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STEM CELLS IN HUMAN GENETIC DISORDERS - CETGEN

Department of Genetics and Evolutionary Biology
Bioscience Institute
University of São Paulo

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Coordinator: Mayana Zatz

2010 REPORT

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Partial results – 2010

a) Main Researchers

Coordination
Mayana Zatz - Coordinator
Peter Pearson – Vice-Coordinator

Managing Committee
Maria Rita Passos-Bueno
Mariz Vainzof
Oswaldo Keith Okamoto
Pericles Assad Hassun Filho

Operational Group
Celia P. Koiffmann
Angela Maria Vianna-Morgante
Regina Célia Mingroni Netto
Eliana Maria Beluzzo Dessen
Fernando Kok

Collaborators	**International collaborators**
Alexandre Busse	Dr. Louis M. Kunkel
Carla Rosenberg	Dr. Jeffery D. Molkentin
Debora Romeo Bertola	Dr. Gary D. Smith
Edmund Chada Bacarat	Dr. Gregory A. Cox
Eduardo Leme Alves da Motta	Dr. Mark R. Hughes
Egberto Reis Barbosa	Dr. Karen Usdin
Flávia de Paula	
Flavia Imbroisi Valle Errera	
Helga Cristina Silva	
Ivo Pavanello Filho	
Krishnamurti de Morais Carvalho	
José Seráfico S. Nóbrega	
Juliana Gurgel-Giannetti	
Luciana Amaral Haddad	
Luiz Augusto Corrêa Passos	
Miriam Fussae Susuki	
Nanci do Nascimento	
Nivaldo Alonso	
Paolo Bartolini	
Paulo Cesar Serafini	
Renato da Silva Freitas	
Ricardo Bento	
Rita de Cassia Mingroni Pavanello	
Silvana Cristina dos Santos	
Sonia Maria Dozzi Brucki	

b) Main research topics

The INCT-CETGEN study involving stem cells derived from individuals affected by genetic disorders is designed to investigate the pathophysiological consequences of gene mutations and other genetic abnormalities on tissue differentiation. Previous basic research has focused on craniofacial malformations, neuromuscular and neurodegenerative disorders, alterations of neurodevelopment and gonadal dysfunction. Research regarding tumor stem cells and influence of genetic alterations related to aging and the stem cell differentiation potential are also being developed. New stem cell therapeutic approaches are being studied in a pre-clinical setting, using transplantation of stem cells into animal models for the genetic disorders under study to establish "proof of principle" for possible therapeutic applications and to predict potential adverse side effects of the treatment procedures. An extensive bank of *de novo* human stem cell lines has been assembled to sustain these researches.

C) Summary of Results

1. **Identification of new sources of stem cells and establishment of cell lines**

At this point in time, 334 stem cell lines have been established, from which 176 are derived from patients affected by different genetic disorders, including craniofacial syndromes, Angelman syndrome, autism and neuromuscular disorders; additionally 158 stem cell lines from normal volunteers have been assembled (table 1). Mesenchymal stem cells (MSCs) have been isolated from several sources, including fat tissue, dental pulp, menstrual blood, umbilical chord, periosteum and muscles. Additionally, we verified that it is possible to isolate mesenchymal stem cells (MSC) from the fallopian tube, and lip obircularis muscle, which are being evaluated for osteoporosis treatment in post-menopause women and bone reconstruction, respectively.

In collaboration with the Huntington Clinics, 77 human embryos with genetic abnormalities have been frozen for future research. Since May 2008, 144 cryopreserved human embryos have been donated for research purposes.

