Chapter 23
Primo Microcell in a Primo Node as a Possible Origin of Adult Stem Cells

Seong-hun Ahn, Sung-won Lee, Sung-Yeoun Hwang, Jae-hyo Kim, and In-chul Sohn

Abstract Adult stem cells have been intensively studied for their cell-therapeutic potential to renew damaged tissues or organs because their use eliminates the ethical, legal, and political concerns with using embryonic stem cells. On the other hand, a microcell contains a micronucleus that has one or a few chromosomes with a small amount of cytoplasm. Recently, microcells have become a possible origin of adult stem cells because of their ability to induce undifferentiated cells. In the early 1960s, after its first observation by Kim, the primo vascular system (PVS) was thought to be an anatomical basis for traditional acupuncture systems (termed *kyungrak* theory or meridians). Kim also claimed that about 1.0-μm-sized DNA-containing granules, named primo microcells or *sanals*, flowed through the PVS consisting of primo vessels and primo nodes to internal organs to proliferate and differentiate into specific cells in the organs in a manner similar to that of adult stem cells. In this study, we harvested primo microcells from Sprague Dawley rats about 250–300 g. The primo microcells were observed to grow into cell-like body entities. These interesting results suggest that Kim's manual pictures from his article 1965 and *Sanal* theory may be true based on experimental studies similar to ours. Also, primo microcells in primo nodes might be one possible origin of adult stem cells. Understanding the primo microcell is thought to be one of the most fundamental studies for cell therapy.

1 Introduction

Stem cells having a self-renewal ability to proliferate in an undifferentiated or unspecialized state and the capability to differentiate or specialize along multiple lineages [1] are classified as mesenchymal stem cells, hematopoietic stem cells, and

I.-c. Sohn (✉)
Department of Meridian and Acupoint, College of Oriental Medicine, Wonkwang University, Jeollabuk, South Korea

Research Center of Traditional Korean Medicine, Wonkwang University, Jeollabuk, South Korea
e-mail: ichsohn@wku.ac.kr

K.-S. Soh et al. (eds.), *The Primo Vascular System: Its Role in Cancer and Regeneration*,
DOI 10.1007/978-1-4614-0601-3_23,

stromal stem cells from bone marrow, umbilical cord blood stem cells, embryonic stem cells, adipose-derived adult stem cells, etc. [2]. Among these, adult stem cells have received much scientific attention and have avoided medical safety and efficacy issues because they eliminate the ethical, legal, and political concerns associated with embryonic stem cells. Recent studies indicate that nascent stem cells exist within other adult tissues, including the brain [3], dermis [4], periosteum [5], skeletal muscle [6], synovium [7, 8], trabecular bone [5], and vasculature [9] tissues, but the most abundant and accessible source of adult stem cells is adipose tissues [1]. So far, the indices and marks common to distinguish nascent stem cells from progenitor cells, as well as the indices and specific characteristics of oneself, have not been examined closely because close study of stem cells is difficult.

In the early 1960s, after its first observation by Kim, the primo vascular system (PVS, also named the Bonghan system) [10], was thought to be the anatomical basis for traditional acupuncture systems (termed kyungrak theory or meridians) [11, 12]. Kim also claimed that about 0.5–1.0-μm-sized DNA-containing granules (named primo microcells or sanals, Bonghan granules) flowed through the PVS consisting of primo vessels and nodes to internal organs to proliferate and differentiate into specific cell in the organs [13] in a manner functionally similar to that of adult stem cells.

Recently, a series of interesting studies of primo microcells were advanced. Baik et al. [14] reported that 2-μm-sized DNA-containing granules observed in primo nodes might be a sort of cell-free DNA. Ogay et al. [15] studied that fragmented 1.7–2.5-μm-sized DNA-containing granules had cytoplasm and that a tri-laminar plasma membrane might be the origin of adult stem cells. Specifically, Kim et al. [16] suggested that Bonghan corpuscles (primo nodes) might be stem cell niches, hinting that the DNA-containing granules might be a new type of stem cell because of integrin ß1, collagen type 1, fibronectin, and Thy1 expressions. Baik et al. [17] studied the morphological features of primo microcells by using scanning electron microscopy (SEM) and atomic force microscopy (AFM) and suggested that primo microcells were a kind of stem cell because of their being joined by two nuclei to each other. In a previous study, we studied the morphology of organ-surface primo vessels and nodes in rabbits [18] and rats [19] to confirm the existence of primo microcells in primo nodes. In this study, we report primo microcell culture results that support Kim's claim on Sanal theory.

