D OI:10.3831 | KPI.2008.11.1.005

원 저

줄기세포의 가능한 원천으로서의 장기표면 봉한소체

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목적 : 봉한소체가 성체줄기세포의 원천이며, 봉한관이 줄기세포 수송로일 가능성을 확인함.
방법 : 쥐의 내부 장기표면에서 봉한소체와 봉한관을 채취했다. 다양한 줄기세포 표지항체를 써서 면역조직학적 분석을 했다.
결과 : mesenchymal 줄기세포에 관한 Integrin β1, Collagen type 1, Fibronectin의 강한 발현을 확인했다. CD54는 발현되지 않았다. 조혈줄기세포에 관련하여 Thy 1의 발현이 있었다.
결론 : 골수조직과 유사하게 mesenchymal과 조혈줄기세포의 표지가 BHC에서 확인되었고, 봉한관에서는 vWF가 발현되어 줄기세포 수송로 가능성을 확인했다.

Bong-Han Corpuscles as Possible Stem Cell Niches on the Organ-Surfaces

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ABSTRACT

Objectives Showing that Bong-Han corpuscles(BHC) are suppliers of the stem cells in adulthood, and the Bong-Han ducts(BHD) are transportation routes of stem cells.

Methods BHC and BHD were obtained from the internal organ-surfaces of rats. The sliced BHC and BHD were immunostained with various stem cell markers. Extracellular matrices were also analyzed by immunohistochemistry.

Result The presence of mesenchymal stem cells was confirmed by the expression of Integrin beta 1, Collagen type 1 and Fibronectin. But CD54 was not expressed. The hematopoietic stem cell marker, Thy 1 was strongly expressed. BHDs showed Collagen type 1, Fibronectin, and vWF expression.

Conclusion Both hematopoietic and mesenchymal stem cell markers were expressed strongly in BHC similarly as in bone marrow. An endothelial cell marker(vWF) demonstrated the possibility of the stem cell transportation routes of BHD.

key words *hematopoietic stem cell, mesenchymal stem cell, Bonghan corpuscle, endothelial cell, extracellular matrix*

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Novel Stem Cell Niches

Ⅰ. Introduction

The hematopoietic stem cell(HSC) is presently the best characterized multipotent stem cell population, and was isolated from mouse bone marrow(BM) in 1988[1,2]. Multipotent marrow stromal cells, which give rise to multiple mesenchymal lineages, can also be isolated from mouse BM[3-5]. Some studies have suggested that brain or muscle-derived stem cells may harbor hematopoietic potential[6,7]. Even though these may not be genuine sources of HSC, being instead derived from circulating HSC, it encourages the search for non-BM sources of HSC or mesenchymal stem cells. In this article we report on the identification of a novel HSC niche at the surface of internal organs in the form of corpuscle-like structures.

Interest on these corpuscle-like structures with their associated threadlike ducts at the surface of the internal organs of rabbits, rats, and mice was only recently revived[8-10]. These structures are called BHC and BHD after their first discoverer[11]. Except for some early confirmations by Fujiwara[12], they have been neglected for a long time, partly because their function was obscure and thus seemed not worthy of any attention. Our investigation into their morphology using scanning and transmission electron microscopes revealed the presence of many immunological cells like macrophages, mast cells, and eosinophils. In addition, the distribution of the extracellular matrix in the BHC looked similar to that in immune organs like the spleen or lymph nodes[13]. Furthermore, there were bundles of sub-ducts in the BHD[10], which suggested a novel circulatory function of the BHD.

According to the reports by Bonghan Kim[14] the BHC/D was a part of the acupunctural circulation system that could enhance immunological cell-therapy and hematopoietic functions after acupuncture treatments. Thus we hypothesized that the BHCs are supplier of some stem cells in adulthood, and the BHDs are the transportation routes of the stem cells. As a first step towards verifying this hypothesis, the sliced BHC and BHD were stained with various stem cell markers. Mesenchymal and hematopoietic stem cell markers were very clearly detected, but neither pluripotent nor neural stem cell markers were expressed. The expressions for the endothelial cell and extracellular matrixes were consistent with this result.

