Embryological Approach to Studies of Stem Cells

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Based on the findings of their own studies on embryonic histogenesis of human tissues and published reports, the authors determined the time limits of the existence of human embryonic stem cells and type of their determination in divergent development of tissues in different organs. Realization of genetic information of embryonic stem cells during tissue embryogenesis was studied. This information is realized by the cambium. Variability of all tissue processes is possible only within their own embryonic primordium; prospects of the mesenchyma development in an adult body is discussed.

Key Words: *embryonal stem cells; cambium; ontogenesis*

It seems that the creation of new methods of cell therapy will be one of the discoveries of the twenty-first century. The need for more profound studies of the cytophysiology of transplanted cell elements, promoting human health and longevity, became obvious.

Embryonic stem cells (ESC) existing in the body at the blastocyst stage have potential for the formation of all three embryonic germ layers (ectoderm, entoderm, mesoderm) [1] and subsequently to the development of definitive tissue. However, we should remember that for transformation of ESC into different tissues after implantation into adult body the same conditions of phylo- and embryogenesis as in the embryo should be created. The development and regeneration processes can change after transplantation of cultured ESC into an adult body. So-called regional stem cells (RSC) can be detected in an embryo at later stages, during the formation of organs and definitive tissues [3].

Embryonic stem cell early realizes the potential of divergent development directed at the formation of definitive tissues. With the appearance of the embryonal shield this cell looses its capacity to be a universal cell for all tissues and organs and acquires a new predetermination to the development of tissues and organs of a certain system. In other words, ESC enters the next phase of its preformation. Hence, cambial cells possess hereditary signs inherited from ESC. Compared to ESC cambial cells are characterized by narrower, but constant capacity to form phylogenetically determined tissues. They protect histophysiology of certain tissue groups and do not allow tissue transformation from one embryonic primordium into another. RSC should be regarded not as a universal cell giving rise to nervous, epithelial, muscular, and other tissues, but just as a cell of organs within its embryonic germ layer. Therefore it is more correct to search for potential of an adult RSC within a certain embryonic germ layer.

The potentialities of mesenchymal cells are wider. Mesenchyma is the first tissue appearing in the body. It appears when the embryo consists of three germ layer: ectoderm, entoderm, and mesoderm. The mesenchyma forms due to migration of cells from all embryonic layers (ecto- ento-, and mesoderm). The greater part of mesenchymal cells originates from the mesoderm. It consists of cells with processes and jelly intercellular substance. This first embryonic tissue performs supporting and trophic functions for all embryonic leaflets giving rise to all embryonic organs; the mesenchyma is heterogeneous by its nature.

The supporting and trophic functions of the mesenchyma in an early embryo leads to differentiation of these two functions. From this moment a series of specialized tissues develop from the mesenchyma: tissues with trophic (blood and vascular system) and

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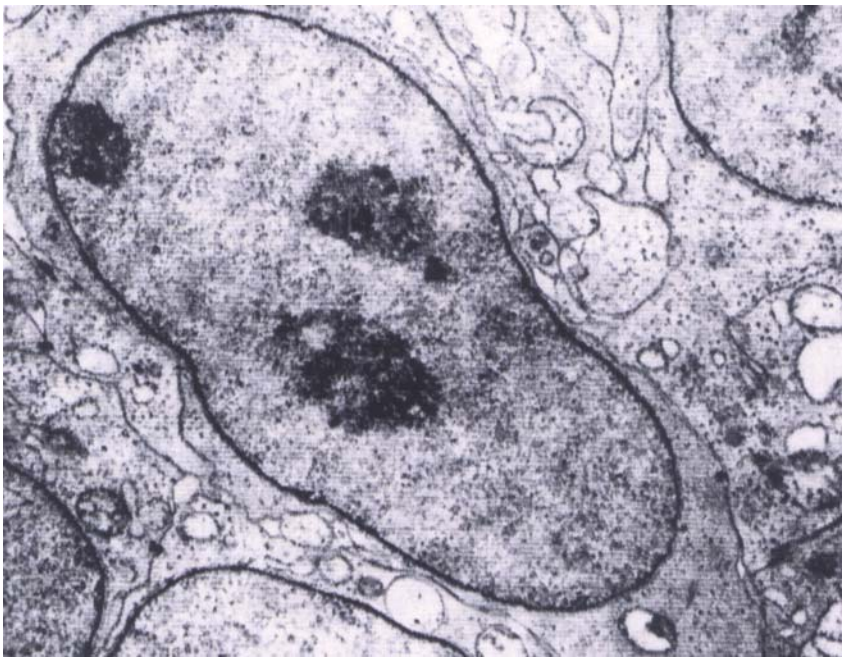

