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REVIEW OF THE 3rd IPLASS MEETING: TOWARD CLINICAL APPLICATIONS OF PLACENTAL AND ENDOMETRIAL STEM CELLS

From 10 to 12 September the 3rd IPLASS Meeting: toward clinical application of endometrial and placental stem cells took place in Granada (Spain). The organizer of the symposium was the International Placenta Stem Cell Society (IPLASS) headed by President Dr. Ornella Parolini (Italy). There were presented 29 oral reports and 25 poster presentations. Scientific and clinical institutions in Italy, United States, Australia, Austria, Netherlands, Sweden, Israel, India, Portugal, Spain, France, Argentina, Brazil, Japan, China, Chile, and Germany are engaged in research of characteristics of stem cells derived from the placenta. Scientists from Ukraine (Institute of Cell Therapy, Kyiv) presented three reports, which scientific value was awarded with a diploma and a prize.

The significance of the placenta, as a organ, is clear; however, many aspects of its functioning became known only during the last 5-7 years. Main research areas included qualitative and quantitative characteristics of the isolated placental cells, evaluation of the effect of their manufacture and storage conditions, as well as the results of pre-clinical and clinical trials of cell preparations. Many studies have demonstrated that placental multipotent stromal cells (*MSCs*) have unique properties: they are able to differentiate in culture into adipocytes, osteocytes and chondrocytes; exhibit immune-regulatory properties and affect many cellular processes. MSCs isolated from chorionic villi (*hCV-MSCs*) and amniotic membrane (*hAMSCs*) have typical for *MSCs* markers (*CD105*, *CD73*, and *CD90* expressing, in the absence of hematopoietic markers). Karyotyping, comparative genomic hybridization, and genotyping testify to high stability of genome, after growing of these cells in culture.

Taking into consideration the co-authorship of the submitted reports, it becomes clear that these are very thorough studies and they were held mainly in joint grants by scientists from various research centers. In particular, J. Patel's report, presented the results of a researchers group from the Center for Clinical Research University of Queensland (Australia) and the School of Medicine of the National University of Singapore (Singapore), has shown that one human placenta can provide the same number of endothelial colony forming cells (*ECFCs*) as 27 donor cord blood units. Cultured cells did not differ from the native by proliferative potential, had minimal differences in the gene expression profile and were capable of restoring limb ischemic injury in mice.

Quite recently it was shown that the placenta is the organ of hematopoiesis because the comparative study of the properties of hematopoietic cells derived from bone marrow, cord blood, fetal liver and placenta of different gestational periods has not only scientific value, but also a great interest in terms of their practical application in clinical practice. These studies were carried out by a team of Ukrainian scientists and presented in the report of M. Kuchma. They state that the placental hematopoietic cells are similar to the fetal liver cells by the expression of surface markers and contain more progenitor cells than the cord blood. In addition, hematopoietic cells isolated from the placenta and frozen by the authors' program do not differ from cells isolated from the non-frozen placenta by their properties and repopulation potential. Furthermore, the placenta can provide more hematopoietic cells than one sample of cord blood. Therefore, the placenta may be an additional source of hematopoietic cells. That is important in the treatment of oncohematological disorders.

To shed some light on the mechanisms of regenerative ability of human mesenchymal stem cells (*hMSCs*) *in vivo* C. Wu and L. Deng (Sichuan University, China) applied long-term monitoring methods for tracking of placental decidua basalis *MSCs* (*PDB-MSCs*) using carbon radioisotope labeling thymidine (^{14}*C-TdR*). Also there were investigated following characteristics of those cells: morphology, colony forming ability, the differentiation potential, karyotype and cell cycle. To study $1 \cdot 10^6$ ^{14}*C-TdR* labeled *PDB-MSCs* were injected intravenously into mice and the organs were examined after 1, 2, 3, 5 and 30 days. Radioactive PDB-MSCs were mainly found in lungs, spleen, liver, stomach, left femur and bone marrow of the recipient. This work argues that ^{14}*C-TdR* labeling does not change the biological characteristics of human *MSCs*, and this method can be applied to the quantitative determination of cell fusion in the long-term preclinical studies.

