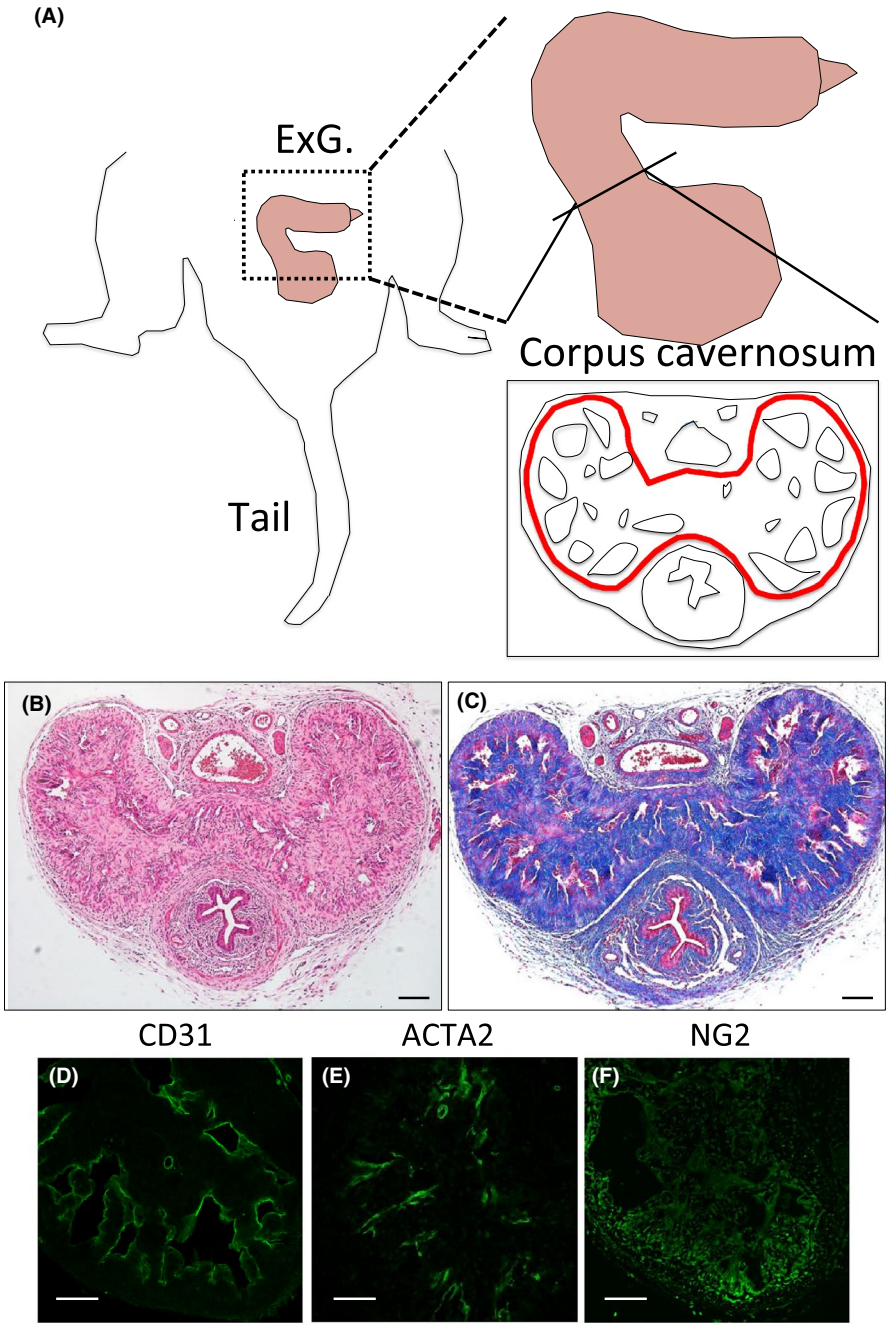

FIGURE 1 The structure of normal mouse corpus cavernosum (CC). (A) A schematic illustration showing the location and structure of the mouse penis in the lower part of adult mice. Dotted square indicates the location of penis in the lower part of mouse. Red lines indicate mouse CC. ExG. external genitalia. (B) The images of hematoxylin-eosin staining of CC. (C) The images of Masson's trichrome staining of CC showing the collagen-rich regions. (D-F) The images of immunofluorescence staining for the following type of cells. (D) CD31-positive endothelial cells, (E) ACTA2- (alpha-smooth muscle actin) positive smooth muscle cells, and (F) NG2-positive pericyte. Scale bar 100 μm [Colour figure can be viewed at wileyonlinelibrary.com]