Table 1. Stem cell banking as of October 2010:

Disease group	**Biological source**	**Nº Cell lines**	**Researchers**
Craniosynostosis	Cranium periosteum	24	MRPB
Cleft lip and/or palate	Orbicular oris muscle	44	MRPB
Cleft palate	Dental pulp	20	MRPB
Autism	Dental pulp	6	MRPB
Other craniofacial syndromes	Cranium periosteum	15	MRPB
Controls	Dental pulp	10	MRPB
Controls	Adipose tissue	28	MRPB
Angelman Syndrome	Dental pulp	3	CPK
Neuromuscular disorders	Muscle fragment	10	MV
	Skin	8	MV
	Adipose tissue/muscle biopsy	10	MV
	Dental pulp	1	MV
	Adipose tissue	35	MZ

Controls	Adipose tissue	6	MZ
	Umbilical cord	6	MZ
	Menstrual blood	88	MZ
	Endometrium	7	MZ
	Fallopian tube	13	MZ
Total		**334**	

2. Stem-cells as tools to understand gene functioning

In addition to the identification of new sources of stem cells, we have been working with MSC or other types of mesenchymal cells, such as fibroblasts, in order to identify important signaling networks involved with genetic disease and for screening of putative drugs to ameliorate the phenotype of some of these conditions. Additionally, stem cells within the cancer context are also being study at the molecular and cellular levels. Genes relevant to cancer stem cell biology have been evaluated in human malignant gliomas and their respective tumoral stem cells.
We also have been working on cultivated cells obtained from Organ of Corti of newborn guinea pigs and mice, in order develop animal models for future cell therapy in deafness. Our aim was the maintenance of progenitor cochlear cells, and in vitro differentiation of those progenitors into hair cells and support cells, fundamental cell types in keeping the hearing function

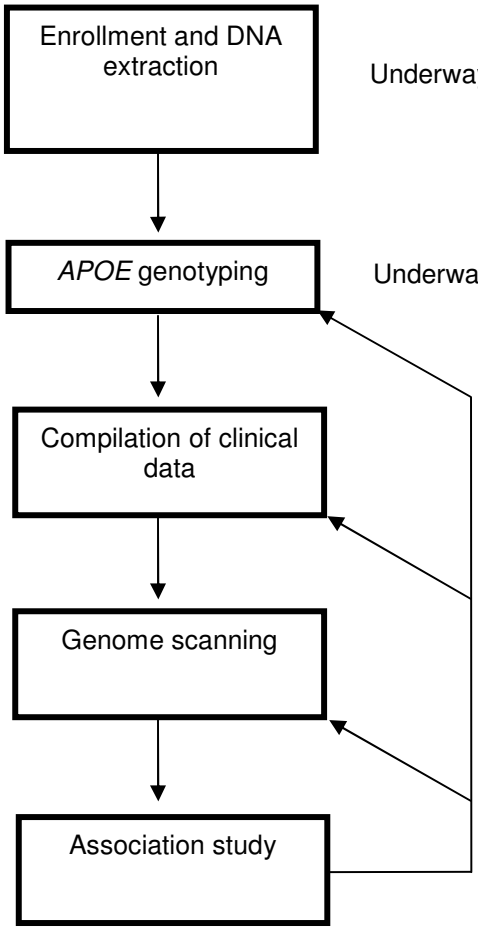
3. Stem-cells aiming future therapies

We have been studying different sources of stem cells and biomaterials in order to evaluate their usefulness in bone reconstruction. The evaluation of a good strategy on cell therapy also depends on the availability of good animal models. Experimental models for studying bone reconstruction and neurogenesis/ neurodegeneration have been established in Wistar rats. Additionally, animal models of muscular dystrophy are being use to evaluate different cell therapy protocols. We studied the transplantation of human stem cells from fat tissue and umbilical cord in the dog and mouse models for muscular dystrophy, and conclude that this therapeutic approach may become very important in pre-clinical and clinical research.

4. Genetic variability in elderly people

The aim of this Project is to map the genetic variability of individuals over 80 years of age without cognitive impairment. A partnership has been established with the School of Public Health (Prof. Maria Lúcia Lebrão) and The School of Nursery (Prof. Yeda A. de Oliveira Duarte), both at the University of Sao Paulo, to recruit 1000 research subjects.