Even though this work is only in the introductory stages of the identification of a new stem cell niche, it may herald the physiological significance of the hitherto ignored corpuscular and threadlike Bonghan systems.

Ⅱ. Materials and Methods

Animals and sample preparation

Wistar rats of about 200g(males, 6 weeks old) were obtained from Jung Ang Laboratory Animal Company. The animals were housed in a constant-temperature controlled environment(23℃) with 60% relative humidity under a 12-h light/dark cycle. All of the animals had ad-libitum access to food and water. The procedures involving the animals and their care were in full compliance with the Seoul National University's Guidelines for Animal experiments. The rats were anesthetized with urethane(1.5g/kg) administered intraperitione and all surgical procedures were performed under general anesthesia. The abdominal sides of the rats were incised. As in previous works[10] on threadlike structures on internal organs of rabbits and rats, we examined the surfaces of the small intestines, livers, and bladder of rats under a stereomicroscope. The threadlike structures and the

connected corpuscles on internal organs were taken out with fine forceps under stereomicroscopy.

Immunohistochemistry

In order to identify the major cell populations that construct the BHC and BHD, isolated rat BHC/D were sectioned using a cryostat and thaw mounted onto gelatin coated glass slides and then fixed with pre-cooled acetone for 2 min. Blocking was performed with 10% normal goat serum for 1h followed by incubation with various cell lineage marker(especially stem cell and endothelial cell marker) primary antibodies overnight. The antibody was visualized using an Alexa555-labeled or Alexa488-labeled secondary antibody. The slides were cover slipped with DAPI containing fade-retardant media and examined using a Carl Ziess fluorescence microscope.

Ⅲ. Results

BHCs and its associated ducts were repeatedly observed on bladder surface(Fig. 1a). For in situ visualization of the organ surface BHCs and their associated ducts, they were stained by Alcian blue solution and showed the bright blue color on their surface which is regarded as membrane of BHC(Fig. 1b-c). Isolated Bonghan structure has corpuscle which is 10 times thicker than its associated ducts with respect to diameter (minor axis 419㎛, major axis 912 ㎛)(Fig. 1d-g).

To know the tissue structure and cellular composition, the isolated organ surface BHCs were stained with various stem cell and extracellular matrix specific antibodies. To identify presence of mesenchymal stem cells, the expression of CD54, Integrin beta1, Collagen type1, Fibronectin in the BHC was analyzed. Integrin beta1, Collagen type1, Fibronectin were clearly expressed, but CD54 was not expressed(Fig. 2). Integrin beta1 was highly expressed in the fibronectin enclosed cells. The fact that collagen type 1 was detected as bright spots shows that collagen type1 existed as fibers which extended from head to tail in BHC. As a hematopoietic stem cell marker, Thy1 antibody was used. Thy1 positive cells were as many as integrin beta1 positive cells, but there were no co-expressing cells(Fig. 2). And the regions where these two markers expressed were

