CRYOPRESERVED CHORIONIC VILLI AS SOURCE OF GENETICALLY STABLE MESENCHYMAL STROMAL CELLS FOR REGENERATIVE MEDICINE

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ABSTRACT

Objectives: This study was designed to explore the potential of human fetal mesenchymal stromal cells derived from chorionic villi (CV-MSC), in view of a possible applications for cellular therapy and regenerative medicine.

Methods: Small amount (5mg) of direct CV samples were GMP-cryopreserved. Following thawing and enzymatic disgregation, cells were in vitro cultured and analyzed for biological endpoints like proliferation rate, immunophenotype and differentiation potential. Genome stability, by karyotype analysis, genome-wide array-CGH and microsatellite analysis, were also explored.

Results: Immunophenotyping of cultured cells showed expression of typical MSC markers and absence of expression of hematopoietic markers. Analysis of multilineage potential showed efficient differentiation into adipocytes, osteocytes and chondrocytes. Potential differentiation toward neural precursors was demonstrated on a subpopulation of CV-MSC by morphologic features and immunocytochemical staining of neuron specific proteins.
Karyotype analysis showed that the frequency of chromosomes aberrations at the different culture passages is not significantly different from the basal frequency found in primary culture. Data obtained from array CGH analysis and microsatellite analysis comparing DNA from early to late passages did not show any copy number variations of DNA segments.

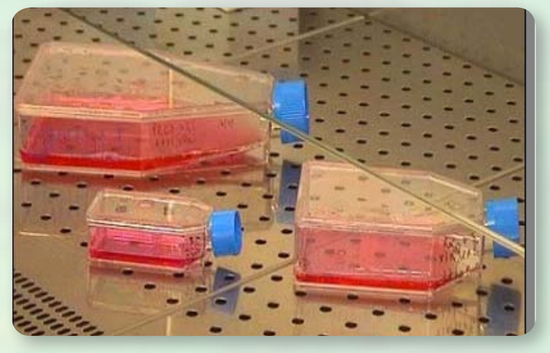
Conclusion: Our findings indicate that it is possible to isolate and extensively expand MSC from cryopreserved direct CV and that the in vitro culture does not interfere with the DNA-repair systems and do not affect genome stability.

AIM OF THE STUDY

This study was designed to explore the potential of human fetal mesenchymal stromal cells derived from chorionic villi (CV-MSC), in view of a possible applications for cellular therapy and regenerative medicine.

MATERIALS and METHODS

Small amount (5mg) of direct CV samples were GMP-cryopreserved. Following thawing and enzymatic disgregation, cells were in vitro cultured and analyzed for biological endpoints like proliferation rate, immunophenotype and differentiation potential. Genome stability, by karyotype analysis, genome-wide array-CGH and microsatellite analysis, were also explored.



RESULTS

CV-MSC CHARACTERIZATION

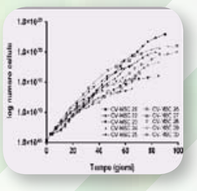
Homogeneous population of fibroblast-like cells were observed in all chorionic villi MSC samples (n=17,) from the beginning until the latest passeges in cultures (A).
CV-MSC generally grown fast. The slow down in the cellular growth due to physiological processes of cellular senescence seems to occour only late in the culture as shown by a DT of 3.2 ± 0.5 days at 15th passage (n=8). At the same time, the trend of the proliferation curve in different samples analyzed was very homegeneous at least until the 10th passage (B).
Immunophenotyping of cultured cells showed expression of typical MSC markers (CD73, CD90, CD105) and absence of expression of hematopoietic markers (CD14, CD19, CD45 CD34, HLA-DR, data not shown). The surface marker profile is consistent with the profile of control human BM-MSC (C).

• Morphology •

A

• Growth curve •

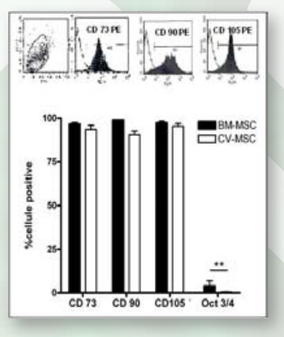
B



TD: 1.4 giorni (5° passaggio)
1.5 giorni (10° passaggio)
3.2 giorni (15° passaggio)
5.1 giorni (20° passaggio)