At present, 471 DNA samples have been extracted in our Center from individuals over 60 years of age, 22% of which corresponding to individuals above 80 years of age. *APOE* genotyping associated with Alzheimer Disease is being performed. Clinical data regarding cognitive and motor function as well as social-economic and habits/behavior aspects are being collected for further correlation studies other genetic variables. About 1 million polimorfisms are expected to be genotyped in this project. A simplified scheme of the study is presented below:


Enrollment and DNA extraction
Underway. N = 471 (06/10/2010)
APOE genotyping
Underway.
Compilation of clinical data
Genome scanning
Association study

Below, we present the abstracts of published/accepted papers in 2010 and the list of publication in 2009.

1- **Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M. Human Genome Research Center, Biosciences Institute, University of São Paulo, São Paulo, Brazil.**

Cell Transplant. 2010;19(3):279-89. Epub 2009 Dec 8.

Abstract

Adipose tissue may represent a potential source of adult stem cells for tissue engineering applications in veterinary medicine. It can be obtained in large quantities, under local anesthesia, and with minimal discomfort. In this study, canine adipose tissue was obtained by biopsy from subcutaneous adipose tissue or by suction-assisted lipectomy (i.e., liposuction). Adipose tissue was processed to obtain a fibroblast-like population of cells similar to human adipose-derived stem cells (hASCs). These canine adipose-derived stem cells (cASCs) can be maintained in vitro for extended periods with stable population doubling and low levels of senescence. Immunofluorescence and flow cytometry show that the majority of cASCs are of mesodermal or mesenchymal origin. cASCs are able to differentiate in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. In conclusion, like human lipoaspirate, canine adipose tissue may also contain multipotent cells and represent an important stem cell source both for veterinary cell therapy as well as preclinical studies.

2- **Human adipose-derived stem cells: current challenges and clinical perspectives. Yarak, S[1] & Okamoto, OK[2]. [1]Departamento de Patologia, Universidade Federal de São Paulo. [2]Departamento de Neurologia e Neurocirurgia, Universidade Federal de São Paulo.**

An. Bras. Dermatol. vol.85 no. 5, 2010.

Abstract

Adult or somatic stem cells hold great promise for tissue regeneration. Currently, one major scientific interest is focused on the basic biology and clinical application of mesenchymal stem cells. Adipose tissue-derived stem cells (ADSC) share similar characteristics with bone marrow mesenchymal stem cells but have some advantages including harvesting through a less invasive cirurgical procedure. Moreover, ADSC have the potential to differentiate into cells of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle, as well as cells of non-mesodermal lineage, such as hepatocytes, pancreatic endocrine cells, neurons, cardiomyocytes, hepatocytes and vascular endothelial cells. There are, however, some inconsistencies in the scientific literature regarding methods for harvesting adipose tissue, as well as isolation, characterization and handling of ADSC. Future clinical applications of ADSC rely on more defined and widespread methods for obtaining ADCS of clinical grade quality. In this review, current methods in ADSC research are discussed with emphasis in strategies designed for future applications in regenerative medicine and possible challenges in the way.

3- **Reduced transcription of TCOF1 in adult cells of Treacher Collins syndrome patients. Masotti C, Ornelas CC, Splendore-Gordonos A, Moura R, Félix TM, Alonso N, Camargo AA, Passos-Bueno MR.**

BMC Med Genet. 2009 Dec 14;10:136.

Abstract

BACKGROUND: Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial disorder caused by frameshift deletions or duplications in the TCOF1 gene. These mutations cause premature termination codons, which are predicted to lead to mRNA degradation by nonsense mediated mRNA decay (NMD). Haploinsufficiency of the gene product (treacle) during embryonic development is the proposed molecular mechanism underlying TCS. However, it is still unknown if TCOF1 expression levels are decreased in post-embryonic human cells.

METHODS: We have estimated TCOF1 transcript levels through real time PCR in mRNA obtained from leucocytes and mesenchymal cells of TCS patients (n = 23) and controls (n = 18). Mutational screening and analysis of NMD were performed by direct sequencing of gDNA and cDNA, respectively.

RESULTS: All the 23 patients had typical clinical features of the syndrome and pathogenic mutations were detected in 19 of them. We demonstrated that the expression level of TCOF1 is 18-31% lower in patients than in controls ($p < 0.05$), even if we exclude the patients in whom we did not detect the pathogenic mutation. We also observed that the mutant allele is usually less abundant than the wild type one in mesenchymal cells.

