원 저

In-Situ Observation of New Extra-Vascular Threadlike Structure of Mouse Using a Fluorescence Stereoscopic Microscope

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Abstract

We report the new threadlike structures outside the blood vessels of mice. For this, we developed an in-situ searching method of the structure by vital staining with the dye of acridine orange and using a fluorescent stereomicroscope designed specifically for this purpose. We consider that the newly found threadlike structure might be rediscovery of the extra-vascular Bonghan duct which was reported in 1963 by Bonghan Kim.

Key words : in-situ, fluorescence stereoscopic microscope, acridine orange, threadlike structure, extra-vascular Bonghan duct

INTRODUCTION

Bonghan Kim claimed in 1960s that there existed threadlike duct structures, so called Bonghan ducts which formed a new circulatory network throughout the body including those inside the blood vessels. However, no others have clearly confirmed his epoch-making discovery except by Fujiwara[1-3]. It is widely accepted in anatomy that there is no threadlike structure afloat running along inside blood vessels. Despite such prevailing knowledge we recently found a novel threadlike structure inside of the blood vessel of rat and rabbits[4-12]. The threadlike structure which we observed might be the rediscovery of the intra-vascular Bonghan duct[13].

In this article, we report on finding of the new threadlike structures at the outside the blood vessel of a mouse. We developed the method to search extra-vascular threadlike structures. The inside body of a mouse was vital stained by the fluorescent dye of acridine orange and in-situ observation was performed to detect the extra-vascular threadlike structure under a fluorescent stereomicroscope. We set up the fluorescent stereomicroscope by attaching the light source of a miniature optical fiber tip which was designed for our experimental purpose to a usual stereo-microscope. The delicately controllable localized illumination through the miniature optical fiber tip had more efficient illuminating effects than epi-fluorescence illumination through the objective when we investigated the very complex shaped three-dimensional live subjects.

In the next section we present the detailed method and procedure to investigate the extra-vascular threadlike structure. The images of microscopes will be presented in the result section, and further research directions will be given in the discussion section.

METHOD

The inside bodies of mice were stained by the fluorescent dye of acridine orange through femoral vein injection and in-vivo observation was performed to seek external Bonghan-ducts under a fluorescent stereo microscope. The acridine orange selectively stains DNA in the tissue and emits the green light of 520nm when it is excited by blue light of 487nm.

We set up the fluorescent stereomicroscope by attaching the light source designed for our experimental purpose to a usual stereo microscope. **Fig. 1** is the schematic of the constructed fluorescent stereomicroscope. The zooming stereomicroscope multiplies the image of a sample by from 10X to 200X. The excitation light source consists of a mercury arc lamp of 200W and a bandpass filter of which passing wavelength is from 450nm to 490nm. The emission filter is attached to the side of objective to pass the fluorescent emitting green light from the sample through the objective but to reject the excitation light scattered from the sample. The emission filter is the long wavelength pass filter of which pass-on wavelength is 515nm and rising edge interval is less than 10nm. The excitation light from the source is guided to the sample by the optical glass fiber bundle and coupling optics. The optical fiber bundle of 3mm diameter is covered by the stainless steel of 0.5mm thick to be the stiff tip of 60mm length at the side of sample. The tip is mounted on the micro-manipulator in order to control the direction and position of the illuminating light. The delicately controllable localized illumination through the miniature optical fiber tip has more efficient illuminating effects than epi-fluorescence illumination through the objective when we investigate the very complex shaped three-dimensional in-vivo samples.

The samples for in-situ in-vivo investigation were prepared as follows. ICR mice of 4 weeks that were obtained from the Laboratory Animal Center of Seoul National University were used. Procedures involving animals and their care conformed with the institutional guidelines, which are in full compliance with current international laws and policies.(NIH guide for the Care and Use of Laboratory Animals, NIH Publication NO. 85-23, 1985)

A mouse was anesthetized with urethane(0.2mg/g) administrated intraperitoneally and all surgical procedures were performed under deep anesthesia. The 0.1% solution of acridine orange(0.02mL/g) was infused into the body of the mouse through the femoral vein. The frontal side of the mouse was incised and stomach intestines were put aside. The perivascular fats were removed such that vascular systems were exposed for easy approach.

RESULTS

In an hour after infusing the acridine orange to the femoral vein, many green lines inside body are observed under the fluorescent stereomicroscope. Among them, we concentrated the bright green lines which run along the spermatic artery. **Fig. 2** shows them. The bright field image of a part of spermatic artery around the middle abdomen is shown in(**A**) and its corresponding fluorescent image in(**B**). Two bright green lines clearly appeared just near the both sides of spermatic artery. Their widths are about 100μm. Observing the lines microscopically, we found threadlike structures in them. **Fig. 3** shows the microscopic images of the threadlike structure which was separated from its original tissue with the spermatic artery : a differential interference contrast microscopic image in(**A**), a fluorescence microscopic image in(**B**), a section of confocal laser scanning microscopic images in(**C**). In all of the three images, we could easily discern the two different parts of the sample, i.e., spermatic artery tissue(left of the sample) and threadlike structure tissue(right of the sample). The widths of the threadlike structures were about 20~50μm. As shown in(**B**) and(**C**) of **Fig. 3** the nuclei of the threadlike structure were of the rod-shape and were regularly aligned to form broken lines of striped pattern. A nucleus size was about 5μm$\times$20μm and the interval between nuclei was 10~15μm.