the tunica albuginea. Several histological studies have been performed for CC and sinusoids by utilizing thin sections with H&E staining.[41,42] Histological analyses with such thin sections of H&E staining generally revealed only part of the CC structures thus not revealing the entire 3D structures.[43-45] The currently identified sinusoid structures showed variations in their sizes revealed by reconstructed 3D histological images. It has been reported that mouse CC contains collagen-rich prominent trabeculae ("island like" central area) adjacent to the deep artery in central region. Such peculiar fibrous structure does not contain sinusoids but mesenchymal-rich ECM region which presumably contribute to erection process for mouse copulation unlike the case of human CC.[36] The prominently developed sinusoids located adjacent to outer of CC may suggest the essential roles of "outer" region for erection during contraction and relaxation in contrast to the central regions of mouse CC.

Several studies implicated the physiological importance of outer CC region adjacent to tunica albuginea.[46] Stem-like cells have been suggested to locate in such region shown by the slow cell cycle labeling to detect immature type of cells.[47,48] As for the connection of such outer region and central region of CC, helicine artery is suggested to deliver blood from deep artery to sinusoidal structures. The storage of blood during erection in sinusoids veno-occlusion by tunica albuginea has been also described as essential for blood storage.[25] Hence, the current observation of the prominent outer sinusoidal structures may suggest the potential importance of such

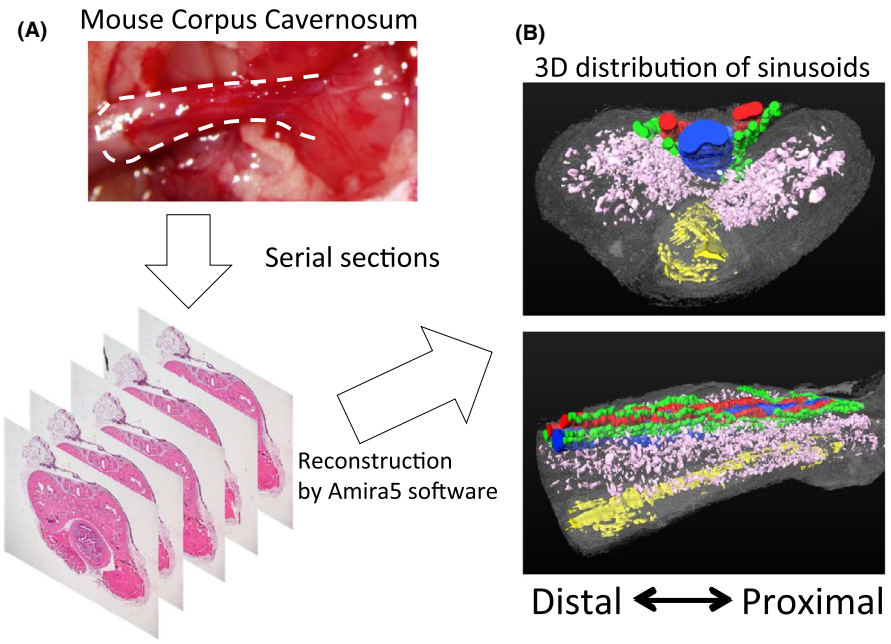

FIGURE 2 Three-dimensional (3D) reconstruction of images covering normal mouse CC sections. (A) The schema showed the 3D reconstruction process by Amira software with approximately 400 serial cross sections of mouse CC. White dotted line indicates CC. (B) The 3D images were reconstructed from serial H&E stained sections. The regions corresponding to proximal and distal end of external genitalia are indicated. Prominently developed sinusoids were detected adjacent to the outer regions of CC (tunica albuginea). Pink color regions indicate sinusoidal spaces. Red color regions indicate dorsal artery. Blue color regions indicate dorsal vein. Green color regions indicate nerve bundle. Yellow color regions indicate urethra [Colour figure can be viewed at wileyonlinelibrary.com]