CONCLUSIONS: This is the first study to report decreased expression levels of TCOF1 in TCS adult human cells, but it is still unknown if this finding is associated to any phenotype in adulthood. In addition, as we demonstrated that alleles harboring the pathogenic mutations have lower expression, we herein corroborate the current hypothesis of NMD of the mutant transcript as the explanation for diminished levels of TCOF1 expression. Further, considering that TCOF1 deficiency in adult cells could be associated to pathologic clinical findings, it will be important to verify if TCS patients have an impairment in adult stem cell properties, as this can reduce the efficiency of plastic surgery results during rehabilitation of these patients.

4- **Human stem cell cultures from Cleft lip/palate patients show enrichment of transcripts involved in extracellular matrix modeling by comparison to controls. Daniela Franco Bueno[1a], Daniele Yumi Sunaga[1a], Gerson Shigeru Kobayashi[1], Meire Aguena[1], Cassio Eduardo Raposo do Amaral[2], Cibele Masotti[1], Lucas Alvizi Cruz[1], Peter Lees Pearson[1], Maria Rita Passos-Bueno[1*]**

Accepted in Stem Cells Reviews and Reports, 2010

Abstract

Nonsyndromic cleft lip and palate (NSCL/P) is a complex disease resulting from failure of fusion of facial primordia, a complex developmental process that includes the epithelial-mesenchymal transition (EMT). Detection of differential gene transcription between NSCL/P patients and control individuals offers an interesting alternative for investigating pathways involved in disease manifestation. Here we compared the transcriptome of 6 dental pulp stem cell (DPSC) cultures from NSCL/P patients and 6 controls. Eighty-seven differentially expressed genes (DEGs) were identified. The most significant putative gene network comprised 13 out of 87 DEGs of which 8 encode extracellular proteins*: ACAN, COL4A1, COL4A2, GDF15, IGF2, MMP1, MMP3* and *PDGFa.* Through clustering analyses we also observed that *MMP3, ACAN, COL4A1 and COL4A2* exhibit co-regulated expression. Interestingly, it is known that *MMP3* cleavages a wide range of extracellular proteins, including the collagens IV, V, IX, X, proteoglycans, fibronectin and laminin. It is also capable of activating other MMPs. Moreover, *MMP3* had previously been associated with NSCL/P. The same general pattern was observed in a further sample, confirming involvement of synchronized gene expression patterns which differed between NSCL/P patients and controls. These results show the robustness of our methodology for the detection

of differentially expressed genes using the RankProd method. In conclusion, DPSCs from NSCL/P patients exhibit gene expression signatures involving genes associated with mechanisms of extracellular matrix modeling and palate EMT processes which differ from those observed in controls. This comparative approach should lead to a more rapid identification of gene networks predisposing to this complex malformation syndrome than conventional gene mapping technologies.

5- **Aberrant signaling pathways in medulloblastomas: a stem cell connection. Carolina Oliveira Rodini I, Daniela Emi Suzuki I, Adriana Miti Nakahata I, Márcia Cristina Leite Pereira I, Luciana Janjoppi I, Silvia Regina Caminada Toledo II, Oswaldo Keith Okamoto I,*. I Department of Neurology and Neurosurgery, Laboratory of Experimental Neurology, Universidade Federal de São Paulo-UNIFESP, São Paulo, Brazil. II Universidade Federal de São Paulo, Division of Genetics of the Department of Morphology - Instituto de Oncologia Pediátrica GRAACC/UNIFESP, São Paulo, Brazil.**

Accepted for publication. Arq. Neuro-Psiquiatr. 2010.

