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Determination of bone marrow cell morphology in rat

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ABSTRACT

The skeletal system of the body is responsible for important functions in the human body. In addition to causing movement, this system also plays a role in the production of blood cells and fat storage. Bone marrow is a spongy or viscous tissue that fills the inside of the body's bones. The basic structure of bone marrow is of two types. Red bone marrow and yellow bone marrow. Red bone marrow contains blood stem cells that can become red blood cells, white blood cells, or platelets. Yellow bone marrow is made mostly of fat and contains stem cells that can turn into cartilage, fat, or bone cells. Human bone marrow mesenchymal stem cells (HBMSCs) are widely used cell sources for clinical bone regeneration. Achieving a therapeutic effect depends on the osteogenic differentiation potential of the stem cells. The purpose of judging the morphology of bone marrow cells is to diagnose leukemia or bone marrow disorders, determine the cause of severe anemia or thrombocytopenia and low platelet count, identify abnormal chromosomes to prevent hereditary diseases, and plan their treatment. In this study, we examined the morphological characteristics of bone marrow cells, mesenchyme cells, and osteoblasts in a laboratory environment. The results of the morphological investigations showed changes such as the change of the position of the nucleus and the rounding of the cytoplasm in the differentiated cells compared to the mesenchyme cells. Therefore, to identify and diagnose as many of these cells as possible, molecular genetic techniques such as network algorithms and fluorescence staining can be used for hematological and cytomorphological investigations.

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Introduction

Bone marrow cancer causes the bone to grow excessively and the tissue replaces the bone (1). Since tissues do not have the resistance of bone, this process leads to bone fracture. Bone marrow is exactly the red marrow inside the bone. This fluid is present in the bones of the pelvis, skull, shoulder, rib, thigh, and spine. There are two types of bone marrow cancer, multiple myeloma, and leukemia. In multiple myeloma, cancer occurs in plasma cells. Plasma cells are located in the bone marrow. In leukemia, the growth of white blood cells is affected. Acute leukemia causes rapid growth of white blood cells and chronic leukemia slows down the growth of blood cells (2). Bone marrow cancer is both benign and malignant. Benign bone tumors are called bone cysts (3), osteochondroma (4), Giant-cell tumors (5), enchondroma (6), and fibrous dysplasia (7). Malignant bone tumors that lead to death are multiple myeloma (8), osteosarcoma (9), Ewing sarcoma (10), and chondrosarcoma (11).

Tests done to diagnose bone marrow cancer include blood and urine tests, bone marrow aspiration, and imaging. Blood or urine tests can detect a specific protein associated with multiple myeloma. Blood tests can also assess kidney function, electrolyte, or other cellular levels. In bone marrow aspiration, doctors use special needles to pierce one of the bones and take a small sample of bone marrow. The specialist examines this sample under a microscope for abnormal or cancerous cells. This test is performed under anesthesia to minimize discomfort or pain. Imaging tests such as X-rays, computed tomography (CT) scans, magnetic resonance imaging (MRI), and positron emission tomography (PET) scans can be used to find abnormal or damaged bones. These tests are used intermittently during treatment to control the progress of the treatment or the progress of the disease (12).

Examining the morphology of bone marrow (BM) cell is an important cornerstone in hematological diagnosis and malignant and non-malignant diseases affecting the hematopoietic system. The use of molecular genetic methods along with cytomorphological examination is an important step in the diagnosis of many diseases. The morphological role of bone marrow cells is still pivotal and it is difficult to automate this method, which is why in a routine clinical workflow, microscopic examination and classification of single-cell morphology is still mainly performed

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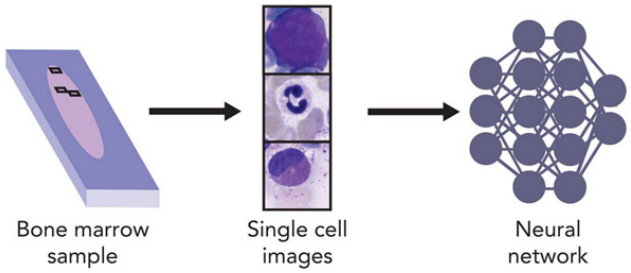

Figure 1. Schematic of convolutional neural networks in bone marrow cells.

by human experts. However, manual assessment of bone marrow smears can be tedious and time-consuming, and highly dependent on the skill and experience of the examiner. In addition, examining the morphology of individual cells is more qualitative, which makes it difficult to combine this method with other diagnostic methods that provide more quantitative data. Biomedical applications of deep learning algorithms rely on large expert-annotated datasets. Natural image classification has made significant progress over the past few years with the widespread use of Convolutional Neural Networks (CNN). The convolutional neural network is a special type of neural network with several layers (Fig 1). It processes data that has a grid arrangement and then extracts important features. A big advantage of using CNN is that it does not need to perform many processing steps on the images (13). This method has been used to detect mitosis in breast cancer tissue sections (14), skin cancer diagnosis (15), mammography evaluations (16), and cytological classifications in peripheral blood (17). However, the successful use of CNNs for image classification usually requires the availability of a sufficient amount of high-quality image and annotation data, which may generally be difficult to access by medical professionals due to the associated costs.

