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Review article

Nonhematopoietic stem cells of fetal origin - how much of today's enthusiasm will pass the time test?

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Abstract: Stem cells originating at fetal age are for many reasons superior as a material for the regenerative medicine purposes, when compared to their adult counterparts. While hematopoietic cells, isolated from fetal liver or cord blood, have been well known for a long time and have passed practical tests as clinical transplantation material, the non-hematopoietic cells are newly recognized, and the knowledge of their phenotype and differentiation potential is rather insufficient. We, and the others, have identified a subpopulation of cord blood cells phenotypically different from hematopoietic cells ($CD34^-$, $CD45^-$, $CD29^+$, $CD44^+$, $CD51^+$, $CD105^+$, SH-2, SH-3), *in vitro* plastic adherent, and capable of multilineage differentiation. The other candidates for multipotential stem cells are cells extracted from umbilical cord or placental tissue. The preliminary observations suggest, that these cells, phenotypically similar to the nonhematopoietic cord blood cells, are capable of extensive replication *in vitro* and of multilineage differentiation into a variety of tissues including cardiac muscle, bone and cartilage, adipocytes, and nerve cells. The other possible medical applications include "rejuvenation" of selected tissues and systems in senile patients, and therapeutical cloning - for both purposes, cells at the fetal stage of genetic regulation may be more useful than cells collected from adult donors. There is still, however, a high level of uncertainty concerning future medical applications of fetal stem cells. Their numbers and characteristics may differ from the preliminary observations, and their behavior *in vivo* may not fulfill the expectations originating from the *in vitro* studies. Finally, the autologous applications of stem cells collected at the stage of birth may need the involvement of technical and financial resources for the storage of frozen cell samples throughout the period of life of their potential user. Such procedure seems possible from technical point of view, but may be inadequately substantiated by the eventual advantages.

Key words: Fetal stem cells - Multipotentiality - Regenerative medicine

Introduction

Stem cells are capable both of self-renewal, and production of the progeny differentiating into a variety of tissues - for that reason they are a promising material for practical clinical applications in so-called "regenerative medicine" or "tissue engineering". Both the characteristics, and the numbers of human stem cells vary depending on the developmental stage of organism, its age, and the health status. For the practical reasons, the selection of stem cells of practical clinical value must be a compromise between their quality, availability, and the extent of medical or ethical contraindications based on the impact of the collection procedure on cell donor.

The most suitable material, considering the biological qualities, are embryo stem cells. They are to the highest extent multipotential (or even totipotential), have almost unlimited proliferative potential, and (by therapeutic cloning) they may be almost identical (HLA compatibility) as patient's cells. Unfortunately, the attempts to use embryonic cells are (at least currently) strongly criticized on the ethical basis. There also exists, although not based on the experimental data, the uncertainty, if the totipotentiality of embryonic stem cells does not increase the risk of uncontrolled growth and differentiation of their progeny in patient's organism.

Stem cells present in adult organism are at the opposite side of the scale, when compared to embryonic stem

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cells. Their number, already low in the baby, decreases several logs along with increasing donor's age. Adult cells are limited to relatively few ways of differentiation, and their proliferative potential is low proportionally to shortening of their telomere lenghts, and inactivity of the telomerase enzyme. The only hope for widening of practical clinical applications of adult stem cells is their putative "plasticity" potential - phenomenon, which after preliminary enthusiastic recognition is now increasingly questioned.

Stem cells of fetal origin (and collected at the fetal phase of development, or immediately after, during delivery), may be located in the middle between embryonic and adult stem cells. Although their density in fetal tissues is much lower than in embryo blastocyst, their total numbers exceed significantly those of embryonic origin. Their "quality" measured by the extent of multipotentiality is inferior when compared to embryonic cells, but exceeds significatly that of adult cells - the longer telomeres, telomerase activity, and much lower frequency of DNA lesions caused by cell replication and division events, or environmental mutagens, make fetal stem cells the "material of choice" for tissue engineering. Strong argument supporting the importance of fetal stem cells is complete lack of medical or ethical contraindications against the collection of those cells which reside in "waste material" - cord blood, umbilical cord, or placental tissues.