H. Kaipe et al. (*Karolinska Institutet*, Sweden) examined the therapeutic potential of decidual stromal cells (*DSCs*) in patients with junctional epidermolysis bullosa, Herlitztype. This disease is caused by a deficiency of laminin-332 and leads to life-threatening damage of skin and mucosa. Infusion of amniotic membrane cells and *DSCs* can improve the healing process of blisters, severe wounds on elbows, in the groin and on the face of the patients. But at the same *DSCs* can cause the formation of multispecific anti-HLA antibodies in patients with a normal immune system, as well as possible risk of alloimmunization of immunocompromised patients.

The report of *V. Shablii* et al. (*Institute of Cell Therapy*, Ukraine) was devoted to the study of phenotype and therapeutic properties of multipotent cells of the trophoblast (multipoten throphoblas cells – *MTCs*), which were characterized by flow cytometry, immunocytochemistry and *qRT-PCR*. Placental tissue, before cells isolation, was cryopreserved according to the program developed by the authors. To study the therapeutic properties of *MTCs* in damaged heart tissue there was used isoproterenol-induced cardiomyopathy model in *FVB* mice. It was first discovered that placental *MTCs* expressed mesenchymal and trophoblast markers simultaneously and contained cytokeratin7-positive and CK7-negative- progenitors. Cells isolated from frozen tissue had an immunophenotype similar to the one of native tissue. After intravenous injection into animals with cardiomyopathy, cells were detected in the myocardium on the 2nd and 28th day. This work is of a great interest, since no one has ever described the presence of trophoblast stem cells in the placenta.

The report of *A. Svitina* et al. (*Institute of Cell Therapy*, Ukraine) presented the results of the effect of human trophoblast stem cells (*hTSCs*), isolated from the placenta, in the treatment of colorectal cancer in rats. There was investigated a phenotype of native and

cryopreserved *hTSCs*. Allogeneic intravenous cell transplantation, in patients with dimethylhydrazine-induced colorectal cancer, suppressed inhibition of tumor growth with a dose-dependent effect. At the same time, xenogeneic transplantation had no effect.

Several reports and posters were devoted to the study of stem cells isolated from amniotic fluid and amniotic tissue. The inner layer of human amniotic membrane (*hAM*) consists of fetal membranes, which are usually utilized after the birth, as a part of the placenta. It is translucent and very flexible structure without nerves, muscles, lymph vessels, consisting of the amniotic epithelium and amniotic mesenchymal stromal cells in avascular mesenchyme. *hAM* has several important properties: bacteriostatic, anti-angiogenic, analgesic, antifibrotic, anti-inflammatory effects, promotes wound healing and epithelialization, exhibiting low immunogenicity.

Some studies demonstrated anti-cancer effect of human amniotic membrane. They are more recent preclinical studies, since the cellular mechanisms responsible for the anti-cancer properties of *hAM*, are still poorly investigated. In the work presented by *A. Mamede* et al. (*University of Coimbra*, Portugal), there was investigated the anti-cancer potential of hAM extract (*hAM-E*) on human hepatocellular carcinoma and found that the cell line HepG2, is more sensitive to *hAM-E* treatment, than cell line HuH7. It appears that the obtained preparation can have multiple targets in cancer cells. It is concluded that the extract has important role, and it is necessary to connect received information with the observed cellular mechanisms in the future, thus figuring out the potential of *hAM* in oncology.

In another study, *S. Guerra* et al. (*University of Coimbra*, Portugal) have shown that human amniotic membrane secretes several cytotoxic cytokines and interleukins. Factors, secreted by *hAM*, can not reduce the metabolic activity of the cell lines. Nevertheless, the conditioned culture medium (*CCM*) AM enhances the effect of 5-Fluorouracil, doxorubicin and sorafenib in *HuH7 cells*. On the other hand, the *CCM* inhibits cytopathic effect of cisplatin on *HuH7cells*. Similar results were also observed in *HepG2* cells. In *Hep3 B2.1-7* cell line *CCM* potentiates the action of doxorubicin, but inhibits the effect of cisplatin and does not have the ability to stimulate or inhibit the effect of 5-Fluorouracil and sorafenib. Apparently, the *CCM* has different effects on cells. It is important not only to prove that *CCM* may increase the effects of conventional chemotherapy, but also to determine the impact pathways of *hAM* on carcinogenesis.