Abstract

Medulloblastoma is a highly malignant primary tumor of the central nervous system. It represents the most frequent type of solid tumor and the leading cause o death related to cancer in early childhood. Current treatment includes surgery, chemotherapy and radiotherapy which may lead to severe cognitive impairment and secondary brain tumors. New perspectives for therapeutic development have emerged with the identification of stem-like cells displaying high tumorigenic potential and increased radio- and chemo-resistance in gliomas. Under the cancer stem cell hypothesis, transformation of neural stem cells and/or granular neuron progenitors of the cerebellum are though to be involved in medulloblastoma development. Dissecting the genetic and molecular alterations associated with this process should significantly impact both basic and applied cancer research. Based on cumulative evidences in the fields of genetics and molecular biology of medulloblastomas, we discuss the possible involvement of developmental signaling pathways as critical biochemical switches determining normal neurogenesis or tumorigenesis. From the clinical viewpoint, modulation of signaling pathways such as TGFβ, regulating neural stem cell proliferation and tumor development, might be attempted as an alternative strategy for future drug development aiming at more efficient therapies and improved clinical outcome of patients with pediatric brain cancers.

6- **Alveolar osseous defect in rat for cell therapy: preliminary report. Raposo-Amaral CE, Kobayashi GS, Almeida AB, Bueno DF, Freitas FR, Vulcano LC, Passos-Bueno MR, Alonso N.**

Acta Cir Bras. 2010 Aug;25(4):313-7.

Abstract

PURPOSE: To study were to reproduce an alveolar bone defect model in Wistar rats to be used for testing the efficacy of stem cell therapies. Additionally, we also aimed to determine the osteogenesis process of this osseous defect in the 1 month period post-surgery.

METHODS: The animals were randomly divided into two groups of 7 animals each. A gingivobuccal incision was made, and a bone defect of 28 mm(2) of area was performed in the alveolar region. Animals were killed at 2 weeks after surgery (n=7) and 4 weeks after surgery (n=7).

RESULTS: The average area of the alveolar defect at time point of 2 weeks was 22.27 +/- 1.31 mm(2) and the average area of alveolar defect at time point of 4 weeks was 9.03 +/- 1.17 mm(2). The average amount of bone formation at time point of 2 weeks was 5.73 +/- 1.31 mm(2) and the average amount of bone formation at time point of 4 weeks was 19 +/- 1.17 mm(2). Statistically significant differences between the amount of bone formation at 2 weeks and 4 weeks after surgery were seen (p=0.003).

CONCLUSION: The highest rate of ossification occurred mostly from 2 to 4 weeks after surgery. This observation suggests that 4 weeks after the bone defect creation should be a satisfactory timing to assess the potential of bone inductive stem cells to accelerate bone regeneration in Wistar rats.

7- An experimental model for the study of craniofacial deformities. Costa Ade M, Kobayashi GS, Bueno DF, Martins MT, Ferreira Mde C, Passos-Bueno MR, Alonso N.

Acta Cir Bras. 2010 Jun;25(3):264-8.

Abstract

PURPOSE: To develop an experimental surgical model in rats for the study of craniofacial abnormalities.

METHODS: Full thickness calvarial defects with 10x10-mm and 5x8-mm dimensions were created in 40 male NIS Wistar rats, body weight ranging from 320 to 420 g. The animals were equally divided into two groups. The periosteum was removed and dura mater was left intact. Animals were killed at 8 and 16 weeks postoperatively and cranial tissue samples were taken from the defects for histological analysis.

RESULTS: Cranial defects remained open even after 16 weeks postoperatively.

CONCLUSION: The experimental model with 5x8-mm defects in the parietal region with the removal of the periosteum and maintenance of the integrity of the dura mater are critical and might be used for the study of cranial bone defects in craniofacial abnormalities.

8- Human multipotent mesenchymal stromal cells from distinct sources show different in vivo potential to differentiate into muscle cells when injected in dystrophic mice. Vieira NM, Zucconi E, Bueno CR Jr, Secco M, Suzuki MF, Bartolini P, Vainzof M, Zatz M. Human Genome Research Center, Institute of Biosciences, University of São Paulo, Rua do Matão, n.106-Cidade Universitária, São Paulo, SP, CEP: 05508-090, Brazil.

Stem Cell Rev. 2010 Dec;6(4):560-6.