Human nonhematopoietic stem cells of fetal origin

For obvious reasons, the cells which are impossible to collect without causing serious health complications for the fetus/baby/mother are not considered as interesting from the practical point of view - this concerns mostly cells residing in fetal organs (liver, lung, brain, *etc*.). The available cell sources are these which do not affect newborn baby or mother: cord blood (blood residing in placenta and placental part of umbilical cord after baby's delivery), and umbilical cord and placenta themselves.

Nonhematopoietic cord blood stem cells

While cord blood (CB) hematopoietic stem cells are well characterized, and have been clinically applied for transplantations for almost 20 years, the existence of CB nonhematopoietic stem cells has been recognized since a few years [3, 5, 6, 22], and their frequency in cord blood is still disputable [2, 12, 13]. We, and the others [1, 3, 7] have detected non-hematopoietic (CD34-, CD45-), plastic-adherent cells, able to grow without addition of cytokines *in vitro* for a period allowing for over 10 passages. These cells are forming fibroblast-like plastic-adherent colonies, express on their surface markers similar to those on adult mesenchymal stem cells (MSC), and under proper stimulation differentiate into a variety of tissues (Tab. 1). The ability of these cells to differentiate towards hematopoiesis is disputable, since it was confirmed only by one group [12], and not reported by the other groups investigating their differentiation potential. Our experience supports the suggestion of inability of MSC-like cord blood cells to differentiate into hematopoietic cells [3], implying the nonexistence of "plasticity" phenomenon in our experimental conditions.

When summarizing the "positive reports" concerning the nonhematopoietic stem cells in cord blood, it seems that these cells are a perfect material for the future clinical applications. They are not only able to differentiate into a large variety of tissues *in vitro* or *in vivo*, exceed adult MSC in their proliferative potential (longer telomeres, telomerase activity), but also are a unique cell population which may be collected in good shape (high numbers, high viability, and low risk of contamination) without any ethical, medical, or technical problems. Following the optimistic conclusions from *in vitro* observations, preliminary reports confirming the beneficial effects of cord blood cells in treatment of brain stroke, heart infarct, and possibly other incurable so far diseases were published [9, 10, 28]

The other, however, not so widely recognized reports, are questioning the applicability, or even existence of nonhematopoietic cord blood cells. Multiple groups failed to identify these cells in mature delivery cord blood [8, 15, 16, 25], reported their presence only in mid-trimester fetal blood [4, 27], or demonstrated the presence of cells in only small faction of cord blood collections. In our experience, since detecting by us the non-hematopoietic CB cells in the year 2000, till now, we have failed to optimize and improve the method of their selection and expansion. Several groups reported the frequency of MSC-like cells in CB samples between 0% and 60% [2, 12, 27], in our experience (unpublished data), the frequency of MSC-like cells in CB collections does not exceed 20%. We hypothesize that the low frequency of the non-hematopoietic cells in CB samples reflects the biological phenomenon, rather than being an effect of inadequate research methods. The mechanism responsible for the phenomenon may be the insufficient mobilization of these cells during delivery. It can not also be excluded that the MSC-like cells are naturally present in circulation in earlier phases of fetal life only (an equivalent of hematopoietic cell migration phase?), and collection of these cells during on-time delivery is possible only due to the last remnants of these cells present in small percentage of cases. Irrespective of the mechanism responsible for the low percentage of CB samples containing MSC-like cells, this phenomenon, if confirmed, may substantially reduce the practical importance of CB nonhematopoietic stem cells for clinical applications.

Table 1. Characteristics of cord blood MSC-like cells, according to selected publications

Parameter	Characteristics [2, 3, 6, 12, 13]
Morphological characteristics	$CD14^-$, $CD33^-$, $CD34^-$, $CD45^-$, $CD49^-$, $CD50^-$, $CD62^-$, $CD106^-$, $CD117^-$, glycophorin A^-, $CD133^-$, $CD135^-$
	plastic-adherent, $CD13^+$, $CD29^+$, $CD44^+$, $CD49^+$, $CD51^+$, $CD73^+$, CD90/Thy-1^+, CD105/SH-2^+, SH-3^+, SH-4^+, telomeres $^{+++}$, telomerase$^+$
Differentiation potential	osteogenesis, adipogenesis, chondrogenesis, neurogenesis (neurons, oligodendrocytes, astrocytes), myogenesis (myocardium), hepatocytes (*in vivo*)*, hematopoiesis*

* reported only by [12]

Table 2. Characteristics of umbilical cord MSC-like cells, according to selected publications

