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BRIEF REPORT

Bonghan System as Mesenchymal Stem Cell Niches and Pathways of Macrophages in Adipose Tissues

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Abstract
A new technique for visualizing Bonghan ducts (BHDs) and Bonghan corpuscles (BHCs) was developed by using a vivi-staining dye, Trypan blue. The dye stains BHDs and BHCs preferentially to adipocytes so that tracking a BHD and a BHC, even inside adipose tissues, is possible. Concerning the functions of the BHD and the BHC in adipose tissues, we propose conjectures: the Bonghan system may be niches for mesenchymal stem cells, which can differentiate into adipocytes, and pathways for macrophages involved in adipogenesis.

Obesity is a major health problem in developed countries as the cause of diabetes or cardiovascular disorders. Adipose tissues are mainly composed of adipocytes, and their genesis and growth are subjects of wide interest [1,2]. In this brief report, we point out that novel threadlike and corpuscular structures, so-called Bonghan ducts (BHDs) and Bonghan corpuscles (BHCs), which are yet unknown to most researchers in this field, are present in adipose tissues. A technique for visualizing these novel structures in adipose tissues was only recently developed and is introduced for the first time in this brief report. Considering their possible physiological roles of these novel structures in connection with tissue regeneration we propose a conjecture that the Bonghan system is a mesenchymal stem cell (MSC) niche which provides adipogenesis and can possibly be regulated by using acupuncture. Another conjecture is that the BHD is a pathway for macrophages, which are closely related to adipogenesis and to acupuncture. Thus, we propose two hypotheses that the Bonghan system in adipose tissues functions as MSC niches and that the BHDs are macrophage pathways.

The recent rediscovery of the Bonghan system, which was claimed by Bongh Han Kim to be the anatomical structure corresponding to the acupuncture points and meridians in the early 1960s [3] has revitalized related research: intravascular BHD in blood vessels [4–6] and lymphatic vessels [7–9], in brain ventricles and the central canals of spines [10], and on the surfaces of internal organs [11–13]. Investigations aiming to elucidate the details of the anatomy and the morphology of the BHD have been performed by using confocal laser scanning microscopy [13],

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various electron microscopies [14,15], and an immunohistochemical technique [16]. The circulatory function of the BHD was investigated by injecting Alcian blue [15]. Catecholamine hormone flow in the BHD was also confirmed [17]. In the tracking of the BHD on the surfaces of internal organs, we often get lost when the BHD enters adipose tissue. In this brief report, we present a new visualizing dye, Trypan blue, that stains the BHD but not adipose tissues, thus making the BHD visible even inside adipose tissues.

Sprague-Dawley rats, 250 g, were housed at a constant temperature (23°C) and humidity (60%). The rats were anesthetized with urethane (1.5 g/kg) administered intraperitoneally, and all surgical procedures were performed under general anesthesia. The procedures involving the animals and their care conformed to institutional guidelines, which were in full compliance with current international laws and policies.

The staining was done with 0.4% Trypan blue (Sigma Aldrich, USA) by spreading and washing with phosphate-buffered saline (PBS). Trypan blue is used as a vivi-staining dye, for example, of vitereoretinal membranes in ophthalmic surgery [18]. The observations of the BHD were done with a stereomicroscope (SZX12, Olympus).

The BHDs often entered the adipose tissues present around internal organs, so we were not able to trace them into adipose tissues because the BHDs were not visible. Figure 1 shows a BHC and a BHD that became blue due to the Alcian blue that had been injected into the femoral vein of a rat and that later appeared in the BHC and the BHD by flowing into them through some unknown paths [9]. Notice that the BHD entered the adipose tissue and disappeared from view.

Using Trypan blue, we were able to visualize the BHD inside the adipose tissue because the BHD is stained preferentially compared with adipose tissues or blood vessels. In Figure 2A, a BHD and its connected corpuscle (arrow) were stained blue inside the adipose tissue around the omentum near the stomach. Figure 2B shows two BHDs (arrows) and a BHC (thick arrow) in adipose tissue around the small

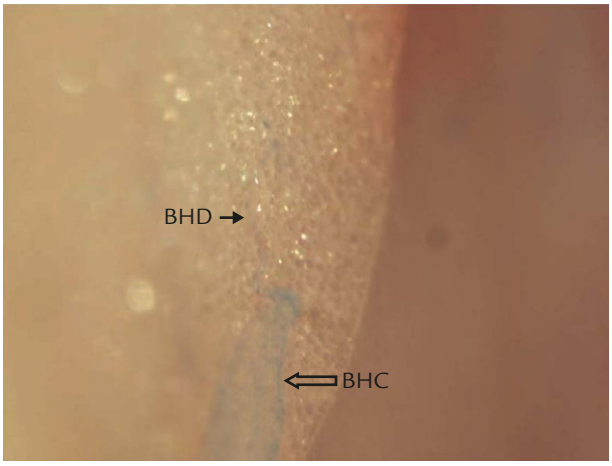

Figure 1 A Bonghan corpuscle (BHC) and Bonghan duct (BHD) on adipose tissue around the small intestine of a rat. Alcian blue that flowed in the BHD and the BHC left them blue. Notice that the BHD entered the adipose tissue, so further *in-situ* tracking of the BHD was not possible.

