Naturally Generated Microcells as One Possible Origin of Adult Stem Cells

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Abstract: Adult stem cells have been intensively studied for their cell-therapeutic potential to renew and replace lost cells in any damaged tissue or organ. Yet, the origin of adult stem cells remains an important question to be resolved. On the other hand, microcells have been used for cell fusion in cancer research. A microcell contains a micronucleus that has one or a few chromosomes with a small amount of cytoplasm. Recently it was reported that microcells can transform into undifferentiated cells, which suggests microcells are a possible origin of adult stem cells.

In the early 1960’s Bonghan Kim claimed that anatomical structures corresponding to acupuncture meridians had been observed and that they formed a circulatory network distributed throughout a body which was entirely different from the blood, lymph, or neural systems. A liquid whose most important component was microcell-like bodies called “Bonghan granules” flowed in this new circulatory system. Granule sizes, shapes, structures and DNA-content were similar to those of microcells. Furthermore, Kim observed that the Bonghan granules grew and regenerated or repaired damaged cells. Recently, after decades of neglect, Kim’s studies are being considered again, and some of his findings, including Bonghan granules, have been reconfirmed.

We put forward a hypothesis on a possible origin of adult stem cells: The Bonghan granules that flow in the Bonghan circulatory network are naturally generated microcells and they are capable of growing and differentiating into tissue cells and also regenerating the tissues of injured organs. Any differentiated tissue cells in mammalian organisms may have a capacity for self-reproduction and self-renewal by specific “microcell-like Bonghan granules”. Understanding of the origin of adult stem cells in animals is one of the most fundamental tasks in modern biomedicine and may result in new approaches to the treatment of many diseases in the future.

Keywords: Bonghan granules, Bonghan corpuscle, microcells, stem cells, acupuncture meridians

Introduction

An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Adult stem cells have been identified in many organs and tissues, such as the brain, bone marrow, peripheral blood, blood vessels, skin and liver [1]. Even though there has been a lot of progress in stem cell research, many important questions about adult stem cells remain to be answered. Among them, two are of particular importance: What are the sources of adult stem cells in the body? Does a single type of stem cells exist that can regenerate the cells of any organ or tissue?

In this article we propose the hypothesis that naturally produced microcells are the origin of the single type of stem cells. As is well known, microcells contain micronuclei composed of one or a few chromosomes together with a small amount of cytoplasm enclosed by a membrane. Microcells are commonly used for cell fusion studies, especially in connection with cancer research [2, 3]. They are a natural component of tumor tissues, metabolically very active and naturally resistant to anticancer agents. The rapidly growing microcells can transform into young undifferentiated cells, which suggests a possible origin of stem cells in pathological situations [4].

There are naturally generated microcell-like granules in the acupuncture meridian system, whereas microcells used in cell fusion and cancer studies were produced by treatment with chemicals like colchicines, colcemid and cytochalasin B. The microcell-like granules in the acupuncture meridian system are referred to as “Bonghan granules” after their discoverer Bonghan Kim or as “Sanal” that literally means vital-some [5]. Kim

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was the first one to pursue a systematic study of the physical substrate for acupuncture stimulation, and he claimed evidence for the granules being an anatomical substrate for acupuncture points, based primarily on work done on rabbits. Corresponding to the acupuncture meridians and collaterals there exist threadlike tissue structures called "Bonghan ducts" that constitute a third circulatory system, which is different from the blood and lymph systems [6]. Bonghan granules are in many respects similar to microcells and they flow through this net of ducts. Unfortunately, his research was forgotten for nearly forty years, and only recently has his work been intensively restudied [7, 8, 9].

Physical reality of acupuncture meridians and Bonghan system

At present it is widely accepted that acupuncture is a viable alternative tool for pain control and anesthesia [10] as well as a medicine for illnesses. Yet, in spite of many scientific efforts, the mechanism of acupuncture's effects remains unresolved. One of the essential questions is whether the acupuncture meridians really exist or are merely functional virtual representations. So far no anatomical-histological structures have been established, except for the Bonghan system claimed by Kim in the early 1960's [6]. He reported finding an anatomical structure corresponding to acupuncture points and meridians. He demonstrated through experiments on rabbits that the acupuncture meridians were part of a new circulatory system which was distinctly different from the blood, lymph, or neural system.

