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Exploration of differentiation standard for primo through the histological common point of threadlike structure found in rats and swine

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ABSTRACT

Background: The novel threadlike structure is called the primo structure, and studies are conducted through many different approaches. Although various ways of differentiation are currently used, a standard for differentiation is deemed necessary in order to identify the primo structure based on the overall form of the structure. This study aims to explore the differentiation standard through the histological common point of the threadlike structure of rat and swine by using the hematoxylin and eosin (H&E) staining method commonly used for histological research in various structures.

Methods: An 8-week old Sprague-Dawley rat and a Yorkshire pig weighing 30–40 kg were used. A total of 65 pieces of rat threadlike structure and 100 pieces of swine threadlike structure were collected after the abdomen was cut and opened. The following three different characteristics were confirmed using the H&E staining method for the collected structures: (1) bright cell availability; (2) cavity availability; and (3) nuclei density.

Results: For the rat threadlike structure, the bright cell (70.5%) and nuclei density (92.6%) were mainly observed; in the swine threadlike structure, however, the bright cell (60.6%) and cavity (67.2%) were mainly observed. The bright cell was confirmed to have been observed in the threadlike structures of both rat and swine.

Conclusion: The bright cell is determined to be the common point in the primo structure. However, further research is deemed necessary in the future as to the functions performed by the characteristic shown by the Primo structure.

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1. Introduction

The Bonghan system or threadlike structure, which is called the primo vascular system, has been suggested as the third circulatory system. To study the primo vascular system as a new tissue, specific morphological and molecular biological indices should be suggested. In particular, primo tissues tend to be distributed atypically compared to other tissues; thus, they are less likely to be present in certain locations and are found less frequently. The specific characteristics of primo tissues are seldom identified as well.

Lee et al[1] visualized primo nodes and primo vessels in the sinuses of rat brain using chromium-hematoxylin (Cr–Hx) solution, which was then suggested as a method for primo visualization that can be distinguished from the blood clots developed during surgery. Lim et al[2] suggested that the rat organ-surface primo vessel can be useful in identifying the primo tissues by morphologically observing with Hemacolor staining followed by simple light microscope for a brief time.

Hematoxylin and eosin (H&E) stain is a commonly used staining method to identify the morphological characteristics in various tissues; hematoxylin stains mainly the cell nucleus and cell membranes, whereas eosin stains the cytoplasm, enabling observing the overall morphology of the cell. With this method, the experimental approach is easy, and the database for most human tissues is already established; hence the process of distinguishing various similar tissues is simplified. As such, the histological differentiation of primo tissues using H&E stain should be conducted prior to other studies.

To date, the specific characteristics of the primo tissue were identified by comparing the primo tissue and similar tissues in studies in various animals including mouse,[3] rat,[4] rabbit,[5] canine,[6] and swine.[7] However the comparison of morphological characteristics between primo tissues observed in different species has not been reported. If the same anatomical and histological characteristics are observed in threadlike structures found in various species of animals, they may be the same tissues possibly found in nonexperimented animals and even in humans. Studies on medium and large-sized animals are insufficient compared to those on mice and rats, with a relatively large amount of study results accumulated. Some studies were conducted on swine and canine primo tissues, but the reliability and reproducibility of those studies leave something to be desired because the studies were not followed up due to the lack of laboratory facilities for medium and large-sized animals, aside from the lack of data itself for reference. Therefore, there is a need to accumulate and analyze study results involving medium and large-sized animals for the comparative study of primo tissues in various species.

In this study, rat threadlike structures were histologically analyzed using H&E staining, which is commonly and widely used in histology. On the basis of such analysis, the histological characteristics of swine threadlike structures were compared. Based on these results, the major differentiation criteria for threadlike structures observed in different animals were considered.

2. Methods

2.1. Rat

2.1.1. Experimental animal

About 8–9 weeks old Sprague-Dawley rats ($n = 100$; DBL, Korea) weighing 250–320 g were used in the experiment. Animals were bred at $22 \pm 1\,^{\circ}C$, $55 \pm 10\%$ relative humidity, and 12-hour dark/light cycle. Animals had free access to feed and water during the acclimation period and experimental period, but only water was provided 24 hours before the operation.

2.1.2. Operation and tissue collection

General anesthesia was performed by injecting 20% urethane (Sigma-Aldrich, USA) 1.5 g/kg intramuscularly. The abdomen was cut open by incising along the linea alba to avoid bleeding. During the operation, the drying of the organ surface was prevented by dropping phosphate-buffered saline (PBS, pH7.4) maintained in the water bath at $40\,^{\circ}C$.

The surface of the organ was observed using stereoscopic microscope (SMZ1500, Nikon Japan); milky-white transparent mass or glandular tissues were stained with 0.4% trypan blue (Sigma-Aldrich, USA) and immediately washed with PBS, with the connection of stained features and surrounding tissues subsequently identified before collecting tissues. We used only the vessels of the threadlike structure. The collected tissues were immediately fixed in 10% neutral buffered formalin (NBF) for 24 hours.

2.1.3. Tissue treatment and staining

The fixed tissues were frozen-sectioned at 8 μm thickness and applied to H&E, Masson's trichrome, and immunohistochemistry staining methods. For Masson's trichrome stain, the mesentery was used as positive control and fibrin as negative control. Tissues other than the mesentery were tested by immunohistochemistry. The LYVE1(Abcam) antibody was used for distinguishing from lymphatic vessels, and the CD31(Abcam) antibody, for distinguishing from blood vessels. It was identified using the von Willebrand factor (Abcam) antibody, which shows positive response in the primo tissue.[8]

A total of 65 tissues were collected based on Masson's trichrome stain and the immunohistochemistry results. The basic structures of the cell nucleus and cytoplasm of the collected tissues were observed through H & E stain.

2.1.4. Tissue differentiation

Based on the H&E stain results and references,[9,10] tissues were differentiated by three tissue characteristics. Specifically, primo tissues were differentiated by the presence of cavity, presence of bright cells, and crowding of nuclei. The cavity, which is made of membranes of elastic fiber, was identified by the shape of the holes of epithelial cells (Fig. 1. R-6); the bright cells were detected by observing cells wherein the cytoplasm was not stained by eosin and the nucleus in the center was stained by hematoxylin (Fig. 1. R-2). The degree of crowding nuclei was based on the observation of more than 30 nuclei in a 50 μm × 50 μm area (Fig. 1. R-5).