The amniotic fluid-derived cells: the biomedical challenge for the third millennium

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Introduction

The definition of stem cells (SC) is actually one of the big debates, far to be solved, in the scientific community. There are, however, some characteristics which are common to all of them: self-renewal, ability to generate at least one daughter cell with characteristics similar to the initial cell, multilineage differentiation potential from a single cell, and capacity for functional reconstitution of a specific tissue (Weissman, 2000). Up to date, nevertheless, the best-known SC is the zygote with the ability to induce the formation of an entire living organism and therefore, due to this capacity to be defined totipotent. Following cell divisions make the fertilized egg lose progressively some of its totipotency. In fact already at the blastocyst stage, embryonic stem cells (ESCs) isolated from the inner cell mass lose the totipotency, but are still able to differentiate into the three germ layers. These SCs are at this stage considered pluripotent (Eckfeldt et al., 2005). An increasing number of SC types have been described in the literature, derived from embryonic, fetal or adult origin. Based on the self-renewal and differentiation abilities, embryo-derived SCs are hierarchically higher than other cell types that have more restricted properties. Taking for a wile apart the scientific knowledge and the importance of stem cells research there are also ethical and political problems among different countries for stem cells research. Considering this latter remarks nevertheless as written by Prof. Lee, professor of molecular biology, at Princeton University, on Nature: "..if genes and genomes represent one of the pillar of the biomedical progresses on the XXI century, the second one is the stem cells biology".

Amniotic fluid-derived stem cells

Human amniotic fluid cells (H-AFC) have been used as a diagnostic tool for the prenatal diagnosis of fetal genetic anomalies for more than 50 years. Furthermore indications that amniotic fluid may contain cells that are not fully differentiated were reported in the early 1990s when small nucleated cells, which were identified as hematopoietic progenitors, were detected (Torricelli et al., 1993). After this evidence several other scientific novelties as been brought out to the attention of the scientific community up to the 1999 when Mosquera and co-workers (Mosquera et al., 1999) established a milestone in the amniotic fluid stem cell history. In fact they demonstrate that these cells possess pluripotent properties due to the telomerase activity. In these brief history of the H-AFC the last but not least evidence, provided in the last 5 years, suggests that they can also harbor a therapeutic potential for human diseases.

Amniotic fluid cells represent a very heterogeneous population that include both cell type derived from fetal membranes and of the fetus itself. In order to give a classification of the different cells populations, the morphological, biochemical and growing features are widely used. According to these latter issues they can be divided into three main groups: epitheloid E-type cells, amniotic fluid specific AF-type cells and fibroblastic-type cells (Prusa and Hengstschlager, 2002; Gosen, 1983; Hoehn and Salk, 1982). Nevertheless, in general, H-AFC can be classified like having mesenchymal stem cells characteristics. In fact these stem cells were the first to be described; they display the higher proliferation and differentiation plasticity of adult mesenchymal stem cells and are able to differentiate towards all the three germ layers cells populations. Amniotic fluid stem cell lines have a typical doubling time of about 36 h and no need for feeder layers, cells maintained for over 250 population doublings retained long telomeres (Mosquera et al., 1999) and a normal karyotype, do not form tumours when injected *in vivo*.

H-AFC have shown to be cells derived from both embryonic and extra-embryonic tissues. Moreover these type of specific cells lineages can be found in a differentiated and undifferentiated step. The types and characteristics of H-AFC can nevertheless varying with parameters such as: gestational age and presence of fetal pathologies. Recently many literature data have demonstrated the presence of fetal mesenchymal stem to possess a great differentiation capacity to induce cellular types derived from the three germ layers. In particular, since 2003, it has been demonstrated that the amniotic fluid contains stem cells which are positive for the pluripotent marker Oct4 (Prusa, 2002) and for mesenchymal markers CD29,CD44, CD73, CD90, CD105, able to differ in a osteogenetic and adipogenetic sense (Anker et al., 2003; Tsai et al., 2004). The demonstration that the differentiation of dissimilar cellular types (adipogenic, osteogenic, miogenic, endothelial, neurogenic ed hepatic) can be obtained by a unique cell which is positive for mesenchymal markers and negative for hematopoietic ones (De Coppi et al., 2007) has been fundamental to