• Immunophenotype

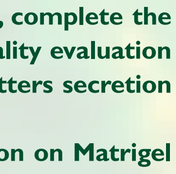
C



DIFFERENTIATION POTENTIAL

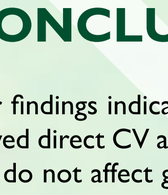
CVS-MSC possess the potential, if stimulated, to differentiate into osteoblasts, adipocytes and chondroblasts. Osteogenic differentiation was demonstrated by positive staining with Alizarin red, that colours red calcium deposits. Cells differentiated toward adipocytes accumulated lipid-rich vacuoles in the cytoplasms and stained red with Oil Red O. Chondrogenic differentiation is demonstrated by developing of chondrogenic pellet. Sectioned pellets stain positive after Alcian blue exposure indicating synthesis of proteoglycans by chondrocytes. No difference are found between BM-MSC and and MSC derived from chorionic villi cultures.

Osteogenic



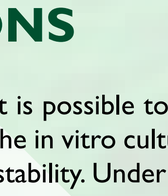
Alizarin Red staining +

Adipogenic



Oil Red O staining +

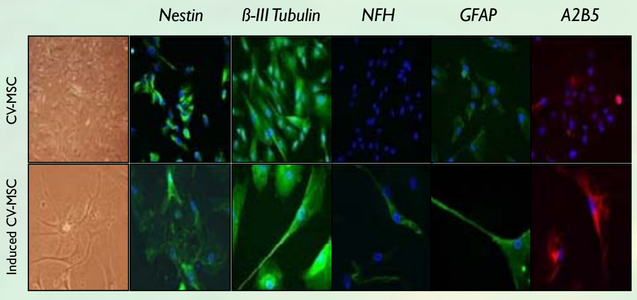
Chondrogenic

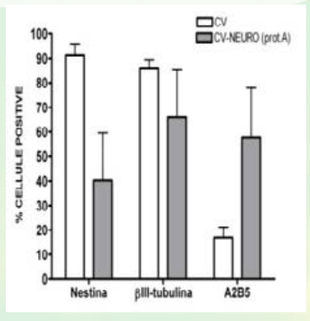


Alcian blue staining +

High percentage of CV-MSCs express costutively neural stem cells marker Nestin and the neuron specific class III b-tubulin. A subpopulation of cells express, additionally, the neural progenitor marker A2B5 and few cells express the glial molecule GFAP. Following exposure to a neural induction medium containing retinoic acid, CV-MSC were rapidly transformed from fibroblastic to neural morphologies. The percentage of A2B5+ cells increased 2.4 fold and a subpopulation of induced cells exihbited a positive immunofluorescence stain for neuron differentiation marker NFH. Based on these data we hypotized a succesfull stimulation of CV-MSC toward neuro-glial lineage by increasing neural committed progenitors A2B5+ and inducing differentiation of same mature neural NFH+ precursors.

Neuro-glial

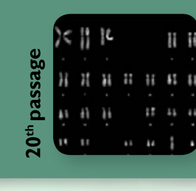




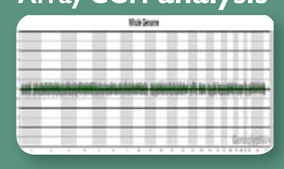
GENOME STABILITY ANALYSIS

No chromosomes alterations were evident by karyotype analysis up to the 20th passage in CVS-MSC in vitro cultures. Preliminary data obtained from array CGH analysis comparing DNA from early to late passages (1rst vs 20th) did not show any significative copy number variations of DNA segments. Also the microsatellite stability analysis did not show any changes in the electropherogram profiles up to the 28°in vitro passages, thus indicating that the in vitro culture did not induce any modification of the genome stability.

Karyotype:



Array CGH analysis



Microsatellite analysis

ONGOING and FUTURE

Optimize of the protocol for the differentiation of CVS-MSC toward neuronal cells, complete the phenotypic caratterization of neuro-glial differentiated cells and perform a functionality evaluation by testing the excitable properties of induced cells and measuring the neurotrasmitters secretion during neurogenesis .
Investigate the angiogenic proprierties of CVS-MSC by analysis of capillary formation on Matrigel coated plates following VEGF stimulation

CONCLUSIONS

Our findings indicate that it is possible to isolate and extensively expand MSC from cryopreserved direct CV and that the in vitro culture does not interfere with the DNA-repair systems and do not affect genome stability. Under these circumstances, CV-MSC could be suitable for therapeutic proposes.

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