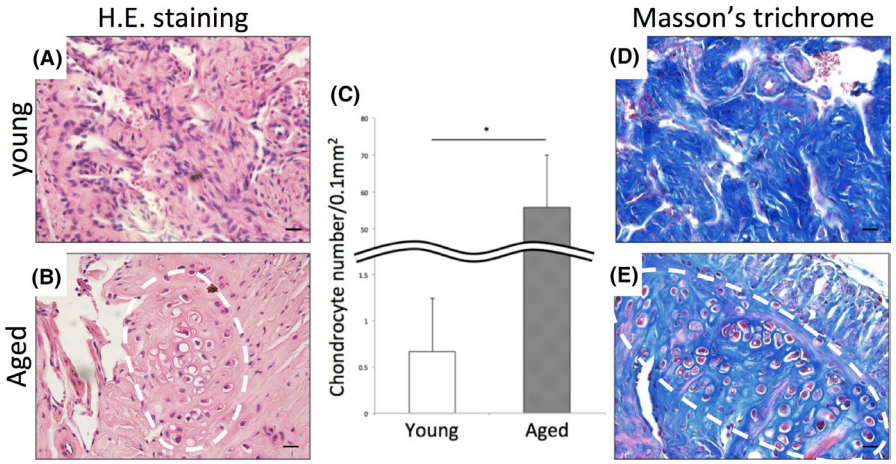

FIGURE 3 Detection of ectopic chondrocytes in aged mouse CC. (A) The H&E staining images of young (2 months old) mouse CC shown by cross sections. Scale bar: 20 μm. (B) The H&E staining images of aged (14 months old age which is regarded as senescence) mouse CC. Dotted line indicates the accumulation of ectopic chondrocytes. Scale bar: 20 μm. (C) The bar graph shows increased number of chondrocytes in the aged mice CC. The number was calculated by 0.1 mm^2 area. *indicates $P < .05$. (D) The Masson's trichrome staining images of young mouse CC. Scale bar: 20 μm. (E) The Masson's trichrome staining images of aged mouse CC. Dotted line indicates the location of ectopic chondrocytes. Scale bar: 20 μm [Colour figure can be viewed at wileyonlinelibrary.com]

sinusoids. Further studies are necessary to examine such sinusoid including the possibility of "mature" sinusoids in the outer region of CC.

Previous study on penile aging processes suggested the importance of outer regions of CC.[49] Abnormal cellular composition such as chondrocytes has been suggested in mouse with augmented androgen production.[31,50] The detection of ectopic chondrocytes was reported in such mouse models. Detection of fibrosis has been also suggested to occur in the outer region of CC in case of aged animal models.[51] In some human clinical studies, reduced number of smooth muscle cells and tissue abnormality adjacent to the tunica region (outer region of CC) has been suggested in certain form of ED. Such organic ED has been also suggested as correlated with reduced veno-occlusive functions in the region adjacent to tunica albuginea. In the case of other penile abnormalities, Peyronie's disease shows curved penis caused by mesenchymal abnormality.[23] Previous report indicated abnormal mesenchyme defects in peri-tunica region including the appearance of chondrocyte.[23] Hence, ectopic chondrogenesis can be associated with various tissue abnormalities such as ED and Peyronie's disease. Onset of such abnormal structures may be due to the sensitivity of prominent or mature sinusoids in the above regions. Further studies with 3D sinusoidal analyses with pathological and aged conditions are necessary. Examination of the pathological conditions with a status of critical signaling was subsequently performed (as below).