prove that the amniotic fluid contains pluripotent stem cells.
The potential of H-AFC to induce the differential toward cell types of the ECTODERMAL layer are coming from studies related to neurogenic lineages, whereas for the MESODERMAL lineage the differentiation into osteoblast, fibroblast, adipocytes, chondrocytes and endothelial cells, were assessed. Currently, instead, very little information exist for what concerns the ENDODERMAL differentiation potential.

ECTODERMAL lineages – For what regards this lineage data on the potential capacity of H-AFC to became ectodermal-type population are still controversy. In fact Tsai and co-workers (2004) demonstrate that H-AFC can be differentiated into ectodermal neurons when culturing under b-mercaptoethanol and bFGF conditioned media together with the data presented by Prusa et al. (2004), which did not observed neuronal differentiation of amniotic fluid-derived cells in response to neurogenic differentiation medium. Nevertheless it should underlined that, when cultured in standard medium, after 3 weeks, the same cells were able to express neuronal markers such as CD133, nestin, neurofilament CNPase, BDNF and NT3 (Prusa et al., 2004).

MESODERMAL lineages – Recently it as been also shown that upon an optimized 30 days differentiation protocol, AF stem cells display a complete expression of osteogenic markers (COL1, ONC, OPN, OCN, OPG, BSP, Runx2) (Antonucci et al., 2009). In order to test the ability of these cells to proliferate on surfaces commonly used in oral osteointegrated implantology, Antonucci and co-workers (Antonucci et al., 2009) carried out cultures onto different test disks, namely smooth copper, machined titanium and Sandblasted and Acid Etching titanium (SLA titanium), showing great adhesion capacity.

ENDODERMAL lineages – Te information regarding the possible application in endodermal cell type is still the missing part of the whole story. Nevertheless De Coppi et al. (2007) have reported that cells, after 3 weeks of culturing, were able to produce urea. These cells, CD117 positive, were seeded onto a collagen sandwich layer and stimulated with a combination of growth factor and cytokines. Together with a clear morphological changes they were expressing an hepatocyte like phenotype.

Recently we have described, also, that H-AFC amniotic fluid, for their ability to differentiate to various lineages, could represent a good candidate for therapeutic applications. In fact a small aliquot of amniotic fluid obtained by amniocentesis could be enough to generate, via clonal expansion, a tissue engineered construct ready to be implantated, immediately after birth, or prenatally, or even later in life, the latter if the cells were frozen and banked (Kaviani et al., 2001; 2003). For gene therapy purposes human cells should be genetically modified with a therapeutic gene and delivered systematically or injected directly into the tissue of interest. In particular, on undifferentiated status, stem cells continued to express both the transgenes and stemness cell markers OCT4 and SSEA4. When cultured under mesenchymal conditions, infected cells could still differentiate into osteocytes and adipocytes expressing lineage specific genes. Adenovirus may be useful to engineer populations of pluripotent stem cells, which may be used in a wide range of gene therapy treatments (Grisafi et al., 2008; Arnhold et al., 2008).
Another evidence for the possibility to use these cells type for gene therapy is coming from the work of Arnhold et al. (2008) in which the Authors were able to demonstrate that an efficient transformation of primary human amniocytes by E1 gene functions of human adenovirus serotype 5 (Ad5) yield in stable cell lines, exhibiting morphological features of epithelial like cells. Moreover a thorough investigation using immunocytochemistry confirmed the expression of epithelial cell markers. The analysis also revealed the expression of neuronal and glial marker proteins, such as nestin, vimentin, A2B5 and GFAP. Using RT-PCR, transcripts of the neurotrophic factors nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial cell line derived neurotrophic factor (GDNF), and neurotrophin 3 (NT-3) could be detected. The results suggest a co-expression of epithelial and neuronal marker proteins in E1-transformed human amniotic fluid derived cells and thus a preferential transformation into neuronal-like cells (Arnhold et al., 2008).
The “easy to handle”, in terms of laboratory procedures and protocols, of the H-AFC is one of the main advantage for the amniotic fluid to be a key factor for the cellular and gene therapy. The phenotypic stability and the possibility to maintain the functionality upon cryo-conservation, in fact suggest that this cell type could have their role in both pediatric and adult regenerative medicine (Schmidt et al., 2008). In the view of the potential use of mesenchimal stem cells, a new issue as been achieved by Macchiarini and coworkers (Macchiarini et al., 2008), which, on Lancet, were able to show that the pre-treatment of a transplanted, cell free, trachea, with chondrocytes-differentiated mesenchymal adult stem cells, derived and isolated from the receiver of the graft, were clearly able to reduce the graft vs host disease, probably due to their immuno-modulatory activity. The paper state in the interpretation of the results that: it could be possible to produce a cellular, tissue engineered airway with properties that allow normal functioning, and which is free from risks of rejection.
The main issue is the concept of utilizing the amniotic fluid or the placenta as stem cell sources for fetal tissue engineering. Regarding this main topic some new publications start to be visible. Recently, fetal tissue engineering has emerged as a promising concept in surgical reconstruction of birth defects in the neonatal period (Fauza, 2003; Fauza et al., 2001).