Abstract

Limb-girdle muscular dystrophies are a heterogeneous group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or deficiency of muscle proteins. The murine model of Limb-Girdle Muscular Dystrophy 2B, the SJL mice, carries a deletion in the dysferlin gene. Functionally, this mouse model shows discrete muscle weakness, starting at the age of 4-6 weeks. The possibility to restore the expression of the defective protein and improve muscular performance by cell therapy is a promising approach for the future treatment of progressive muscular dystrophies (PMD). We and others have recently shown that human adipose multipotent mesenchymal stromal cells (hASCs) can differentiate into skeletal muscle when in contact with dystrophic muscle cells in vitro and in vivo. Umbilical cord tissue and adipose tissue are known rich sources of multipotent mesenchymal stromal cells (MSCs), widely used for cell-based therapy studies. The main objective of the present study is to evaluate if MSCs from these two different sources have the same potential to reach and differentiate in muscle cells in vivo or if this capability is influenced by the niche from where they were obtained. In order to address this question we injected human derived umbilical cord tissue MSCs (hUCT MSCs) into the caudal vein of SJL mice with the same protocol previously used for hASCs; we evaluated the ability of these cells to engraft into recipient dystrophic muscle after systemic delivery, to express human muscle proteins in the dystrophic host and their effect in functional performance. These results are of great interest for future therapeutic application.

9- **Retention of progenitor cell phenotype in otospheres from guinea pig and mouse cochlea. Oiticica, J; Barboza-Júnior, LCM, Batissoco, AC, Lezirovitz, K; Mingroni-Netto RC, Haddad LA , Bento RF., 2010.**

Submitted to publication in Journal of Translational Medicine:

Abstract

Background: Culturing otospheres from dissociated organ of Corti is an appropriate starting point aiming at the development of cell therapy for hair cell loss. Although guinea pigs have been widely used as an excellent experimental model for studying the biology of the inner ear, the mouse cochlea has been more suitable for yielding otospheres *in vitro*. The aim of this study was to compare conditions and outcomes of suspension cultures of dissociated organ of Corti in either mouse or guinea pig at postnatal day three (P3), and to evaluate the guinea pig as a potential cochlea donor for preclinical cell therapy. **Methods:** Organs of Corti were surgically isolated from P3 guinea pig or mouse cochlea, dissociated and cultivated under non-adherent conditions. Cultures were maintained in serum-free DMEM:F12 medium, supplemented with epidermal growth factor (EGF) plus either basic fibroblast growth factor (β-FGF) or transforming growth factor alpha (TGF-α). Immunofluorescence assays were conducted for phenotype characterization. **Results:** The TGFα group presented a number of spheres significantly higher than the bFGF group. Although mouse cultures yielded more cells per sphere than guinea pig cultures, sox2 and nestin distributed similarly in otosphere cells from both organisms. **Conclusions:** Dissociated guinea pig cochlea produced otospheres *in vitro*, expressing sox2- and nestin similarly to mouse otospheres. Our data is supporting evidence for the presence of inner ear progenitor cells in the postnatal guinea pig. However, there is limited viability for these celld in neonatal guinea pig cochlea when compared to the differentiation potential observed for the mouse organ of Corti at the same developmental stage. .

List of Publications in 2009

1. Bueno, DF, Kerkis, I, Costa, AM, Martins, MT, Kobayashi, GS, Zucconi, E, Fanganiello, RD, Salles, FT, Almeida, AB, do Amaral, CE, Alonso, N and Passos-Bueno, MR. "New Source of Muscle-Derived Stem Cells with Potential for Alveolar Bone Reconstruction in Cleft Lip and/or Palate Patients". Tissue Eng Part A **15**(2): 427-35.(2009)

2. Jazedje, T, Perin, PM, Czeresnia, CE, Maluf, M, Halpern, S, Secco, M, Bueno, DF, Vieira, NM, Zucconi, E and Zatz, M. "Human fallopian tube: a new source of multipotent adult mesenchymal stem cells discarded in surgical procedures". J Transl Med **7**: 46.(2009)

3. Jazedje, T, Secco, M, Vieira, NM, Zucconi, E, Gollop, TR, Vainzof, M and Zatz, M. "Stem cells from umbilical cord blood do have myogenic potential, with and without differentiation induction in vitro". J Transl Med **7**(1): 6.(2009)