The Bonghan network consists of several subsystems: The superficial Bonghan ducts (BHDs) are classical acupuncture meridians and collaterals. They extend in such a manner that a web like network is distributed throughout the body. Parts of these ducts form a subnet like BHDs on the various internal organ surfaces; these threadlike semi-transparent BHDs exist even inside blood vessels, as floating small ducts [7, 8].

Confirmation of Bonghan Theory

Despite strong interest in Far Eastern countries, Bonghan theory was not confirmed by other research groups. The main reason was that the critical technique of staining was not disclosed, without which it is extremely difficult to observe the claimed tissues. In Japan Fujiwara and Yu [11, 12], however, reproduced the anatomical and histological results of Kim by observing BHDs on the surface of internal organs and inside of blood vessels in rabbits. These studies were met with skepticism and failed to get wide attention. The principal reason for the skepticism was that string-like structures formed by fibrin might be mistaken for the claimed threadlike structure, that is, BHDs. This skepticism was overcome only very recently [8].

The intravascular BHDs were extensively studied using rabbits, rats and mice, applying very slow perfusions with dextrose solution [7]. The critical problem of differentiating the BHDs from fibrin strings was resolved by the fluorescence technique of acridine-orange staining of DNA [8]. This staining revealed rod-shape nuclei, of 15-20 μm in length, distributed on broken-line stripes, whereas the strings formed by fibrin had only spherical white blood cells scattered around. These features are in good agreement with Bonghan’s original description. Histological examinations using hematoxylin-eosin staining, Malory’s triple, and Verhoeft’s elastic stain also showed positive results [13], and further study with silver impregnation demonstrated the presence of argyrophilic fiber which is another hallmark of the BHDs. The most important morphological characteristic of the BHD is its bundle-like structure that is formed by tubules, i.e. the duct is not a single tube but a cable of multiple tubules. This bundle structure has also been confirmed by separation of the tubules by applying an electric field [14].

Another subsystem of the BHD network is the organ-surface Bonghan ducts (organ-surface BHD) that are distributed over the surface of various internal organs. The organ-surface BHDs are freely movable and fixed sparsely onto the peritonea of various organs, such as the small and large intestine, stomach and liver. They are about 100 μm in diameter, and milky white and semi-transparent. There are Bonghan corpuscles interconnected by BHDs; they are 0.5-3.0 mm in diameter and have an oval or long cucumber shape.

Observations of organ-surface BHDs in rats, rabbits and swine were reported by three independent groups [9, 15, 16]. The difficulty of recognizing the organ-surface BHDs is multifold: First, their transparency and small diameter (less than 100 μm) make them almost invisible to the naked eye, and a good stereo microscope and a strong intention to search for them are necessary. Second, fibrin released in bleeding during surgery has a strong affinity for the BHDs, and thus they are easily disguised as blood coagulated fibrin strings. Third, organ-surface BHDs are not regularly distributed in the same location; their position and size vary depending upon the subject animals and also on their physiological states. Fourth, and most importantly, it is difficult to distinguish the organ-surface BHDs from small lymph vessels. Distinguishing these two requires examination of their histological and morphological structures [9, 15].

Bonghan granules flowing in the Bonghan ducts
The discovery of the Bonghan network as the third circulatory system was followed by the observation of a liquid that flows through the ducts. Biochemical analysis revealed large amounts of hyaluronic acid, hormones, and granules of DNA (called BH-granules hereafter) among others. The BH-granules are spherically shaped and their diameter is from 0.8 to 2.4μm. They are enclosed by a thin membrane whose chemical components include lipoprotein. Two parts can be distinguished under a phase-contrast microscope: The central part looks dark and has various shapes. It is basophilic, Feulgen reaction positive, and includes one chromosome amount of DNA. This central part is surrounded by a bright plasma.

The BH-granule originates from micro-nucleation of some cells in organs and tissues. In the first stage, point-like small granules appear and move around within the nuclei. The movement becomes faster and granules leave the nucleus after its membrane is ruptured. The granules move more vigorously in the cytoplasm, and finally the membrane disintegrates and the BH-granules are scattered around. According to Kim the BH-granulization process appears in normal tissue cells and can also be induced by various chemical and physical factors [5]. But Kim did not clearly state what the conditions for normal cells to be micro-nucleated were, nor did he give the explicit nature of the physical-chemical factors.