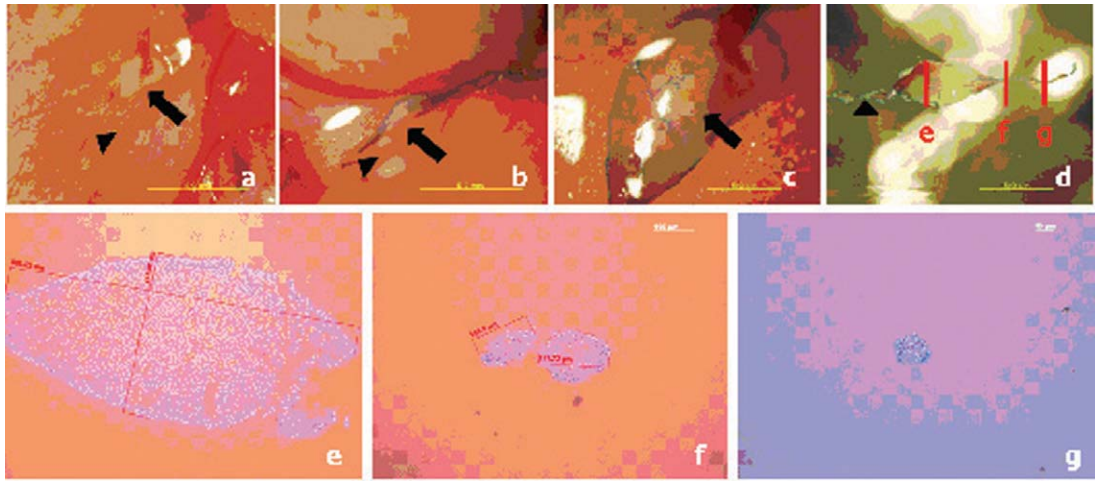

Fig. 1. Isolation of organ surface Bonghan structure. Large size OSBHC(arrow) is detected on bladder surface(B) of Wistar rat(a). OSBHC(arrow) and connected OSBHD(arrowhead) are stained by Alcian blue(b, c). Isolated OSBHC/D was serially sliced to know the inner structure(d). Each part of the slices were stained with DAPI and measured their size with respect to diameter(e cf f, g).

clearly separated. Other markers(SSEA1, Tra1, Nestin, sox2, Glycopropin A) were tested but not detected (data not shown).

In case of organ surface BHCs associated ducts, they had much of collagen type1 and Fibronectin, but there were no Thy1 positive cells. BHD showed dull signal of integrin beta1(Fig. 3a-b, e-f, i-j). When the expression of endothelial cell marker vWF was examined, the high level of expression was detected in both of the organ surface BHCs and BHD.(Fig. 3c-d, g-h, k-l).

To characterize organ surface BHD, more fully several endothelial cell markers were tested for BHD from various organ surfaces. There was no consistency between different BHDs, but all of the BHDs showed high level of vWF expression. And some regions which were close to BHCs(a,c)-and therefore thicker than others-expressed small amount of Reca1 and Flk1(Fig. 4).

Ⅳ. Discussion

In order to establish the unique nature of the BHC/D and to elucidate their detailed structure we have been performing a series of investigations on: (1)The anatomy and basic histology, (2)Their ultrastructure using electron microscopy, (3)Their