2 Materials and Methods

2.1 Animals

The Sprague Dawley rats of about 250–300 g used in this study were obtained from Samtaco Laboratory Animal Company (Osan, Korea). The animals were housed in a temperature-controlled environment (23°C) with 60% relative humidity, a 12-h

light/dark cycle, and ad-libitum access to food and water. All the procedures involving the animals and their care conformed to institutional guidelines, which were in full compliance with current international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996). This study was approved by the Institute of Laboratory Animal Resources at Wonkwang University.

2.2 Surgical Procedures

The rats were injected with urethane (1.5 g/kg) i.m. before the surgical procedure. All surgical procedures were performed under general anesthesia. We incised the skin along the medial alba of the abdomen very carefully and opened the abdomen. The suprahepatic vena cava was clipped with forceps before the hepatectomy. The liver was isolated from the abdominal cavity and was rapidly moved to a phosphate buffer saline (PBS, pH 7.2) pool and washed 3–5 times very carefully. We carefully searched for primo nodes on the liver-lobe surfaces by using small surgical instruments, such as iris scissors, microforceps, and needles, for manipulation. The search for the nodes and the vessels was carried out under a stereoscopic microscope (SZX10, Olympus, Japan).

2.3 Cell Culture

The primo nodes isolated on a clean bench were moved to a cell culture medium. After transfer, the samples were cut 3–4 times and spun down at 2,000 rpm for about 5 min to separate the red blood cells (RBCs). The supernatant was cultured in a CO_2 incubator. The results were pictured with a microscope (Olympus, Japan) by using NIS-Elements software (Nikon, Japan).

3 Results and Discussion

3.1 Organ Surface Primo Vessels and Nodes in Rat Liver

The PVS suggested with kyungrak by Kim [10, 11] is well known to be different from the vascular system, the nerve system, and the lymph system [20]. Until now, the observation methods for the PVS have varied. Among them, staining methods were generally used, and the staining dyes that were mainly used were trypan blue [21, 22], acridine orange [23], janus green B [20], and alician blue [24–27]. However, until now no specific antibody has been observed on primo nodes and primo vessels, the appearances of the observed primo nodes have varied, and the locations where they have been observed have not been the same.

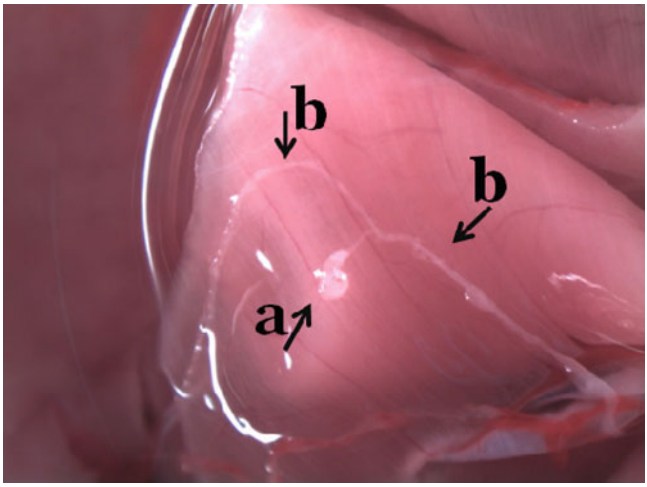

Fig. 23.1 The prominent type of primo node and vessels on the liver surface in rats: (*a*) primo node and (*b*) primo vessel

We formed two hypotheses. First, the PVS existed uniformly but observation methods were not sufficient to observe primo nodes and primo vessels regularly. Second, we did not know what conditions caused primo nodes and primo vessels to be in an activation state or in an inactivation state. Furthermore, we did not know if the PVS to be observed was in an activation state or in an inactivation state. For example, in the living body, the fatty tissue volume and the adipocyte number become larger in nutritive sufficient conditions, but in nutritive insufficient conditions, the volume and the number become smaller.

The primo node and primo vessels in Fig. 23.1 were observed on the surface of a rat's liver by using a visual observation method with stereomicroscopy. The observed primo node and primo vessels were milky in color and semi-transparent, with no blood vessels for nutrition. This is a unique characteristic that distinguishes the PVS from the lymph and the nerve systems.

