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Large-scale erythrocyte production method established using erythrocyte progenitor cells

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By transducing two genes (c-MYC and BCL-XL) into iPS cells and ES cells, a Kyoto University research team led by Prof. Koji Eto at CiRA has succeeded in producing erythrocyte progenitor cells with almost unlimited ability to replicate in vitro, which they then differentiated successfully into mature erythrocytes. Although these erythrocytes consisted mostly of fetal-type hemoglobin, they were confirmed to have oxygen-carrying capacity and to have circulatory capacity following transfusion into mice. This technology is expected to contribute to a more reliable transfusion system by offering a new option that allows a stable supply of erythrocytes to be produced without depending on the availability of donor blood. The findings of the research team have been published in the online edition of the *Stem Cell Reports*.

Erythrocytes, which have the function of transporting oxygen within the body, have no nucleus and are therefore not capable of self-replication. As a result, patients with blood diseases that cause serious anemia are currently dependent on the transfusion of blood products prepared from donated blood. Unfortunately, the number of blood donors is on the decline, mainly due to demographic changes in Japan. Hopes for the creation of a more stable transfusion system have centered on the establishment of a technology for large-scale erythrocyte production in vitro, using iPS cells or ES cells. Up to now, however, there have been no reports of erythrocyte production in sufficient quantities for use in transfusion.

Erythrocytes are produced in the body by progressive evolution of

hematopoietic stem cells into hematopoietic progenitor cells and then erythroblasts. During the differentiation process from hematopoietic progenitor cells, chromosomes in the cell nucleus aggregate and the cell becomes a mature erythrocyte by subsequently losing this nucleus (denucleation). Hematopoietic progenitor cells, however, still have self-replication ability, and if the factors involved in this self-replication could be identified, it might become possible to induce virtually unlimited replication of progenitor cells. The research team had already succeeded in producing megakaryocytes (cells from which platelets develop) with almost unlimited replication ability, but in this latest research, aimed at creating erythrocyte progenitor cells which would have a similar almost unlimited replication ability, they transduced two genes (c-MYC, BCL-XL) into iPS cells and ES cells, which resulted in the production of erythrocyte progenitor cells with virtually unlimited in vitro replication ability.

The research showed that an increase in the expression of the c-MYC gene led to an enhancement of the replication ability of erythrocyte progenitor cells. It was also found that the expression of the BCL-XL gene was higher during the maturation process of erythrocyte progenitor cells than during their replication process. These findings indicate that these genes are important for the maturation of erythrocytes. As the c-MYC gene and BCL-XL gene are present within erythrocyte progenitor cells, methods that make use of them actually mimic the body's own system and can therefore be thought of as safer with a view to future clinical application than the previously developed systems for large-scale erythrocyte production using oncogene-derived from human papillomavirus that are not normally expressed in the human body. According to rough calculations, the amount of culture medium needed to produce enough erythrocytes for a normal transfusion pack (containing one trillion units) is 1,000 - 2,000 liters when using direct differentiation from undifferentiated iPS cells, whereas 50-100 liters is sufficient with our newly developed method. The same results were

achieved with cells placed in frozen storage and then thawed. If a more efficient denucleation method can be established along with a method for generating erythrocytes containing only adult-type hemoglobin, progress will have been made toward a more reliable supply of erythrocytes for transfusion in the future.

More information: "Immortalization of erythroblast by c-MYC and BCL-XL enables large-scale erythrocyte production from human pluripotent stem cells" Sho-ichi Hirose, Naoya Takayama, Sou Nakamura, Kazumichi Nagasawa, Kiyosumi Ochi, Shinji Hirata, Satoshi Yamazaki, Tomoyuki Yamaguchi, Makoto Otsu, Shinya Sano, Nobuyasu Takahashi, Akira Sawaguchi, Mamoru Ito, Takashi Kato, Hiromitsu Nakauchi, and Koji Eto, *Stem Cell Reports*, 2013.

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