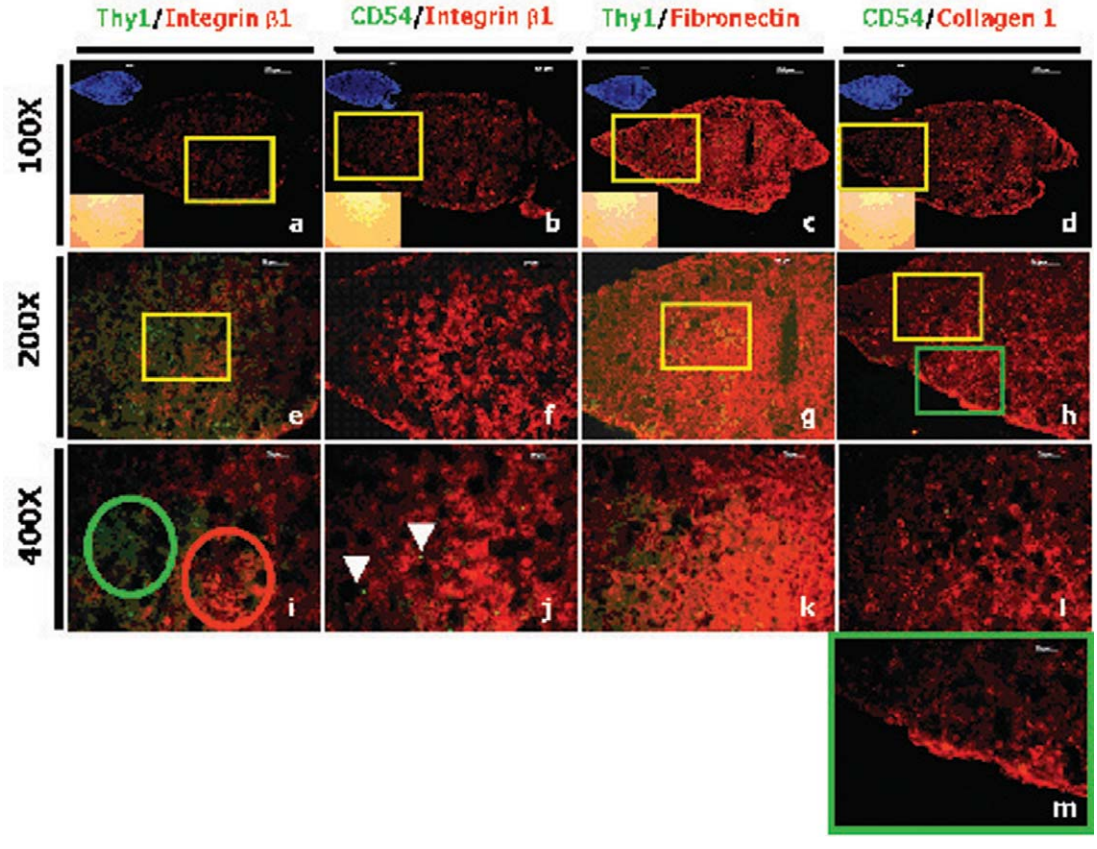

Fig. 2. Stem cell marker expression in the OSBHC. To identify the stem cell candidates in the OSBHC, immunohistochemistry against Thy1(HSCs marker), CD54, Integrin β1(MSCs marker), Fibronectin and collagen type1 (ECM constructed by MSCs) was performed. Double labeling of Thy1 and integrin β1 shows positive cells of Thy1 (green circle) and Integrin β1(red circle) were located at separate regions of the OSBHC(a, e, i). CD54 signals were rarely expressed as bright small spots, but the signal patterns were correct(b, f, arrowhead in j). Fibronectin enclosed most cells of OSBHC(c, g, k), whereas collagen type1 was detected as separated bright spots(d, h, i, m). TRA1, SSEA1(ESCs), Sox2, Nestin(NSCs), Glycopropyrin A(erythroid cells) were not detected(data not shown).

cellular nature by immunohistochemistry, and (4)Their circulatory function by injection of nanoparticles. The present work is concerned with the third category of investigations. In our previous works that were mostly concerned with the first category, conventional staining methods were used for histological studies with light microscopes and confocal laser scanning microscopes[10, 15-17]. In these anatomical studies the BHC/D were shown to be distinct from known tissues such as nerves, blood capillaries or lymph vessels. But the detailed study on their structure at the cellular level needs electron microscopy, which revealed many immune function cells[13]. For example, macrophages have a deep connection with inflammation through the receptor and acetylcholine, which raised the $\alpha 7$ possibility of explaining the therapeutic effects of acupuncture on inflammation[18]. The current work in the third category suggests even more significant function of the BHC, namely, a novel stem cell niche.

To know whether the stem cell marker expressing cells are in the organ surface BHC, we performed immunohistochemistry with various well known stem cell markers. Even though marker expression itself is not sufficient evidence for the presence of stem cells, detection of stem cell marker expression and their surrounding extracellular matrix composition gave us insight about the organ surface BHC as a novel stem cell niche candidate. In this analysis, Thy1 (hematopoietic stem cell marker) or integrin beta 1 (mesenchymal stem cell marker) positive cells were major cell types which composing BHC, and the cells were surrounded by fibronectin or collagen type 1 fibers(Fig. 2). But the fact that there were no

