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Perinatal Stem Cells Revisited: Directions and Indications at the Crossroads Between Tissue Regeneration and Repair

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Perinatal stem cells research attracted great interest worldwide in recent years. Foetus-associated tissues contain various populations of stem cells, most of which are comprised within the category of mesenchymal stem cells (MSCs). This special issue collects both reviews and original reports on all the perinatal stem cell types which are currently under investigation. These cells have multiple promising features: differentiative capacity towards mature cell types of all the three germ layers, hypoimmunogenicity *in vitro* and *in vivo*, ease of sourcing, *ex vivo* culture and storage. In particular, immune modulation is viewed as a promising feature of many MSCs populations, since these cells, once administered therapeutically, may be able to overcome, or at least evade, the host immune response which may lead to acute or chronic rejection of the transplant [1].

In the first paper of the issue, Silini *et al.* discuss the therapeutic potential of amnion-derived cells for the treatment of inflammation and fibrosis [2]. In particular, the authors did analyze the published literature on the potential effects of the amnion and its cellular components within the inflammatory-fibrotic scenarios which are featured in several diseases, and the factors that could be involved in the observed immunomodulatory actions. Moreover, based also on their previous seminal works, the authors compared the different disease indications of human amnion so far, as well as describing the major anti-inflammatory and anti-fibrotic molecules secreted by amniotic cells.

Between the disease indications of amnion-derived cells, liver disorders occupy a preminent position. Orthotopic liver transplantation is still the obliged clinical treatment for end-stage diseases, even if the shortage of donor livers and the lack of a reliable intra- or extra-hepatic cellular source to derive mature hepatocytes, are key elements which push ahead the research on this topic [3, 4]. To this regard, Vaghjani *et al.* comprehensively reviewed the use of placenta-

derived stem cells for hepatocyte-like cells derivation and transplantation [5]. The authors summarized the literature related to the differentiation of human placental stem cells into hepatocyte-like cells (both *in vitro* and *in vivo*) and on the characterisation of the differentiated cells. In addition, conspicuous room is dedicated to the experiments aimed to testing the functionality of the hepatocyte-like cells in pre-clinical animal models of liver disease. The authors also analyzed the biomaterials used for culturing and transplantation of these cells into extra-hepatic sites.

Regenerative medicine approaches for central and peripheral nervous system diseases represent a challenging and viable option for perinatal stem cells [6, 7]. In this issue, Vawda and Fehlings reviewed the current state of the use of mesenchymal stem cells in spinal cord injury (SCI) therapy, and the future perspectives [8]. In particular, the authors analyzed *in vitro* studies and *in vivo* models which collectively suggest that mesenchymal or stromal cells, regardless of derivation, may act through the provision of trophic support and inflammatory modulation. The authors also summarized the potential molecular mechanisms behind these effects. As recognized by the authors, the use of perinatal stem cells in the most recent clinical trials for SCI is growing reaching that of BM-MSCs which currently top the list. As also suggested by the authors, the potential of perinatal MSCs has to be fully exploited in terms of definition of their secretome, since the bioactive compounds secreted by these cells are viewed as the main agents of their beneficial effects in SCI and other central nervous system diseases [8].

Cardiovascular diseases are another key clinical option for perinatal stem cells. Post-infarct chronic heart failure and myocardial infarction represent complex diseases where not only the number of functional myocytes is reduced, but also the biology of other key populations, as endocardial endothelial cells, is noticeably modified, contributing to the so-called "endothelial dysfunction" [9, 10]. Despite the great research efforts of the last years, which allowed the characterization of heart-resident stem/progenitor cells, also with features of MSCs [11, 12], the vast range of biological actions of perinatal stem cells strikingly emerges as a valuable option. In the

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last review paper of this special issue, Corrao *et al.* analyzed the literature on the use of different stem cell populations for cell therapy of heart diseases, making the case for Wharton's jelly cells (WJCs) as capable to provide key support to the failing organ in terms of expression of immunomodulatory molecules and ability to differentiate towards cardiomyocyte-like cells [13]. The consensus of most studies and clinical trials is that the effects of MSCs administration in heart diseases are mainly of brief duration: to this regard, in the first original paper of this special issue, Lopez and co-workers did compare the long-term effects of cellular therapy in a myocardial infarction (MI) rat model, by using both BM-MSCs and WJ-MSCs [14]. In particular, WJ-MSCs were able to form beating cells after coculture experiments *in vitro*, and when applied *in vivo*, a significant improvement in ejection fraction was seen in animals that received MSCs at 25 to 31 wks time-points after injection. Collectively, the authors presented results which are in agreement with previous reports, with the additional promising evidence that the beneficial effects on cardiac functions are durable, thus increasing the potential importance of WJ-MSCs in cell therapy for MI.

A further research paper by Balci and Can explored the efficiency of different cryopreservation conditions for WJ-MSCs storage [15]. This is an important point since the main prospected use for these cells is as allogeneic "off the shelf" therapeutic option, when stored in dedicated tissue banks [16]. The authors analyzed the outcomes of different methods, as computer-controlled multi step slow freezing and vitrification, and assessed the maintenance of the key phenotypical features of WJ-MSC (expression of "core" MSC markers and HLA-DR) upon de-freezing and culture. Their data strongly point to slow freezing as the best method with the addition of dimethylsulfoxide or sucrose depending on the rate chosen for the freezing program [15].