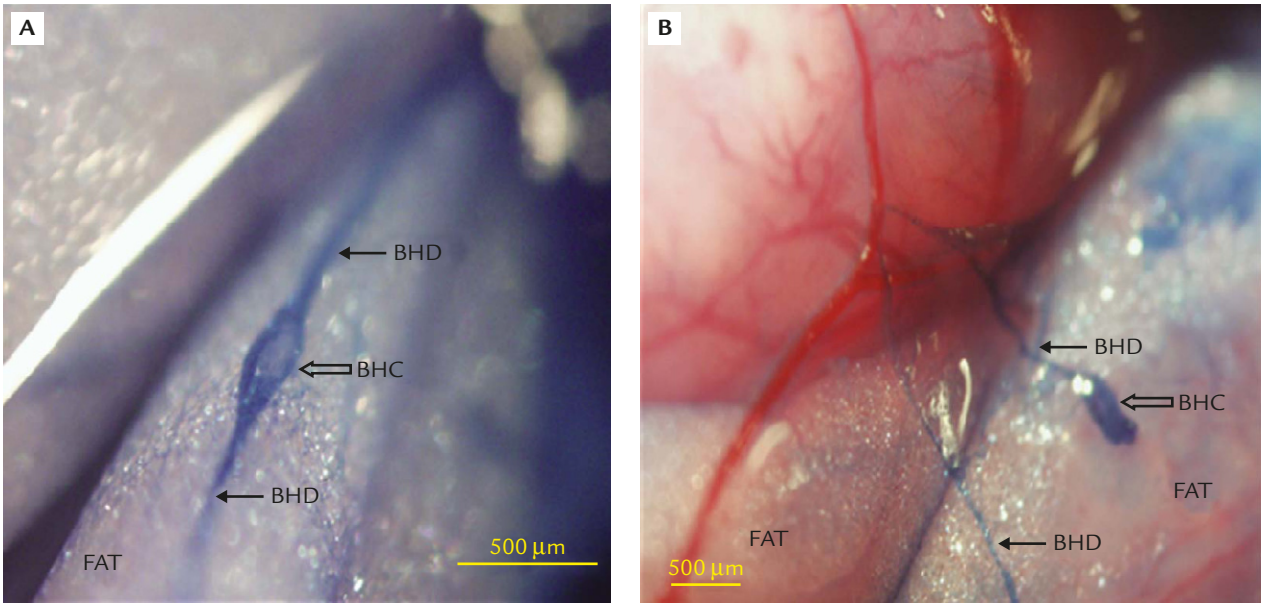

Figure 2 Trypan blue staining of a Bonghan duct (BHD) and a Bonghan corpuscle (BHC) inside adipose tissues. (A) A BHC and connected BHD inside adipose tissue around the small intestine of a rat. (B) A BHC and two BHDs near a small intestine of the same rat. Notice that blood vessels and adipose tissues were not stained.

intestine of a rat. Verification of these threadlike structures as parts of the Bonghan system by using a histological examination will be given elsewhere.

We propose two hypotheses on the possible roles of the BHC and the BHD in adipose tissues. The first hypothesis is that the BHC may be a MSC niche. The presence of MSCs in the BHC on the surfaces of the internal organs of rats was indicated by the expression of Integrin beta 1, collagen type 1, and fibronectin [19]. In addition, in a proteomic analysis of the BHD, the existence of proteins related to the recruitment of MSCs, the cell process in MSC, and to MSC/myofibroblast differentiation was seen in the protein profile from the BHD of a rabbit [20]. Considering these immunohistochemical and proteomics data, we conjecture that the BHC in adipose tissues may be a MSC niche. MSCs can differentiate into a variety of cell lineages [21,22], and adipogenesis is of particular interest among their diverse differentiation possibilities [23,24]. In the research on stem cells in connection with adipose tissues, such as adipocyte-derived stem cells, one should pay more attention to the BHD and the BHC as novel structures.

The second hypothesis is that the BHD may be a pathway for macrophages secreting angiogenic factors and cytokines. As reported by Cho et al. [25], these macrophages infiltrate in large numbers the tip portion of the dense microvasculature in adult adipose tissue of mice, suggesting that macrophages might be involved in the formation of vascular plexuses and in adipogenesis. Thus, macrophage-assisted angiogenesis precedes adipogenesis/lipogenesis in the tip of adult epididymal adipose tissue. Since we found an abundant presence of macrophages in the BHD [14], we naturally hypothesized that, in addition to the circulation in the blood, the BHD might be an unobserved path for the macrophages. We notice the close relation between macrophages and acupuncture for which improved immune function and beneficial effects on inflammation are often cited [26]. There is a report on the abundance of mast cells, typical immune cells, at acupuncture points [27]. Macrophages are known to have a deep connection with inflammation through the $\alpha 7$ receptor and acetylcholine, which presents a possible explanation for the therapeutic effects of acupuncture on inflammation [28]. In conclusion, the novel circulatory system consisting of the BHD and the BHC in adipose tissues is worth investigating in connection with MSCs, macrophages, and adipogenesis.

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