Fig. 1. Embryonic thymus on weeks 4-5 of development. Polypotent colony-forming hemopoietic cell, ×9000. (Preparation by I. I. Kalinina).

supporting functions (cartilaginous tissue, bones, smooth muscles, and all types of the most prevalent connective tissue). Hence, the mesenchyma is transformed into the corresponding differentiated tissues, retaining all its functions in the adult organism.

The cambial cells are poorly differentiated elements of any tissue capable of division. They maintain the pool of cambial cells and enable physiological regeneration with maintenance of histophysiology of all tissues [4,5,7]. Determination is a multi-step process. Hereditary information of tissues is realized by the cambium and serves as the phylogenetic defense of the organism from heterotopic growth.

Hence, cambial cells are the youngest elements of tissues. They are present in all tissues and differ from each other by their potentialities. Cambial cells of tissues developing from the mesenchyma are characterized by maximum potentialities. These tissues are called the internal medium tissues, or tissues of the mesenchymal origin. In supporting and trophic tissues cambial cells are situated among small blood vessels. Studies by A. Ya. Fridenshtein *et al.* [6] demonstrated that under appropriate conditions cambial cells (as exemplified by stromal cells) provide mutual transfer of tissues, but only within their germ layer. Therefore, the opinion that connective tissue cells can transform into hepatocytes during cell therapy of liver cirrhosis is unjustified. We found that these transformations are impossible for the epithelial stroma in the thymus. Human fetal thymus on the 4th-5th weeks of development contains polypotent colony-forming hemopoietic cells close to ESC, but not ESC. Transplantation of thymic cells from mouse embryo aged 11 days (corresponding to human 4-5 weeks) to lethally irradiated mice leads to the appearance of different types of colonies in the recipient mouse spleen: mixed, erythrocytic, megakaryocytic, and granulocytic. In mouse embryonic thymus this cell disappears on day 15 of the development, while in human thymus by weeks 10-11 [2]. Human embryonic thymic hemopoietic cell at the stage of 4-5 weeks is a large oval cell with cytoplasmic processes, basophilic cytoplasm, and numerous ribosomes (Fig. 1). This cell contains a nucleus with low content of condensed chromatin, which looks dispersed. Several nucleoli are clearly seen in the nucleus [8]. This cell, a candidate for ESC, was isolated by D. W. van Bekkum *et al.* [9] from mouse bone marrow. This structure seems to be characteristic of common ESC, which deserves a detailed study and characterization.

Thus, we can say that ESC are absent in the adult organism. The blastocyst stage with the appearance of embryonic germ layer is followed by divergent development of organs and tissues. Simultaneously, ESC enters the next stage of its determination, when its hereditary information is realized in definitive tissues to which it is transferred through cambial cells. These latter cells exhibit lability: under certain conditions they can realize structural tissue rearrangement, but only within their own germ layer. This is true for all tissues, including tissues of the mesenchymal origin. The fundamental studies of the true ESC at the earliest stages of embryogenesis should be carried out on a wider scale for effective tissue therapy. This cell is to be photographed and its cytological characteristics determined. Tissue therapy now enters into medical

practice, which necessitates studies of cambial cells and their potentialities with consideration for possible differentiation inductors. Simulation of tissue processes in animals and studies with participation of embryologists and morphologists seems to be promising.

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