Among cell sources, MSCs are widely used due to their unique properties including paracrine effects, immune system modulation, low immunogenicity, and the ability to differentiate into multiple cell lines such as cardiomyocytes (18). Mesenchymal stem cells are found in various sources such as bone marrow, placenta, umbilical cord blood, and adipose tissue. Meanwhile, mesenchymal stem cells derived from fat have many advantages, including accessibility, easy harvesting, and few complications. Recently, combined strategies such as genetic induction and the use of biological agents have been developed to increase the therapeutic efficacy of MSCs (19).

In several studies using genetic induction, mesenchymal stem cells have been differentiated into induced cardiomyocyte-like cells (iCMs), in which different genes and microRNAs are used (20-23). Various studies have shown that various miRNAs are involved in cardiomyogenesis, known as cardiac miRNAs, such as miR-133, miR-499, and miR-1(24). MiR-1 is one of the miRNAs that induce the expression of Gata4 and Nkx2-5 genes and activate the Wnt/β-Catenin signaling pathway, causing the differentiation of mesenchymal stem cells into pseudocardiac cells (25).

Materials and Methods

After extracting rat bone marrow mesenchyme cells and culturing them, these cells were cultured in a culture medium containing beta-glycerol phosphate, dexamethasone, and ascorbic acid compounds for 21 days. After 21 days of cultivation, the morphology of the nucleus in osteogenic and non-osteogenic cells was examined using Hoechst's fluorescent dye (1 µg/ml), and the cytoplasm's morphology using acridine orange fluorescence dye (0.01 µg/ml). The morphological study of the cells was done using a fluorescent microscope (Olympus IX70-Japan) and after photographing with a digital camera, the diameter of the nuclei was measured and compared with the help of Motic software in both osteogenic and non-osteogenic groups.

Results

After staining the nuclei of the cells with Hoechst dye at the end of 21 days of cultivation, the size of the diameter of the nuclei in the osteogenic group (osteoblast cells) with an average of 10.05±0.28 µm and in the non-osteogenic group (mesenchymal cells) with an average of 10.01±0.39 µm did not show a significant difference at the 5% level. (Fig 2. A, B). Staining the cytoplasm of cells with acridine orange at the end of the culture period showed that non-osteogenic cells have a series of recognizable features compared to osteogenic cells. Also, the results showed that the cytoplasm of mesenchyme cells is multifaceted and their nucleus is in the central position. While the nucleus in osteoblast cells is out of the central position and stretched around, and on the other hand, the cytoplasm is also out of the multifaceted state and looks almost round (Fig 2. C, D).

Human bone marrow mesenchymal stem cells (HBMSCs) are widely used cell sources for clinical bone regeneration. Achieving a therapeutic effect depends on the osteogenic differentiation potential of the stem cells. The normal range of bone marrow morphology depends on its cellular estimation. In this way, the cellularity of the bone marrow is evaluated by estimating the ratio of hematopoietic cells to fat. A general rule of thumb is that a normal cell is between 25 and 75% hematopoietic cells. When bone marrow cells adhere to a surface and spread, their morphology changes from the initial state to a range of potential morphologies. For example, in human mesenchymal stem cells (MSC), the cells are transformed from elongated spindles into cubic or polygonal mesenchymal stem cells. In general, mesenchymal stem cells that show several large processes are called stellate morphologies. Also, the differentiation of stem cells includes changing a cell into more specialized types of cells. These changes include changes in cell morphology, membrane potential,

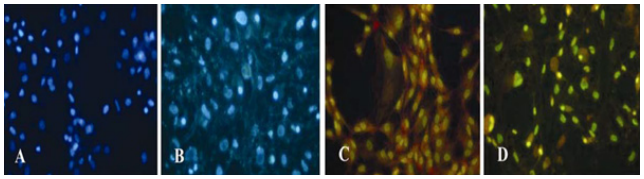

Figure 2. Examining the morphology of the nucleus and cytoplasm by fluorescent staining - staining of nuclei in mesenchyme cells in the non-osteogenic environment (A) and the osteogenic environment (B) by Hoechst dye. Staining of mesenchyme cells in the non-osteogenic environment (C) and the osteogenic environment (D) by acridine orange dye (X400 magnification).