Ethical considerations

Since the discovery of the possibility to use these cell types for gene or cellular therapy, the use of fetal tissue/cells has always been object of ethical debates in the scientific community. The main reason for the controversies is mainly coming from the fact that the primary source of fetal tissue is induced abortion. The National Institutes of Health, the American Obstetrical and Gynecological Society, have long regulated the use of fetal specimens from this latter source in the United Sta-

tes (Report of the Human Fetal Tissue Transplantation Panel, 1988; Annas and Elias, 1989; Greely et al., 1991). Nevertheless is actually not so difficult to understand the big efforts of numerous national and international ethical committees and governmental bodies, to date a consensus, which nowadays has not yet been reached, either in the US or in the European Community. This polemic is less evident instead when arguing on the cryo-conservation and following isolation of stem cells from the amniotic fluid and placenta, as a novel development in fetal tissue processing. These new procedures, with optimized and standardized protocols, add a new discussion concerning the use of fetal tissue for therapeutic or research purposes. In fact the key factor for the non-ethical problems raised by the use of amniotic fluid-derived stem cells, is coming from the fact that these specimens can be obtained through routine prenatal screening testing, without the need for invasive fetal biopsy.

If amniotic-derived stem cells from a diseased fetus could/should be used for autologous therapeutic application, no ethical discussion would be anticipated, as long as the procedure is shown to be a valid therapeutic option for a given perinatal condition. In this scenario, the ethical considerations are the very same that apply to any fetal intervention. The other side of the coin is that the use of these stem cells, in an allogeneic fashion, makes ethical issues analogous to the ones involving fetal tissue/organ transplantation, regardless of whether the original specimen comes from a live or deceased fetus.

At the same time, apart from whether an autologous or allogeneic application is being considered, should the amniotic fluid or the placenta be confirmed as reliable sources of embryonic-like stem cells, the ethical objections to embryo disposal now plaguing the progress of amniotic fluid-derived stem cells research could be completely avoided.

Conclusion

These characteristics, together with the absence of ethical issues concerning their employment, suggest that stem cells present in the amniotic fluid might be promising candidates for tissue engineering and stem cell therapy of several human disorders. Moreover taking into account what as been above discussed, it should only be a matter of time until amniotic fluid-derived stem cells will be a relevant tool in tissue engineering, gene therapy, research field.