L. Verbeeck et al. (*University of Antwerp*, Belgium) studied the interaction of hAM with stem cells, derived from human adipose tissue (hASCs), which are important for tissue engineering. There were tested various methods for isolating stem cells from amniotic membrane, and the first joint culture of hAM with hASCs was created.

Since 2007, the Red Cross Blood Transfusion Service of Upper Austria has been providing cryopreserved amniotic membrane according to several indications from tissue banks to some hospitals. In preclinical studies of *S. Hennerbichler et al.* (*Linz*, Austria) cryopreserved *hAM* was tested as anti-adhesive coating by eye disease. Antifibrotic properties have been shown in a model of liver fibrosis in rats. There were positive results in clinical studies of amniotic membrane for coverage of split skin donor sites covering instead of burns, as well as other promising areas of *hAM* application (e.g. treatment of orbital wall adhesions, oronasal fistulas etc.).

To reduce the potential risk of postoperative infections after transplantation of amniotic membrane *I. Lindlbauer et al.* (*Red Cross Blood Transfusion Service of Upper Austria, Linz*, Austria) proposed parallel microbiological testing with different methods and materials to increase the sensitivity by minimizing the sampling error.

T. Gualdi and al. (*University of Franche-Comté*, France) tried to use human amniotic membrane as a promising therapeutic drug for the bone restoration. It is concluded that its osteogenic differentiation has no significant effect on the immunogenicity; and native *hAM* can be used to restore the bones. Also, the authors have defined the criteria for amnion banking according to influence of storage conditions on the properties of the cells.

In the *E. Menni Research Centre* (*Brescia*, Italy) there was analyzed the structural and ultrastructural *hAM* level to better understand the morphology, function, and the possibility of using its cells for tissue engineering. In particular, from the centre toward the periphery of *hAM*, the surface epithelium changed from squamous stratified, with a exfoliating cells, to a monolayer of cubic and cylindrical cells with secretory activity. Toluidine-blue staining was shown the presence of lipid droplets in both epithelial and mesenchymal cells.

M. Marongiu et al. (*Università degli Studidi Cagliari*, Italy) showed that amniotic epithelial cells (human amnion epithelial cells – *hAECs*), derived from human placenta, may be used for the liver regeneration. Previous studies on the model of rat liver repopulation showed that they can differentiate into hepatocyte-like cells and mature hepatocytes after transplantation. The authors suggested that this process may occur without fusion of donor cells with host cells.

Stem cells, derived from amniotic epithelium, showed high recovery potential in the treatment of various pathological conditions, including spinal cord injury (*S. Venkatachalam et al.*, *University of Madras*, India). As the cells of the fetal origin, hAECs demonstrate high speed division and plasticity, as well as immune tolerance and anti-inflammatory properties. There were proved their immunomodulating properties in suppressing of lymphocyte proliferation.

However, according to *GMP*, *hAECs* for clinical use should be cultured in medium without xenobiotics and immunosuppressants. The report of *S. Zannini et al.* (*University of Bologna*, Italy) showed that serum-free culture conditions did not change the characteristics of *hAECs*. In addition, the immunomodulatory properties of *hAECs* do not depend on the conditions of cultivation, and 3D-protocol does not affect the differentiation of cells in culture. These observations suggest that serum-free medium can simplify the *hAECs* transition from laboratory studies to clinical application.

The work *G. LaRocca* et al. (*University of Palermo*, Italy) demonstrated that mesenchymal stem cells of Wharton's jelly (Wharton's jelly *MSC – WJ-MSCs*) can differentiate into various cell types, and have anti-inflammatory and immunomodulatory properties, which makes them promising tool for regenerative medicine. There was studied the applicability of stem cells from umbilical cord Wharton's jelly for the treatment of encephalopathy in preterm rats. The report stated that intracranial transplantation of human *WJ-MSCs* suppresses accompanying histologic changes in the damaged brain presumably due to the mechanisms of neurotrophic factors secretion and suppression of the inflammation, accompanying a hypoxic-ischemic brain damage.

Organizing Committee has set up a commission that analyzed all the reports and poster presentations. At the closing session three best presentations of young scientists were awarded with diplomas and prizes. In particular, one of the best reports was acknowledged the report of Volodymyr Shablii from Ukraine.

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