Parameter	Characteristics [17, 18, 22-24]
Morphological characteristics	$CD34^-$, $CD45^-$
	plastic-adherent, $CD29^+$, $CD44^+$, CD51+, CD90/Thy-1^+, CD105/SH-2^+, SH-3^+
Differentiation potential	osteogenesis, adipogenesis, chondrogenesis, neurogenesis, myogenesis (myocardium)

Table 3. Characteristics of placental MSC-like cells, according to selected publications

Parameter	Characteristics [26]
Morphological characteristics	$CD14^-$, $CD34^-$, $CD45^-$, $CD117^-$, $CD133^-$
	plastic-adherent, $CD29^+$, $CD44^+$, CD90/Thy-1^+, CD105/SH2^+, SH-3^+, SH-4^+, ESC-associated CM^+(SSEA-4, TRA-1-60, TRA-1-18)
Differentiation potential	osteogenesis, adipogenesis, neurogenesis, myogenesis (myocardium)

Nonhematopoietic stem cells from umbilical cord or placental tissues

The presence of fibroblast-like cells, extracted from umbilical cord Wharton's jelly, and their capability to grow *in vitro* in plastic-adherent manner, was described relatively long time ago [11, 17]. The early finding, reported and patented [19, 21], was the observation confirming that these cells are able to differentiate into cartilage. More recently, cells extracted from Wharton's jelly or perivascular region of umbilical cord were reported as multipotential stem cells, capable of differention into many tissues both *in vitro* and *in vivo* [18, 23, 24]. These cells, similarly to CB nonhematopoietic stem cells, are of fibroblast-like morphology, and both their phenotype, and the differentiation capabilities, are very similar to their counterparts present in cord blood (Tab. 2).

The slightly different, than umbilical cord, nonhematopoietic stem cell source is also the human placenta - a successful isolation of such cells from 10 out of 16 specimens (62.5%) was reported [26]. Both umbilical cord, and fetal-origin tissue from placenta, may be a source of very similar stem cells - it may be hypothesized, if these cells are migrating from fetal circulation, or are remnants of the stem cells responsible for the umbilical cord and placenta formation. The phenotype and differentiation potential of placenta-derived cells (Tab. 3) do not differ substantially from umbilical cord or CB cells - the detailed comparison is impossible due a scarcity of the published data.

Will the nonhematopoietic fetal stem cells "behave" in future according to the preliminary hopes?

Currently there is a high dose of official optimism concerning the future medical applications of stem cells in general, and, among them, the nonhematopoietic fetal stem cells. These cells, being probably the fetal version of MSCs residing in adult bone marrow [14, 20], seem to be capable of multilineage differentiation (at least into bone, cartilage, hematopoietic microenvironment (adipocytes), muscle, nerve cells (neurons, astrocytes, oligodendrocytes), and possibly more other cells and tissues. The experimental data are, however, preliminary, mostly deriving from *in vitro* experiments, and frequently being obtained rather from the research of mixed cell populations than of single cells or cell clones. The other uncertainty results from conclusions drawn from the analysis of morphology and phenotype of maturing cells, rather than from the analysis of their functions. The early stage of research does not exclude several risks of incorrect interpretation of the experimental data: (1) some results may be flawed due to erroneous interpretation of some cell features (morphology, markers), and the cells being the object of the study may not be able to function properly *in vivo*; (2) some effects of *in vivo* application of stem cells may result not from proper repair of the injured tissue by the stem cells themselves, but by regulatory role played by them - this may not decrease the value of therapy, but may change the role of the applied stem cells from "tissue producer" to some kind of "cellular vaccination"; (3) the critical data concerning stem cell existence, frequency, numbers, function, *etc*., are in some way discriminated - the common feeling is that authors getting "worse" results are using "worse" techniques. This "over-enthusiasm", accompanying regularly all new and important scientific breakthroughs, needs to take longer time for data verification. Finally - if fetal cells have to be used for autologous treatment, the long-term storage (probably throughout all the lifetime of their donor) should be con-

sidered - the task being technically possible, but disputable on the basis of the cost/effect ratio. All the uncertainties mentioned above do not change, however, the fact, that for many reasons fetal stem cells seem to be superior to their adult counterparts, and, proportionally to the overall importance of non-embryonic stem cells, will play significant role in medical applications.

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