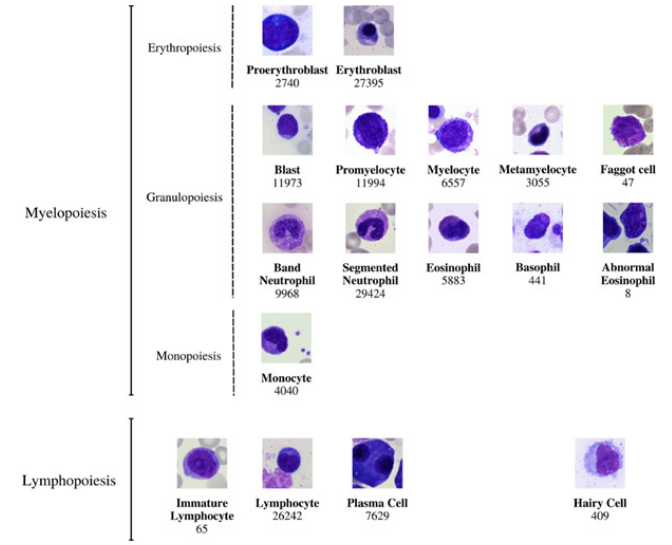

Figure 3. Morphological classification of bone marrow cells. All cells were stained with May-Grunwald-Giemsa/Pappenheim stain and photographed at 340 magnifications (27).

metabolic activity, and responsiveness to specific signals. The result of imaging to judge the morphology of bone marrow cells showed that bone marrow-derived cells show the ability to change their fate and differentiate into liver cells, endothelial cells, muscle cells, cardiomyocytes, and even neurons (26). Long et al. 2019 showed that the rapid coverage of a surface by stem cells along with the stimulation of bone differentiation minimizes the opportunity for biofilm formation while increasing the rate of integration of the device with the surrounding bone tissue (26).

Tripathi et al. in 2022 used the CoAtNet pipeline and model for the morphological classification of bone marrow cells. In this morphological classification, bone marrow cells had a similar structure. The classes were arranged into blood lineages (27). According to the standard method, the main physiological classes of myelopoiesis and lymphopoiesis, as well as common pathological classes, were included in the classification (Fig 3).

Many types of research have shown the differentiation of bone marrow cells and especially mesenchymal stem cells, which is considered one of the important advantages of these cells (28). The importance of this factor in mesenchymal cells has caused a significant increase in treatment studies and transplantation in bone lesions (28). Since the process of differentiation of bone marrow mesenchyme cells into osteoblasts is frequently done in laboratories, it is necessary to have morphological information on bone marrow cells and differentiated cells.

Discussion

In this study, cells isolated from rat bone marrow were placed in an osteogenic environment for 21 days. Examination of the appearance of the differentiated cells with fluorescent staining showed a distinct morphology of the mesenchymal stem cells in terms of the cytoplasm and the position of the nucleus, which indicated the occurrence of the differentiation of these cells. In terms of the size of the nucleus, no change was observed in these cells. In many cells, especially young cells, the nucleus is usually located in the middle of the cell, and when cells differentiate, the nucleus moves to the sides of the cell and is placed in a special place. In this study, in the differentiated osteogenic cells, the cells have changed from multifaceted to round and the position of the nucleus in mesenchymal stem cells has been pushed out of the central position and to the side. The significant difference in the appearance of the cells in the mesenchymal and differentiated state can be attributed to the difference in the organization of the cytoskeleton in these two cells. In cells of mesodermal origin such as myocytes, osteoblasts, and endothelial cells, the functional and internal structural differences of these cells cause their morphological differences after cell differentiation (29). The presence of thick bundles of actin filaments with stretch fibers in mesenchymal stem cells that are spread throughout the cytoplasm on one hand and the presence of compact and fine actin networks in osteoblasts on the other hand cause the morphological differences of these cells from each other (29, 30).

The results of this study showed that during osteogenic treatment, the metabolic activity of differentiating cells increased, which can be attributed to the protein synthesis involved in the differentiation process. In the process of osteogenic differentiation, 8 groups of proteins have been identified, and the largest group of proteins identified in the process of differentiation was related to cell metabolisms, such as Krebs cycle enzymes, amino acid metabolism, protein biosynthesis, and glycolysis (31).

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