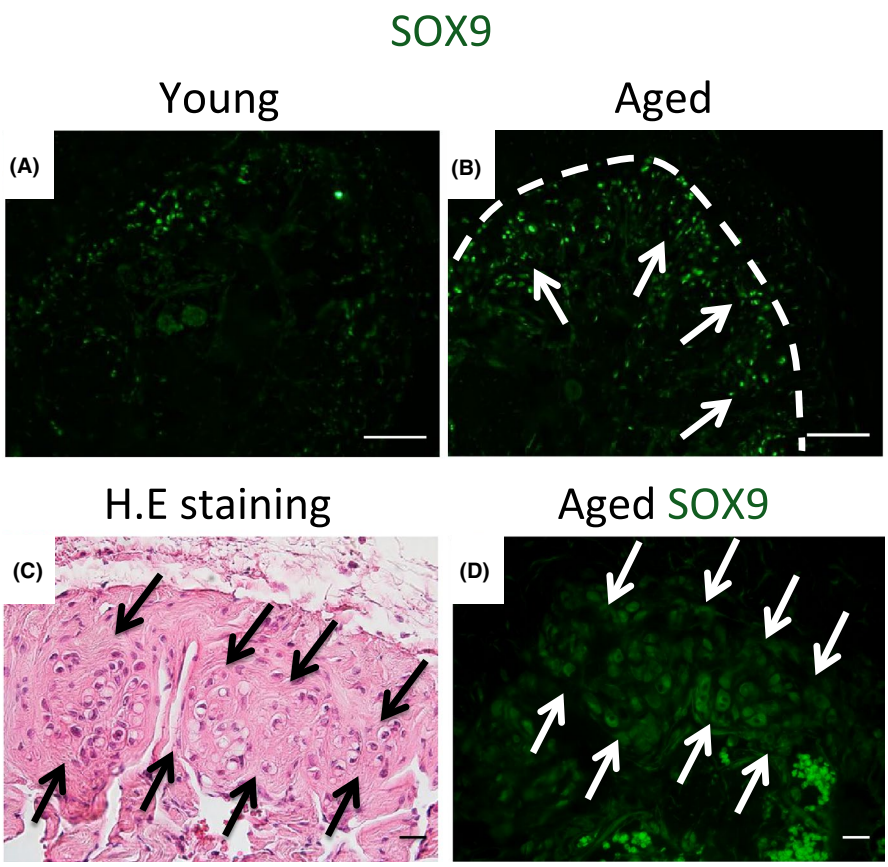

FIGURE 4 Detection of ectopic SOX9 expression in the aged mouse CC. (A) Low level of SOX9 expression in the control young (2 months old) mouse CC. Scale bar: 100 μm. (B) Ectopic SOX9 expression in the aged (14 months old) mouse CC. Dotted lines indicate part of the outer region of CC (tunica albuginea) in the aged mouse CC. White arrows indicate augmented SOX9 expression. Scale bar: 100 μm. (C) Magnified H.E staining image of aged mice CC. The section locates adjacent to SOX9 immunostaining section (D) in the range of 300 μm. Black arrows indicated chondrocytes. Scale bar: 20 μm. (D) Magnified SOX9 staining image of aged mice CC. The section is adjacent to magnified H&E staining section (C) in the range of 300 μm. White arrows indicated chondrocytes. Scale bar: 20 μm [Colour figure can be viewed at wileyonlinelibrary.com]

4.2 | Detection of ectopic chondrogenesis with altered expression of SOX9 and RBPJK in the aged CC

In order to examine the conditions of aged sinusoids, H&E staining analysis was performed in normal and aged mouse CC. Chondrogenic cells were mainly observed in the region adjacent to tunica albuginea (Figure 3A and B). In order to examine the chondrogenic conditions of such regions, ECM staining by Masson's trichrome was performed in both specimens. Prominent chondrogenic cells with production of collagen fibers were detected (Figure 3C and D). In order to examine the cellular conditions associated with such states during aging, the expressions of essential chondrogenic regulators were examined.

Sox9 is one of the Sox family genes, transcription factor which possess high-mobility-group (HMG) domain.[52,53] Sox9 has been suggested as one of the central genes regulating chondrogenesis.[37,38] Many reports have indicated that it is expressed in chondrocyte precursors regulating its differentiation and cartilage formation.[54] It is also suggested that its abnormal expression induces ectopic chondrogenesis. Prominently augmented SOX9 expression was detected in the aged mouse CC compared with normal CC (Figure 4A and B). These data suggest the ectopic chondrogenesis may adapt the augmented chondrogenic programs containing Sox9.

Human penis is known as not including penile bone (baculum). Unlike human penises anatomy,[55] numerous numbers of mammalian species contain baculums which are necessary for copulation processes in addition to CC.[56] Hence, the coexistence of bone and chondrogenic structure inside the erectile tissues such as CC is an intriguing topic from evolutional and comparative biological viewpoints.[57] Ectopic chondrogenic cells detected in aged mouse model might be associated with the existence of penile bone in mouse. Further studies are necessary to compare human and mouse pathogenic conditions and mesenchymal developmental programs in terms of ectopic chondrogenesis of CC.

In order to examine the status of regulatory signals for such ectopic chondrogenesis, the expression of chondrogenic regulators was examined. Notch signaling is one of the essential signals regulating chondrogenesis.[27] In the case of vascular smooth muscle progenitor cells, sustained Notch/Jag1 signaling suppresses the expression of chondrogenic genes including Sox9.[28] It has been also reported that RBPJ/Notch intracellular domain (NICD) complex represses Sox9 transcription.[58] When such cascades are aberrated, detection of ectopic chondrocytes has been reported.[29] Therefore, the correlation between Notch signaling and chondrogenesis is reported in case of animal models and cell line experiments.[59-61]

To get implications for ectopic chondrogenesis in CC, one of the key components for Notch signaling pathway, RBPJK, was examined. Reduced level of RBPJK expression was detected in aged CC samples with ectopic chondrogenesis. RBPJK protein binds DNA to modulate downstream transcription by protein-protein interaction regulating Notch signaling activity.[62] Because of key roles of RBPJK protein for the regulation of Notch signal