3.2 Primo Microcell Growth to a Cell-Like Body

The sanal theory of Kim BongHan's hypothesis was that the sanal (a living seed in Korean, renamed primo microcell in 2009), which flows through the primo vessels, differentiated into a specific cell for proliferation and reproduction [12, 13]. However, sanal theory as claimed by Kim BongHan was denied by Keller [28], who reported that the PVS really consisted of lymph vessels that had been misidentified.

In our results, the primo microcells gained from primo nodes grew into cells or cell-like bodies after 8–9 days of culture, similar to Kim's claim (Fig. 23.2) [13]. From this interesting result, a few doubts occurred. In Kim's observations, a sanal,

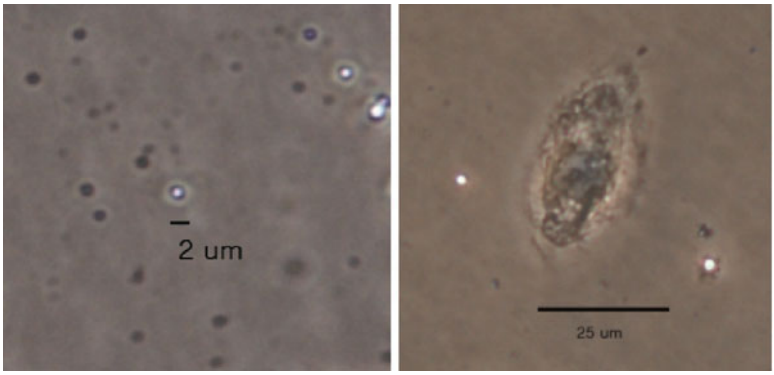

Fig. 23.2 The Kim BongHan's first step result of Sanal: a primo microcell size is about 1.5 μm in diameter (*left*). Eight days later, the primo microcell had grown to be cellbody like, about 30 μm in length and 15 μm in width (*right*). This result is very similar to Kim's results

now renamed primo microcell, had one chromosome, indicating that the DNA in a primo microcell was not perfect. In our observation, the nucleus of a primo microcell was not stained with the DAPI (4′,6-diamidino-2-phenylindole) stain method (data not shown), but the primo microcell grew into a cell-like body without a perfect nucleus. This is similar to an RBC, although an RBC is differentiated without a nucleus.

On the other hand, in Kim's theory [13], a primo microcell produces a daughter cell at the beginning of cell differentiation, and this is repeated so that a primo microcell grows into a cell or tissue. If this hypothesis is correct, how does a primo microcell gain complete DNA. And in our studies, the individual size of some cell-like bodies that have grown from primo microcells is outside the range for normal cell size: ~100 μm in diameter, so we are not convinced whether the cell-like bodies that grow from primo microcell are normal cells or not. Despite these doubts, our conclusion is that the primo microcell grows into cell-like body, and this experimental result is very similar to Kim's observation in the 1960s.

The most critical limit of the current work is that the DNA content in our primo microcells and cell-like body was not tested. In future work, the experiments will be repeated with DNA staining dyes.

4 Conclusion

The primo microcells we observed grew into cell-like bodies. This experiment result is very similar to Kim BongHan's observation in the 1960s and suggests that the primo microcell might be the origin of stem cells or a new type of stem cell. The present report is only preliminary, and further experiments addressing DNA content are needed.