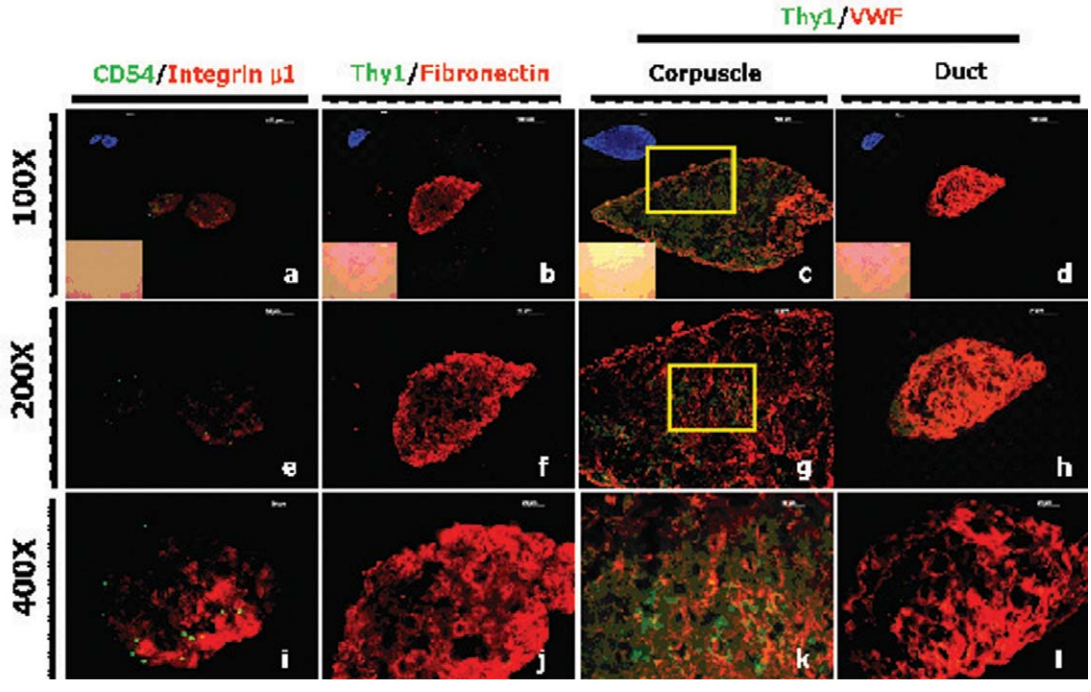

Fig. 3. Distinct marker expression patterns between OSBHC and its associated duct. Immunohistochemistry analysis of OSBHC associated duct shows relatively low level of integrin $\beta 1$ expression(a, e, i). Double labeling of Thy1 and fibronectin shows high level of fibronectin composition of OSBHD, but there were no Thy1 expressing cells(b, f, j). Whereas the OSBHC has mixed cell population of Thy1 positive cells and vWF positive endothelial cells, the major cell type which compose the OSBHD was vWF positive endothelial cells and Thy1 positive cells were not detected(c-d, g-h, k-l).

CD56(mesenchymal stem cell marker) positive cells means the BHC doesn't have the mesenchymal stem cells. We also tried to find the cells which express pluripotent or neural stem cell markers(SSEA1, Tra1, Nestin, sox2, Glycopropin A), there were no reliable marker expressing cells(data not shown).

When the organ surface BHC was compared to its associated BHD through the double labeling of vWF(endothelial cell marker) and Thy1, Thy1 was only expressed in the BHC but not in the BHD. And the Thy1 positive cells co-express neither mesenchymal cell markers(Fig. 2) nor endothelial cell markers(Fig. 3). This distinct cell composition between BHC and OSBHD supports the hypothesis that BHC acts as a stem cell supplier and the stem cells migrate to the organs through the connected BHD.