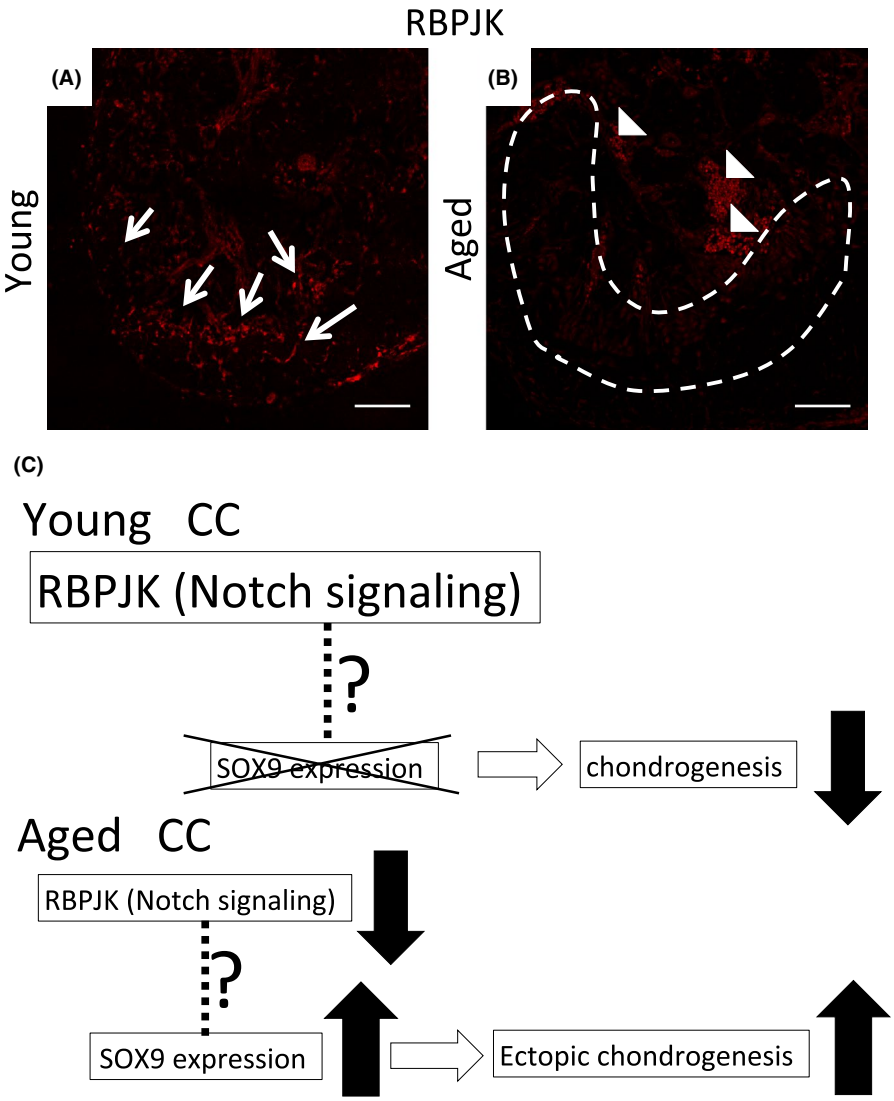

FIGURE 5 The possibility of the reduced Notch signaling in aged mouse CC. (A) The expression of RBPJK, the regulator of Notch signaling, in young (2 months old) mouse CC. White arrows indicate the expression of RBPJK in the CC region. (B) The reduced expression of RBPJK in aged (14 months old) mouse CC. Dotted line indicates the CC region of reduced expression of RBPJK. Arrowheads indicate autofluorescence of red blood cells included in sinusoids. Scale bar: 100 μm. (C) The schema shows possible signaling pathways leading to the augmented chondrogenesis in the aged mouse CC [Colour figure can be viewed at wileyonlinelibrary.com]

pathway, the current expression data suggest the involvement of such signaling for the ectopic chondrogenesis in aged conditions (Figure 5C). It has been suggested that reduced level of Notch signaling is detected in aged vasculature animal model.[63] Thus, aging conditions might directly or indirectly modulate Notch signal content in various biological processes. Such age-related connections between modulated Notch signal and androgen content should be considered and analyzed in the future. Modulation of the Notch signal pathway such as inducing Notch signaling pathway might be considered to treat ectopic chondrogenesis in aged CC. Further examination using the 3D structural analysis for CC and detailed analyses of such signaling are necessary to explore ectopic chondrogenesis and aging of CC.