References

1. Gimble J, Guilak F (2003) Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy 5(5):362–369
2. Weissman IL, Anderson DJ, Gage F (2001) Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Ann Rev Cell Dev Biol 17:387–403
3. Klein C, Butt SJB, Machold RP, Johnson JE, Fishell G (2005) Cerebellum- and forebrain-derived stem cells possess an intrinsic regional character. Development 132(20):4497–4508
4. Li L, Fukunaga-Kalabis M, Yu H, Xu X, Kong J, Lee JT (2009) Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci 123:853–860
5. Zheng Y-X, Ringe J, Liang Z, Loch A, Chen L, Sittinger M (2006) Osteogenic potential of human periosteum-derived progenitor cells in a PLGA scaffold using allogeneic serum. J Zhejiang Univ Sci B 7(10):817–824
6. Bellayr IH, Gharaibeh B, Huard J, Li Y (2010) Skeletal muscle-derived stem cells differentiate into hepatocyte-like cells and aid in liver regeneration. Int J Clin Exp Pathol 3(7):681–690
7. Fan J, Varshney RR, Ren L, Cai D, Wang DA (2009) Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration. Tissue Eng Part B Rev 15(1):75–86
8. Hui J, Ouyang H, Hutmacher D, Goh J, Lee E (2005) Mesenchymal stem cells in musculoskeletal tissue engineering: a review of recent advances at the National University of Singapore. Ann Acad Med Singapore 34:206–212
9. Winter EM, Gittenberger-de Groot AC (2007) Epicardium-derived cells in cardiogenesis and cardiac regeneration. Cell Mol Life Sci 64:692–703
10. Kim B (1961) The Kyungrak system. J Acad Med Sci DPR Korea 108:1–38
11. Kim B (1963) On the Kyungrak system. J Acad Med Sci DPR Korea 90:1–35
12. Kim B (1965) Theory of Kyungrak. J Acad Med Sci DPR Korea 108:1–38
13. Kim B (1965) Sanal theory. J Acad Med Sci DPR Korea 6(108):39–62
14. Baik KY, Sung B-K, Lee B-C et al (2004) Bonghan ducts and corpuscles with DNA-containing granules on the internal organ-surfaces of rabbits. J Int Soc Life Inf Sci 22(2):598–601
15. Ogay V, Baik KY, Lee BC, Soh KS (2006) Characterization of DNA-containing granules flowing through the meridian-like system on the internal organs of rabbits. Acupunct Electrother Res 31(1–2):13–31
16. Kim MS, Hong JY, Hong S et al (2008) Bong-Han corpuscles as possible stem cell niches on organ surfaces. J Pharmacopunct 11(1):5–11
17. Baik KY, Ogay V, Jeoung SC, Soh K-S (2009) Visualization of Bonghan microcells by electron and atomic force microscopy. J Acupunct Meridian Stud 2(2):124–129
18. Ahn SH, Kim MS, Lee SH et al (2009) The morphology study of organ surface BongHan ducts and corpsucle. J Meridian Acupoint 26(1):79–84
19. Lee SH, Ryu Y, Yun Y et al (2010) Anatomical discrimination of the differences between torn mesentary tissue and internal organ-surface primo-vessels. J Acupunct Meridian Stud 3(1):10–15
20. Lee BC, Yoo JS, Baik KY, Kim KW, Soh KS (2005) Novel threadlike structures (Bonghan ducts) inside lymphatic vessels of rabbits visualized with a Janus Green B staining method. Anat Rec B New Anat 286(1):1–7
21. Lee B-C, Kim KW, Soh K-S (2009) Visualizing the network of Bonghan ducts in the omentum and peritoneum by using Trypan blue. J Acupunct Meridian Stud 2(1):66–70
22. Lee B-C, Soh K-S (2010) Visualization of acupuncture meridians in the hypodermis of rat using Trypan blue. J Acupunct Meridian Stud 3(1):49–52
23. Lee B-C, Baik KY, Johng H-M et al (2004) Acridine orange staining method to reveal the characteristic features of an intravascular threadlike structure. Anat Rec B New Anat 278B:27–30
24. Lee C, Lee B-C, Soh K-S (2006) Alcian blue staining method to visualize the lymph intravascular Bonghan duct in the rabbit lymph vessel. J Korean Soc Jungshin Sci 10(1):70–76

25. Lee C, Seol SK, Lee BC, Hong YK, Je JH, Soh KS (2006) Alcian blue staining method to visualize bonghan threads inside large caliber lymphatic vessels and X-ray microtomography to reveal their microchannels. Lymphat Res Biol 4(4):181–190
26. Bk S, Kim MS, Ogay V, Kang DI, Soh KS (2008) Intradermal Alcian-blue injection method to trace acupuncture meridians. J Pharmacopunct 11(2):5–12
27. Yoo JS, Kim MS, Ogay V, Soh KS (2008) In vivo visualization of bonghan ducts inside blood vessels of mice by using an Alcian blue staining method. Indian J Exp Biol 46(5):336–339
28. Kellner G (1966) Bau und Funktion der Haut. Deutche Zeitschrift fur Akupunktur 15:1–31