References

1. Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, Willemze R, Fibbe WE, Kanhai HH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood. 2003;102:1548-9.
2. Annas GJ, Elias S. The politics of transplantation of human fetal tissue [see comments]. New England Journal of Medicine 1989;320:1079-1082.
3. Antonucci I, Iezzi I, Morizio E, Mastrangelo F, Pantalone P, Mattioli-Belmonte M, Gigante A, Salini V, Calabrese G, Tetè S, Palka G, Stuppia L. Isolation of osteogenic progenitors from human amniotic fluid using a single step culture protocol. BMC Biotechnology 2009;9:9-16.
4. Arnhold S, Post C, Glüer S, Hoopmann M, Wenisch S, Volpers C, Addicks K. Neuronal characteristics of amniotic fluid derived cells after adenoviral transformation. Cell Biol Int. 2008;32:1559-66.
5. De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol. 2007 Jan;25(1):100-6. Epub 2007 Jan 7.
6. Eckfeldt CE,Mendenhall EM, Verfaillie CM. Themolecular repertoire of the 'almighty' stem cell. Nat Rev Mol Cell Biol 2005;6:726-37.
7. Fauza DO, Marler JJ, Koka R et al. Fetal tissue engineering: diaphragmatic replacement. Journal of Pediatric Surgery 2001;36:146-151.
8. Fauza DO. Tissue engineering: current state of clinical application. Current Opinion in Pediatrics 2003;15:267-271.
9. Gosen CM. Amniotic fluid cell types and culture. Br Med Bull 1983;39:348-354.
10. Greely HT, Hamm T, Johnson R et al. The ethical use of human fetal tissue in medicine. Stanford University Medical Center Committee on Ethics. New England Journal of Medicine 1989;320:1093-1096.
11. Grisafi D, Piccoli M, Pozzobon M, Ditadi A, Zaramella P, Chiandetti L, Zanon GF, Atala A, Zacchello F, Scarpa M, De Coppi P, Tomanin R. High transduction efficiency of human amniotic fluid stem cells mediated by adenovirus vectors. Stem Cells Dev. 2008;17:953-62.
12. Hoehn H, Salk D. Morphological and biochemical heterogeneity of amniotic fluid cells in culture. Methods Cell Biol 1982;26:11-34.
13. Kaviani A, Guleserian K, Perry TE et al. Tissue engineering from amniotic fluid. Journal of the American College of Surgeons 2003;196:592-597.
14. Kaviani A, Perry TE, Dzakovic A et al. The amniotic fluid as a source of cells for fetal tissue engineering. Journal of Pediatric Surgery 2001;36:1662-1665.
15. Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, Dodson A, Martorell J, Bellini S, Parnigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA. Clinical transplantation of a tissue-engineered airway. Lancet. 2008;372:2023-30.
16. Mosquera A, Fernandez JL, Campos A, Goyanes VJ, Ramiro-Diaz J, Goselvez J. Simultaneus decrease of telomere lenght and telomerase activity with ageing of human amniotic fliuds cells. J Med Genet 1999;36:494-496.
17. Prusa AR, Hengstschlager M. Amniotic fluid cells and human stem cell research: a new connection. Med Sci Monit 2002;8:253-257.
18. Report of the Human Fetal Tissue Transplantation Panel. Bethesda: National Institutes of Health; 1988.
19. Schmidt D, Achermann J, Odermatt B, Genoni M, Zund G, Hoerstrup SP. Cryopreserved amniotic fluid-derived cells: a lifelong autologous fetal stem cell source for heart valve tissue engineering. J Heart Valve Dis. 2008;17:446-55.
20. Torricelli F, Brizzi L, Bernabei PA, et al Identification of hematopoietic progenitor cells in human amniotic fluid before the 12th week of gestation. Ital J Anat Embryol 1993;98: 119-126.
21. Tsai MS, Lee JL, Chang YJ, Hwang SM. Isolation of human multipotent mesenchimal sem cells from second trimester amniotic fluid using a novel two-stage culture protocol. Hum Reprod 2004;19:1450-1456.
22. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science 2000;287: 1442-6.1662-1665.