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DISCLOSURES

Conflict of interest: Daiki Hashimoto, Mizuki Kajimoto, Yuko Ueda, Taiju Hyuga, Kota Fujimoto, Saaya Inoue, Kentaro Suzuki, Tomoya Kataoka, Kazunori Kimura, and Gen Yamada declare that they have no conflict of interest. *Human rights statements and informed consent*: This work does not contain human subjects. *Animal studies and Approval by Ethics Committee*: All institutional and national guidelines for the care and use of laboratory animals were followed. All procedures and protocols were approved by the committee on animal research at Wakayama Medical University, Wakayama, Japan (approval number: 867).

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REFERENCES

1. Dean RC, Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. *Urol Clin North Am*. 2005;32(4):379-395.
2. Kubin M, Wagner G, Fugl-Meyer AR. Epidemiology of erectile dysfunction. *Int J Impot Res*. 2003;15:63-71.

3. Chu NV, Edelman SV. Erectile dysfunction and diabetes. *Curr Diab Rep*. 2002;2:60-66.
4. Musicki B, Hannan JL, Lagoda G, Bivalacqua TJ, Burnett AL. Mechanistic link between erectile dysfunction and systemic endothelial dysfunction in type 2 diabetic rats. *Andrology*. 2016;4:977-983.
5. Tsujimura A, Hiramatsu I, Aoki Y, et al. Atherosclerosis is associated with erectile function and lower urinary tract symptoms, especially nocturia, in middle-aged men. *Prostate Int*. 2017;5:65-69.
6. Zamorano-Leon JJ, Segura A, Lahera V, et al. Relationship between erectile dysfunction, diabetes and dyslipidemia in hypertensive-treated men. *Urol J*. 2018;15:370-375.
7. Hannan JL, Blaser MC, Oldfield L, Pang JJ, Adams SM, Pang SC, Adams MA. Morphological and functional evidence for the contribution of the pudendal artery in aging-induced erectile dysfunction. *J Sex Med*. 2010;7(10):3373-3384. http://dx.doi.org/10.1111/j.1743-6109.2010.01920.x
8. Yafi FA, Jenkins L, Albersen M, et al. Erectile dysfunction. *Nat Rev Dis Primers*. 2016;2(1):16003.
9. Tsujimura A, Hiramatsu I, Nagashima Y, et al. Erectile dysfunction is predictive symptom for poor semen in newlywed men in Japan. *Sex Med*. 2020;8:21-29.
10. Nakano Y, Miyake H, Chiba K, Fujisawa M. Impact of penile rehabilitation with low-dose vardenafil on recovery of erectile function in Japanese men following nerve-sparing radical prostatectomy. *Asian J Androl*. 2014;16:892-896.
11. Albersen M, Fandel TM, Lin G, Wang G, Banie L, Lin C-S, Lue TF. Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. *J Sex Med*. 2010;7(10):3331-3340. http://dx.doi.org/10.1111/j.1743-6109.2010.01875.x
12. Warmke N, Griffin KJ, Cubbon RM. Pericytes in diabetes-associated vascular disease. *J Diabet Comp*. 2016;30:1643-1650.
13. Palese MA, Crone JK, Burnett AL. A castrated mouse model of erectile dysfunction. *J Androl*. 2003;24:699-703.
14. Jin HR, Kim WJ, Song JS, et al. Intracavernous delivery of a designed angiopoietin-1 variant rescues erectile function by enhancing endothelial regeneration in the streptozotocin-induced diabetic mouse. *Diabetes*. 2011;60:969-980.
15. Shin SH, Kim WJ, Choi MJ, et al. Aberrant expression of Wnt family contributes to the pathogenesis of diabetes-induced erectile dysfunction. *Andrology*. 2014;2:107-116.
16. Ferrer JE, Velez JD, Herrera AM. Age-related morphological changes in smooth muscle and collagen content in human corpus cavernosum. *J Sex Med*. 2010;7:2723-2728.
17. Tao M, Tasdemir C, Tasdemir S, Shahabi A, Liu G. Penile alterations at early stage of type 1 diabetes in rats. *Int Braz J Urol*. 2017;43:753-761.
18. Cho MC, Song WH, Paick JS. Suppression of Cavernosal Fibrosis in a Rat Model. *Sex Med Rev*. 2018;6:572-582.
19. Traish AM, Toselli P, Jeong SJ, Kim NN. Adipocyte accumulation in penile corpus cavernosum of the orchiectomized rabbit: a potential mechanism for veno-occlusive dysfunction in androgen deficiency. *J Androl*. 2005;26:242-248.