Kim claimed that any tissue cell in any mammalian organism had a capacity for being renewed through the process of micro-nucleation and maturing to undifferentiated cells from the micro-nucleated Bonghan granules. He proposed essentially a toti-potent source of adult stem cells in early 1965 [5]. It should be noted that the first papers on stem cell concepts were published at approximately the same time [17, 18].

We recently obtained BH-granules from BH-corporcles using a method of differential centrifugation [unpublished data]. A three-dimensional projection of a BH-granule obtained with confocal microscopy revealed that the granule has a roundish form, is about 1.7-2.5 μm in diameter and contains DNA molecules localized predominantly in the central part of the BH-granule. The presence of DNA was detected by specific fluorescent dyes such as Hoechst - 33258 and Propidium Iodide. In addition the granules exhibited a jittering motion, as was described by Kim. Their DNA content has not been analyzed yet; knowing this will be an important step towards verifying their physiological functions.

**Micronucleate cells and microcells**

Observations of micronucleate cells have been recorded sporadically for many years, especially in relation to the study of abnormal mitosis. In normal mitosis the daughter chromosomes that have separated during the anaphase become surrounded by nuclear membranes, either directly as a group or first as single chromosomes with the subsequent fusion of the chromosome containing vesicles. This is followed by decondensation of the chromosomes to form interphase nuclei. Interference with the separation of chromosomes into an anaphase configuration or with the fusion of the chromosomes-containing vesicles can cause the formation of cells containing many micronuclei, each of which contains a different part of the genome [19]. The best known examples of agents that interfere with mitosis, by hindering the assembly of microtubules and the mitotic spindle, are colchicine and its deacetyl derivative, colcemid.

Microcells are cytoplasmic fragments that contain micronuclei composed of one or a few chromosomes surrounded by a thin rim of cytoplasm and a cell membrane. They are produced by enucleation of micronucleate cells [20] and they have also been directly generated without the necessity of forming micro-nucleated cells by first placing mitotic cells directly into cytochalasin B and centrifuging in the Percoll gradient [21]. Another source of microcells is tumor tissues. The relative numbers of microcells in tumor tissue markedly increase after chemotherapy, irradiation or immunotherapy [4].

Microcells have been widely used for cell-fusion studies, especially for microcell-mediated chromosome transfer. The use of somatic cell hybridization to elucidate the effect of the genome of normal cells on the phenotype of a malignant cell was the first demonstration that regulatory genes in normal cells controlled the growth of hybrid cells. Microcell transfer provides a direct way to transfer a single whole chromosome from a donor cell to a recipient cell. The development of microcell-mediated chromosome transfer techniques has allowed for a less laborious examination of the effects of a single chromosome on the phenotype of a cell line without the need to detect phenotypic regression after random chromosomal elimination.

Approaches involving microcell-mediated chromosome transfer led to the study of cell senescence genes, which provide a tool for investigating carcinogenesis [22] and Downs's syndrome [23]. Microcell development was observed in perinuclear bodies of interphase nuclei in damaged macrocells of the Jungarian hamster fibroblast cell line HT-1080 after the application of the cytotoxic drug, thiophosphamidum [4].
Bonghan granules as one possible origin of adult stem cells

Based upon Bonghan Kim’s discovery of BH-granules and recent progress on microcells we are putting forward a hypothesis on the origin of adult stem cells. First, we consider the possibility of BH-granules as a type of microcells that are naturally generated in organs and tissues and flow through the network of the Bonghan system, i.e., acupuncture meridians and collaterals. This “natural” generation is in contrast to the artificial or pathological origin of microcells that have been produced in vitro by chemical agents such as colchicine, colcemid, thiophosphimide and cytochalasin B, or observed in a pathological situation such as large inter-phase sarcoma cells.

BH-granules and microcells are similar in their sizes (diameters are 1.2-4.2μm), their roundish or oval shapes, and intensely stained nuclei. In addition both of them have a thin outer membrane, and one chromosome amount of DNA inside. One difference is the amount of cytoplasm: BH-granules have a fair amount of cytoplasmod around the DNA, but microcells have only a scanty amount. This difference might reflect the natural and artificial or pathological conditions, of their generation, and should be further investigated in the future.

Next, we consider the possibility of BH-granules developing into undifferentiated cells. According to Kim BH-granules were produced in the nuclei of normal cells, and migrated into small BHDs after disintegration of the nuclei and cell membranes [5]. Flowing through the BHD the granules passed along the cutaneous acupuncture points where they received external light to initiate the photochemical reactions that were necessary for their development into cells. Although Kim did not study undifferentiated cells, he observed that BH granules were able to grow and regenerate cells in the damaged tissues of an intentionally inflicted injury.