DISCUSSION

Through the vital staining of acridine orange and observing under a fluorescent stereomicroscope, we could report the first time the threadlike structures outside the blood vessel

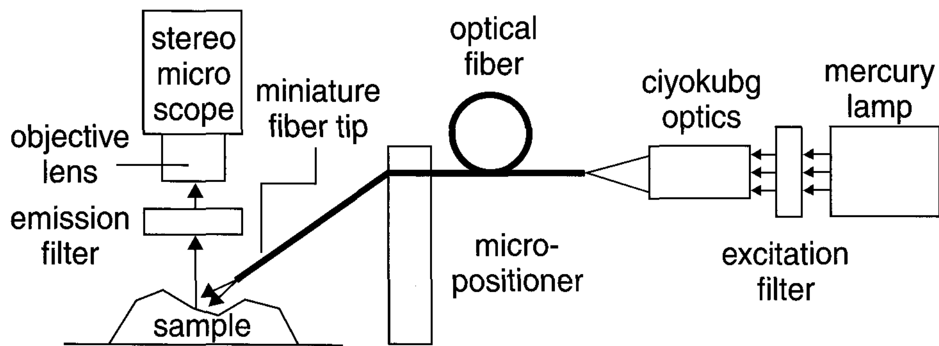

Fig. 1 Schematics of Fluorescence Stereoscopic Microscope(FSM)

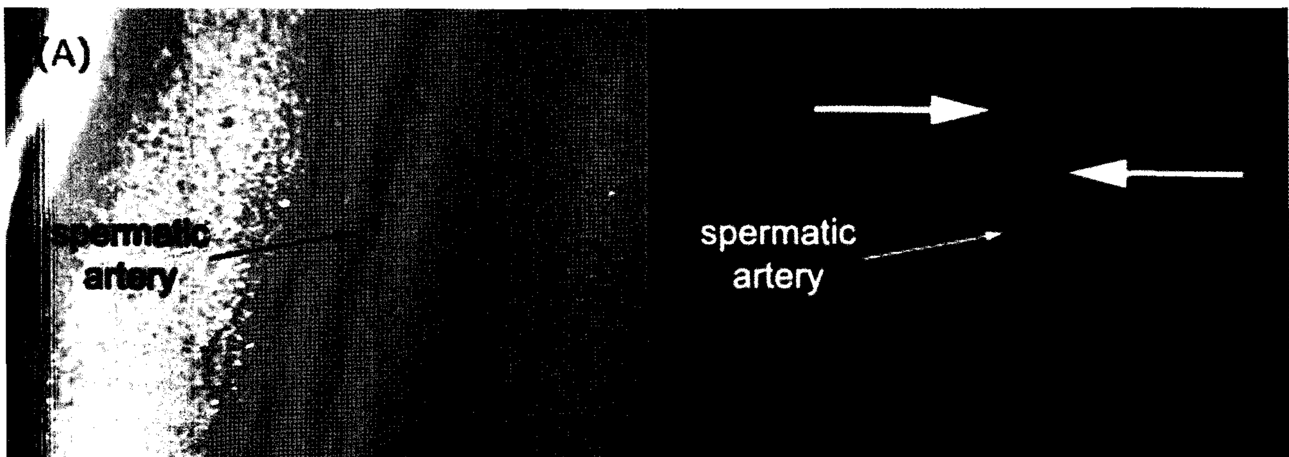

Fig. 2 In-Situ Stereoscopic Images of Extra-Vascular Bonghan Ducts(EVBHD)

(A) Original unfiltered image. The dark line in center of the image is the spermatic artery.

(B) Fluorescence image. The EVBHDs are shown by thick arrows. Both scale bars represent 100μm

Fig. 3 Microscopic Images of Extra-Vascular Bonghan Ducts(EVBHD)

(A) Differential interference contrast image of EVBHD. The scale bar represents 40μm.

(B) Fluorescence image of EVBHD. The scale bar represents 40μm.

(C) Confocal laser scanning microscopic image of EVBHD and spermatic artery. The nuclear arrangement structures of EVBHD and the artery are clearly discernable from each other. The scale bar represents 20μm.

near at the spermatic artery of a mouse. The nucleic morphology of the structure was very similar to those of the intra-vascular threadlike structures of the rats and rabbits[7-8] but different from those of capillaries, nerve fibers and lymphatic ducts. The morphology of the structure highly corresponded to that of the Bonghan duct described by Bonghan Kim[13]. Thus we guess that the threadlike structures reported here is possibly the extra-vascular Bonghan duct. According to Bonghan Kim the network of Bonghan duct consists of intra-vascular ducts, extra-vascular ducts, and superficial ducts under skin. The superficial ducts are the anatomical basis of the acupuncture meridians. However, the Bonghan ducts have not been confirmed yet because the observing method was not known. The nuclei shape and distribution pattern of the threadlike structures are found to be similar regardless of species such as a mouse, rat, and rabbit. The threadlike structures mentioned above may be common to all vertebrates.

Nevertheless, as there are much collagen fibers and elastic fibers around blood vessels, it will be necessary to observe the structure reprted here through a trasmission electron microscope in order to prove whether it is a duct or a cord. Furthermore, it is necessary to find how the structure is related to the acupuncture meridians and collaterals.

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