20. Mihara Y, Maekawa R, Sato S, et al. An integrated genomic approach identifies hoxc8 as an upstream regulator in ovarian endometrioma. *J Clin Endocrinol Metab*. 2020;105.
21. Tamura I, Takagi H, Doi-Tanaka Y, et al. Wilms tumor 1 regulates lipid accumulation in human endometrial stromal cells during decidualization. *J Biol Chem*. 2020;295:4673-4683.
22. Vinay J, Sarquella J, Sanchez J, et al. Adipocyte accumulation in corpus cavernosum: First clinical evidence and pathophysiological implications in erectile dysfunction. *Actas Urol Esp*. 2017;41:97-102.
23. Lucattelli M, Lunghi B, Fineschi S, et al. A new mouse model of Peyronie's disease: an increased expression of hypoxia-inducible factor-1 target genes during the development of penile changes. *Int J Biochem Cell Biol*. 2008;40:2638-2648.
24. Banya Y, Ushiki T, Takagane H, et al. Two circulatory routes within the human corpus cavernosum penis: a scanning electron microscopic study of corrosion casts. *J Urol*. 1989;142:879-883.
25. Hsieh CH, Huang YP, Tsai MH, et al. Tunical outer layer plays an essential role in penile veno-occlusive mechanism evidenced from electrocautery effects to the corpora cavernosa in defrosted human cadavers. *Urology*. 2015;86:1129-1135.
26. Hosaka Y, Saito T, Sugita S, et al. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc Natl Acad Sci U S A*. 2013;110:1875-1880.
27. Dong Y, Jesse AM, Kohn A, et al. RBPjkappa-dependent Notch signaling regulates mesenchymal progenitor cell proliferation and differentiation during skeletal development. *Development*. 2010;137:1461-1471.
28. Briot A, Jaroszewicz A, Warren CM, et al. Repression of Sox9 by Jag1 is continuously required to suppress the default chondrogenic fate of vascular smooth muscle cells. *Dev Cell*. 2014;31:707-721.
29. Mead TJ, Yutzey KE. Notch pathway regulation of chondrocyte differentiation and proliferation during appendicular and axial skeleton development. *Proc Natl Acad Sci U S A*. 2009;106:14420-14425.
30. Li MQ, Yao MN, Yan JQ, et al. The decline of pregnancy rate and abnormal uterine responsiveness of steroid hormones in aging mice. *Reprod Biol*. 2017;17:305-311.
31. Hai L, Hiremath DS, Paquet M, Narayan P. Constitutive luteinizing hormone receptor signaling causes sexual dysfunction and Leydig cell adenomas in male mice. *Biol Reprod*. 2017;96:1007-1018.
32. Haraguchi R, Suzuki K, Murakami R, et al. Molecular analysis of external genitalia formation: the role of fibroblast growth factor (Fgf) genes during genital tubercle formation. *Development*. 2000;127:2471-2479.
33. Haraguchi R, Mo R, Hui C, et al. Unique functions of Sonic hedgehog signaling during external genitalia development. *Development*. 2001;128:4241-4250.
34. Suzuki H, Suzuki K, Yamada G. Systematic analyses of murine masculinization processes based on genital sex differentiation parameters. *Dev Growth Differ*. 2015;57:639-647.
35. Acebedo AR, Suzuki K, Hino S, et al. Mesenchymal actomyosin contractility is required for androgen-driven urethral masculinization in mice. *Commun Biol*. 2019;2:95.
36. Hyuga T, Suzuki K, Acebedo AR, et al. Regulatory roles of epithelial-mesenchymal interaction (EMI) during early and androgen dependent external genitalia development. *Differentiation*. 2019;110:29-35.
37. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. *Nat Genet*. 1999;22:85-89.
38. Akiyama H, Chaboissier MC, Martin JF, Schedl A, de Crombrugghe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. *Genes Dev*. 2002;16:2813-2828.
39. Fortini ME, Artavanis-Tsakonas S. The suppressor of hairless protein participates in notch receptor signaling. *Cell*. 1994;79:273-282.
40. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science*. 1999;284:770-776.
41. Xie X, Du X, Li K, et al. Construction of engineered corpus cavernosum with primary mesenchymal stem cells in vitro. *Sci Rep*. 2017;7:18053.
42. Chen S, Zhu J, Wang M, et al. Comparison of the therapeutic effects of adipose-derived and bone marrow mesenchymal stem cells on erectile dysfunction in diabetic rats. *Int J Mol Med*. 2019;44:1006-1014.

