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RESEARCH ARTICLE

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The Origin of Endothelial Cells in Novel Structures, Bonghan Ducts and Bonghan Corpuscles Determined Using Immunofluorescence

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Abstract
Bonghan ducts (BHDs), and their associated Bonghan corpuscles (BHCs), which are novel threadlike structures, were recently observed in rats and rabbits by using various methods. As further support for the putative circulatory function of the novel threadlike structures (NTS), we investigated the presence and the origin of the endothelial cells within these structures. We immunostained the NTS with anti-CD146, an endothelial cell marker, and with anti-podoplanin, a lymphatic cell marker. Positive expression of CD146 in the BHDs was obtained, and the distribution of endothelial cells showed that the inner boundaries of the channels in the subducts branched from the BHDs and curled around, in a complicated manner, inside a BHCs. The negative expression of podoplanin implies that the endothelial cells in the BHDs are likely to be of vascular and not of lymphatic origin.

1. Introduction

Although there have been many attempts to elucidate the physical basis of acupuncture points [1–3], the identification of anatomical structures corresponding to acupoints and meridians has not yet been achieved. The only clear anatomical claim was made by Bonghan Kim in the early 1960s [4], and Fujiwara subsequently confirmed part of the Bonghan theory [5]. After being neglected for some time, new interest in Bonghan theory has been aroused by recent developments in methods to observe and identify Bonghan ducts (BHDs). BHDs are novel threadlike structures (NTS) in blood vessels [6], lymphatic vessels [7,8], and in the brain ventricles of rabbits and rats [9]. BHDs and Bonghan corpuscles (BHCs) have also been observed on the surfaces of various mammalian internal organs by three independent groups of researchers [10–12].

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The BHDs on the surfaces of internal organs are thin, semi-transparent, freely-movable strands [12] and have intermittent thickened parts called BHCs. The ultra-structures of BHDs and BHCs were studied by using scanning transmission electron microscopes [13]. The BHD has a bundle structures that consists of many subducts, with each subduct having a diameter of about 10 μm. Through these subducts, liquid flows at a slow rate, measured at 0.3 ± 0.1 mm/sec [14].

According to Kim, the network of BHDs connects to form a circulatory system [4], an observation strongly supported by the flow of liquid. The presence of endothelial cells in the BHD would be another critical factor in identifying the BHD as part of a circulatory system, but no investigations have been conducted to identify and characterize these endothelial cells. In the present work, we found CD146 and podoplanin antibodies to be effective for this purpose. CD146, which is involved in the control of endothelial cohesion and permeability, is currently used as a marker for endothelial cell lineage [15–18]. CD146 is located at endothelial junctions but outside adherent junctions [16]. Podoplanin is a lymphatic endothelial cell marker that is highly expressed in proliferating lymphatic endothelial cells [19,20]. Podoplanin expression has been reported in the choroid plexus in rat brain and in the ciliary epithelium of the rat eye [21]. Schacht and colleagues reported podoplanin in salivary gland myoepithelial cells and testicular fibromyocytes, as well as in many other types of cells [20].

The NTS are thought to exist and are presumably found in the body (as shown by previous studies), however, no one has investigated whether the NTS originate from vascular or lymphatic vessels. Therefore, we used CD146 and podoplanin antibodies to determine whether the endothelial cells in the BHDs are of vascular or lymphatic origin.

2. Materials and Methods

2.1. Animal preparation and surgical procedures

New Zealand White female rabbits (1.5 kg) were used in this study. The animals were housed at an appropriate temperature (23ºC), with a controlled humidity of 60%, a 12 hour light and 12 hour dark cycle, with free access to food and water. The procedures for handling and caring for the animals complied with the guidelines of current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). The rabbits were anesthetized with intraperitoneal administered urethane (1.5 g/kg/5 mL), and all surgical procedures were performed under general anesthesia. The abdominal wall was dissected under deep anesthesia. The hair of the abdominal region was removed before surgery by using clippers, and hemostasis was performed on the large skin vessels around the incision in order to avoid contaminating the abdominal and thoracic surfaces with blood.

The BHDs and the BHCs on the organ surfaces were identified using surgical instruments, such as iris scissors and microforceps, under a stereoscopic microscope (Olympus, Japan). The images of the threadlike structures were obtained with a digital camera (Olympus, Japan).

2.2. Histological findings

The threadlike structures were fixed in a 4% paraformaldehyde solution for 3 hours for histological procedures. The overall morphology of the BHDs was determined by examining 10 μm tissue sections cut using a cryotome (Leica CM1850, Germany) and stained with hematoxylin and eosin (HE; Sigma, Steinheim, Germany). Sequential HE stainings were performed with adjacent immunofluorescence slides. Frozen 10 μm sections of BHDs, including BHCs, were pretreated with phosphate-buffered saline (PBS) and blocked with 5% normal horse serum for 30 minutes. The abdominal aorta and the lymphatic vessels in the portal area of the rabbit were used as positive controls. Samples were incubated overnight with anti-CD146 (diluted 1:500, Chemicon, USA) and anti-podoplanin (diluted 1:500, Abcam, Cambridge, UK) antibodies at room temperature. The tissues were exposed to FITC-conjugated anti-mouse IgG at a 1:200 dilution (Jackson Immunoresearch Lab, PA, USA) for 2 hours at room temperature for anti-CD146 antibodies. Tissue used for podoplanin studies were exposed to Cy3-labelled goat anti-mouse IgG at a 1:200 dilution (Amersham Biosciences, UK) for 2 hours at room temperature. Incubation of tissue in mouse IgG, at the same concentration as that used for primary antibodies, created negative controls for immunofluorescence. The negative controls had no immunoreactivity in the identified structures. After the final wash, the samples were mounted using the VECTERSHIELD™ fluorescence system with 4′,6-diamidino-2-phenylindole (DAPI) mounting medium.

3. Results

3.1. Novel threadlike structure

NTSs had a thick corpuscle-like body called a BHC, as shown in the dotted circle in Figure 1A. The BHC is connected by BHDs at both ends, as indicated by