Several reports highlighted that stem cells can be found in the human amniotic fluid throughout gestation. Amniotic fluid stem cells (AFSCs) are free of ethical constraints, can be readily isolated during prenatal diagnostic procedures, and showed promising features for regenerative medicine applications [17, 18]. In this issue, Moschidou *et al.* presented novel results on the molecular profile of AFSCs. In particular, authors investigated whether 1st and 2nd trimester c-KIT⁺ AFSCs could show similar gene expression profiles [19]. As reported, even if both populations are clearly related, differences have been recorded in cell-specific gene expression signatures, which comprise 366 genes for 1st trimester and 340 genes for 2nd trimester AFSC. These data suggest that the phenotype of AFSCs populations is evolving during fetal development, with a reverse correlation between lower levels of genes associated with the undifferentiated state and higher levels of genes involved in the specification of cells and tissues [19]. In our opinion, banking of these cell populations and the development of future applications will benefit from these new data, which may push forward the research of other groups involved in the same topic.

Multipotent MSCs (along with the CD34+ hematopoietic stem cells) are also present within human umbilical cord blood (hUCB). The adherent fraction of hUCB cells constitutes the subpopulation enriched in MSCs. In this issue,

Grabowska *et al.* tested the myogenic potential of adherent fraction of human umbilical cord blood cells (adhUCBs) [20]. Interestingly, adhUCBs cells expressed Pax7 and myogenin, suggesting their myogenic potential. The authors also showed that muscles injected with adhUCBs cells had higher muscle mass when compared with controls. In conclusion, this paper represents a first evidence of the potential use of adherent fraction of hUCB cells as myogenic cells.

Immune features of MSCs evidenced these cells as potential off the shelf therapeutics for many diseases. The range of immune-related molecules which are expressed by these cells grows constantly [21, 22]. However, only few reports do exist in the literature concerning the immunogenicity of differentiated cells with respect to the features of the undifferentiated parental cells. To this regard, Tee *et al.* present a research paper which deals with the differentiation of amniotic epithelium cells (AECs) towards hepatocyte-like cells (HLCs), and the functional and immune features of the same [23]. Naïve hAEC have low immunogenicity and exert immunomodulatory effects that may facilitate allogeneic transplantation [24]. The authors found that AEC-derived hepatocyte-like cells showed immunomodulatory properties and inhibited mitogen induced PBMCs proliferation *in vitro*, similarly to undifferentiated cells. The authors also reported that, similarly to what happens in AECs, IFN- γ challenge results in elevation of class I HLA and CD40, as demonstrated for other stem cell populations, modifying the *in vitro* immunogenicity of HLCs [23]. Therefore as also the authors stated, *in vivo* studies on animal models of acute and chronic liver diseases will be needed to assess the functionality, immunomodulatory effects and immune responses by the recipient to the HLCs in animal models of acute and chronic liver failure to explore the utility of HLCs for allogeneic transplantation. Moreover, in our opinion, further studies may be encouraged to assess the maintenance of the immunomodulatory features of undifferentiated stem cells also in the differentiated progeny of other stem cell types.

To this regard, Anzalone *et al.* [25] present an *in vitro* study on the expression of immune-related molecules in undifferentiated WJ-MSCs and their differentiated progeny, after osteogenic, adipogenic and chondrogenic differentiation. This is a further seminal work on this topic where the authors demonstrated for the first time that differentiated WJ-MSCs do maintain the HLA setting of the parental cells, also for the non-classical type I MHC molecules as HLA-E, HLA-F and HLA-G. In addition, the differentiated cells did not upregulate the expression of the B7 co-stimulators CD80 and CD86, while maintaining the expression of the immunomodulatory molecule CD276 (B7-H3). The findings are useful in better defining the immune phenotype of WJ-MSCs-derived cells, and in particular for the use of these cells in musculoskeletal diseases therapy [26].

In conclusion, the papers of this special issue do provide key references to the scientists in the field by both critical revision of the literature in selected topics, and providing new advances which further encourage research on the potential of perinatal stem cells.

In our opinion, what emerges from these reports is that on one hand there is the need of pushing on the *in vitro* char-

acterization of these cells, in order to satisfy the requirement for safety in cell therapy applications. On the other hand, these cells demonstrated maturity also for further challenging *in vivo* applications which could provide the definitive proof of their usefulness in regenerative medicine applications. Moreover, the emerging evidence that differentiated cells may express the same immunomodulatory molecules sets as their undifferentiated counterpart, opens new scenarios in the “side branch” of regenerative medicine which is called reparative or support medicine. It can be prospected that perinatal stem cells effects may be also related not only to direct organ repopulation, but to the supportive action on diseased organs. A greater understanding of the bioactive components of the secretomes of undifferentiated and differentiated stem cells will enable a more informed use of these cells and/or their therapeutic derivatives to target specific diseases. It is expected that an immunomodulatory differentiated cell, *in vivo*, may better overcome host responses, provide a functional bridging action by replacing the function of local diseased cells, and act on the organ microenvironment by modifying the underlying pathological condition (e.g. chronic inflammation) at the basis of disease. Searching for this cell may be the target of researchers worldwide, and we are convinced that the perinatal tissues may host stem populations with these features.

We are glad and thankful that a panel of renowned scientists, comprising pioneers in the field, has joined our project contributing to this special issue on perinatal stem cells contributing reviews on the most recent data on different topics that may have a strong impact on the future of regenerative medicine, as well as original papers which further move on the targets of the use of these cellular populations. It is our hope that science reported in this special issue may contribute to further key developments within this field.

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CONFLICT OF INTEREST

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