43. Yang F, Zhao JF, Shou QY, et al. Phenotypic modulation of corpus cavernosum smooth muscle cells in a rat model of cavernous neurectomy. *PLoS One*. 2014;9:e105186.
44. Chen S, Huang X, Kong X, et al. Hypoxia-induced phenotypic transformation of corpus cavernosum smooth muscle cells after cavernous nerve crush injury by down-regulating P38 mitogen-activated protein kinase expression. *Sex Med*. 2019;7:433-440.
45. An G, Guo F, Liu X, et al. Functional reconstruction of injured corpus cavernosa using 3D-printed hydrogel scaffolds seeded with HIF-1α-expressing stem cells. *Nat Commun*. 2020;11:2687.
46. Hsu GL, Hsieh CH, Wen HS, Chen YC, Chen SC, Mok MS. Penile venous anatomy: an additional description and its clinical implication. *J Androl*. 2003;24:921-927.
47. Hwang I, Lee HS, Yu HS, Kim ME, Lee JS, Park K. Testosterone modulates endothelial progenitor cells in rat corpus cavernosum. *BJU Int*. 2016;117:976-981.
48. Lin G, Alwaal A, Zhang X, et al. Presence of stem/progenitor cells in the rat penis. *Stem Cells Dev*. 2015;24:264-270.
49. Akkus E, Carrier S, Baba K, et al. Structural alterations in the tunica albuginea of the penis: impact of Peyronie's disease, ageing and impotence. *Br J Urol*. 1997;79:47-53.
50. Hiremath DS, Geerling EC, Hai L, Narayan P. High levels of androgens cause chondrocyte accumulation and loss of smooth muscle in the mouse penile body. *Biol Reprod*. 2020;102:1225-1233.
51. Gonzalez-Cadavid NF. Mechanisms of penile fibrosis. *J Sex Med*. 2009;6(Suppl 3):353-362.
52. Wilhelm D, Hiramatsu R, Mizusaki H, et al. SOX9 regulates prostaglandin D synthase gene transcription in vivo to ensure testis development. *J Biol Chem*. 2007;282:10553-10560.
53. Matoba S, Hiramatsu R, Kanai-Azuma M, et al. Establishment of testis-specific SOX9 activation requires high-glucose metabolism in mouse sex differentiation. *Dev Biol*. 2008;324:76-87.
54. Mori-Akiyama Y, Akiyama H, Rowitch DH, de Crombrugghe B. Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. *Proc Natl Acad Sci U S A*. 2003;100:9360-9365.
55. Matsushita S, Suzuki K, Murashima A, et al. Regulation of masculinization: androgen signalling for external genitalia development. *Nat Rev Urol*. 2018;15:358-368.
56. Schultz NG, Lough-Stevens M, Abreu E, Orr T, Dean MD. The baculum was gained and lost multiple times during mammalian evolution. *Integr Comp Biol*. 2016;56:644-656.
57. Kamikawa-Miyado M, Ogi H, Ogino Y, et al. The morphological and histological characters of the male external genitalia of the house musk shrew. *Suncus murinus*. *Zoolog Sci*. 2005;22:463-468.
58. Chen S, Tao J, Bae Y, et al. Notch gain of function inhibits chondrocyte differentiation via Rbpj-dependent suppression of Sox9. *J Bone Miner Res*. 2013;28:649-659.
59. Watanabe N, Tezuka Y, Matsuno K, et al. Suppression of differentiation and proliferation of early chondrogenic cells by Notch. *J Bone Miner Metab*. 2003;21:344-352.
60. Hardingham TE, Oldershaw RA, Tew SR. Cartilage, SOX9 and Notch signals in chondrogenesis. *J Anat*. 2006;209:469-480.
61. Tian Y, Xu Y, Fu Q, et al. Notch inhibits chondrogenic differentiation of mesenchymal progenitor cells by targeting Twist1. *Mol Cell Endocrinol*. 2015;403:30-38.
62. Castel D, Mourikis P, Bartels SJ, Brinkman AB, Tajbakhsh S, Stunnenberg HG. Dynamic binding of RBPJ is determined by Notch signaling status. *Genes Dev*. 2013;27:1059-1071.
63. Wu X, Zhou Q, Huang L, et al. Ageing-exaggerated proliferation of vascular smooth muscle cells is related to attenuation of Jagged1 expression in endothelial cells. *Cardiovasc Res*. 2008;77:800-808.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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