Thus it is not clear whether the BH-granules regenerate cells in damaged tissues via undifferentiated stem cells or through some other processes. We conjecture that BH-granules, being the same without regard to the organs and tissues in which they were produced, may develop into undifferentiated stem cells. This is only a conjecture made in line with microcell phenomena: Buikis, et al. [4] observed that microcells are metabolically active, and intensively accumulate nucleoproteins into their nuclei and cytoplasm. The microcells grow rapidly and transform into young undifferentiated cells, beginning propagation via mitosis. This implies that stem cells can arise from microcells. They called this cytological mechanism for the immortality of tumor cell populations “sporosis”. Our hypothesis states that “sporosis” could happen in normal physiology with the BH-granules that flow in the acupuncture meridian system.

Finally we put forth the supposition that BH-granules are the origin of a single type of adult stem cells which can regenerate the cells of any organ or tissue. Various kinds of stem cells have been found [1], but their origins and totipotent stem cells have remained an unresolved question for which BH-granules could offer the right solution. In his last paper [24] Kim studied the hematopoietic process of BH-granules, which can be compared to the well known hematopoietic stem cells that give rise to all types of blood cells [25, 26]. This hematopoietic process could be the first step in proving the hypothesis proposed in this article when the technique to obtain BH-granules is well developed.

It is most remarkable that Kim claimed to have observed a phenomenon corresponding to microcells and adult stem cells in the early 1960’s when no such concepts were even conceived of among Western biomedical researchers. Through the combination of these modern concepts with BH-granules, a new insight into centuries-old acupuncture phenomena in terms of current biomedical terms could evolve. The mechanism for the development of undifferentiated cells from microcells and BH-granules would be one of the most important contributions to the field of cytogenetics and molecular biology.

Concluding remarks

We can summarize the hypothesis as follows: Bonghan granules are naturally generated microcells that flow in the network of Bonghan ducts. They grow to become undifferentiated adult stem cells and are able to renew the tissues in any damaged organs.

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Naturally Generated Microcells as One of Possible Origins of Adult Stem Cells
成熟幹細胞ができる可能性があるうちの1つとしての Naturally Generated Microcells (NGM)

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要旨: 傷害をうけた組織や臓器の細胞新生や入れ替えを行う成熟幹細胞の治療力が研究されている。しかし、まだその成熟幹細胞の働き方について重要な疑問が残っている。
一方、マイクロセルは癌研究で細胞融合のために使われているが、そのマイクロセルにはわずかな量の原形質に数個のクロモソーム含む小核がある。最近、そのマイクロセルが成熟幹細胞になっていく可能性を示し、マイクロセルが未成熟細胞へと変化するという報告があった。

1960年代初めに、キム・ボンハンは、鍼灸経絡と一致する解剖学的構造物を観察し、それは、血液、リンパあるいは神経のシステムとは全く違った、全身に分布する網目状の循環する構造物であると主張した。この新しい循環系のなかをマイクロセル様の「ボンハン顆粒」と呼ばれる重要な構造物が流れ、それらのサイズ、形状と構造、DNA含有量等は、マイクロセルと類似している。さらに、キムはボンハン顆粒が傷害を受けた細胞を再生させ、修復することをも観察した。

このような彼の業績は10年の間、無視されていたが、最近になって、ボンハン顆粒の概念を含む彼の業績が再び脚光をあび、その内容の一部が再確認された。

我々は、本稿で成熟幹細胞が形成される仮説を提案する。ボンハン循環網目系を流れるボンハン-顆粒は、マイクロセルへと成長し、さらに組織細胞へと分化し、傷ついた臓器の組織を再生させることができる。哺乳類でみられる再生型の組織細胞は、特殊なマイクロセルのような「ボンハン-顆粒」によって自己の再生と自己複製の能力を持っている。

成熟幹細胞がどうして出来てくるのかが明らかになることは、現代の生物医学の中でも最も基本的な研究の1つで、将来多くの疾患の治療に新しいアプローチを提供するであろう。

キーワード: ボンハン顆粒、ボンハン小体、(microcells) マイクロ細胞、幹細胞、鍼灸経絡