So, to examine the property of the organ surface BHD as a cell migration pathway, the isolated BHDs which connected corpuscle to corpuscle and corpuscle to organ surface were analyzed by several endothelial cell markers. Immunohistochemistry results show the OSBHDs were composed by Flk1, vWF and RECA1 positive cells(Fig. 4). But the expression levels of these markers were not consistent in the BHDs from various organ surfaces. Though it is not enough to prove the hypothesis of BHD as a transportation route between BHC and BHC, and from BHCs to organs, the existence of endothelial cells is a strong evidence of this hypothesis.

Various organ surface BHCs were observed on internal oragans of rats, mice and rabbits, and similar structures were also detected in blood or

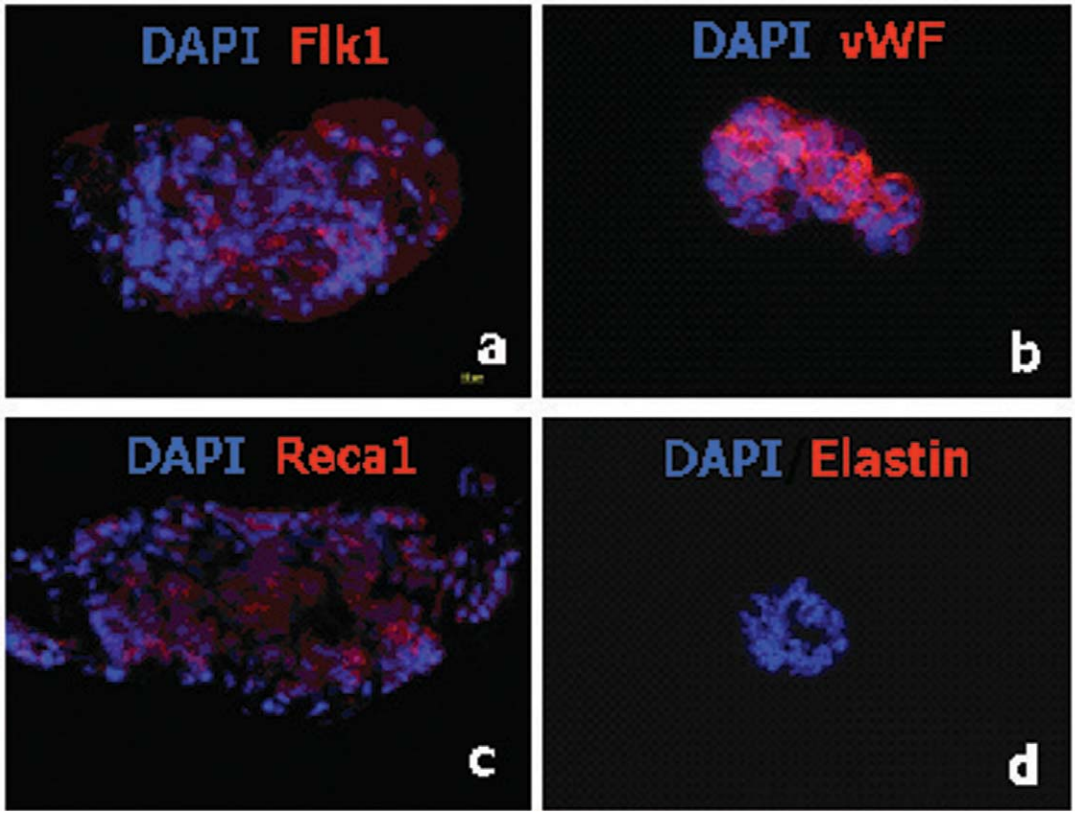

Fig. 4. Flk1, vWF and RECA1 showed the high level of endothelial cells marker expression(a-c). But the elastin was not detected(d).

lymphatic vessels[16,17]. It will be worth further attempts to continue trying to isolate stem cell marker specific cells from these other BHCs. The present work is only a first step in such a study, and provides a strong clue for finding new stem cell niches. Newly identified stem cell niches provide an insight into potential stem cell sources.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation(KOSEF) grant funded by the Korea government(MOST)(No. R0A-2003-000-10371-0)

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