4. Masotti C, Ornelas CO, Splendore-Gordonos A, Moura R, Felix TM, Alonso N, Camargo AA, Passos-Bueno MR. "Reduced transcription of TCOF1 in adult cells of Treacher Collins syndrome patients". BMC Medical Genetics, dez 14:10:136, 2009

5. Okamoto OK. "Cancer stem cell genomics: the quest for early markers of malignant progression". Expert Rev Mol Diagn. 2009 Sep;9(6):545-54. Review.PMID: 19732002.

6. Secco M, Moreira YB, Zucconi E, Vieira NM, Jazedje T, Muotri AR, Okamoto OK, Verjovski-Almeida S, Zatz M. "Gene expression profile of mesenchymal stem cells from paired umbilical cord units: cord is different from blood". Stem Cell Rev. 2009 Dec;5(4):387-401.

7. Zatz, M. "Stem Cell Researches in Brazil: Present and Future Challenges". Stem Cell Rev.(2009)

8. Zucconi, E, Vieira, NM, Bueno, DF, Secco, M, Jazedje, T, Ambrosio, CE, Passos-Bueno, MR, Miglino, MA and Zatz, M. "Mesenchymal Stem Cells Derived from Canine Umbilical Cord Vein - A Novel Source for Cell Therapy Studies". Stem Cells Dev.(2009)

Abstracts presented in International and Brazilian meetings

- **8TH ISSCR ANNUAL MEETING of the ISSCR. June 16-19th, San Francisco, CA, USA.**

HUMAN FALLOPIAN TUBE STEM CELLS ARE ABLE TO PRODUCE BONE IN VIVO
Jazedje, Tatiana1, Bueno, Daniela F.1, Czeresnia, Carlos E.2, Perin, Paulo M.3, Halpern, Silvio2, Maluf, Mariangela3, Martins, Marilia T.1, Passos-Bueno, Maria R.1, Zatz, Mayana1
1University of Sao Paulo, Sao Paulo, Brazil, 2Celula Mater, Sao Paulo, Brazil, 3CEERH Specialized Center for Human Reproduction, Sao Paulo, Brazil.

Our group described recently human fallopian tube mesenchymal stem cells (htMSCs) as a new source of multipotent stem cells obtained from women submitted to hysterectomies or sterilization surgeries. Since women post menopause are the most affected by osteoporosis, our objective was to evaluate, in vitro and in vivo, the osteogenic capacity of htMSCs. Three different stem cells lineages of htMSCS were used. All chosen lineages were characterized by cytometry and showed good osteogenic differentiation in vitro prior to the in vivo experiments. We performed two symmetric full-thickness cranial defects (5 x 8 mm) on each parietal region of eight nonimmunosuppressed (NIS) rats. The left side (LS) of six animals was covered with CellCeram (Scaffdex) only and the right side (RS) with the CellCeram and stem cells. In two animals, the RS had CellCeram only and nothing was added at the left side (controls) to ensure that the provoked damage does not regenerate naturally. Animals were euthanized at 90 days postoperatively and cranial tissue samples were taken from the defects for histological analysis. We observed bone formation in both sides, but a more mature bone was present in the RS. Human DNA was polymerase chain reaction-amplified only at the RS, indicating that this new bone had human cells. The use of htMSCs in NIS rats did not cause any graft rejection. Our findings suggest that htMSCs are a promising source of stem cells that might be useful in the future to treat osteoporosis or other bone defects.

NEURONAL DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS FROM EXFOLIATED DECIDUOUS TEETH OBTAINED FROM ANGELMAN SYNDROME PATIENTS
Cruvinel, Estela M., Secco, Mariane, Almeida, Camila NA, Zatz, Mayana, Koiffmann, Celia P.
University of São Paulo, São Paulo, Brazil

Angelman syndrome (AS) is a neurodevelopmental disorder caused by the loss of function of the maternal UBE3A gene localized on chromosome 15. UBE3A encodes an ubiquitin ligase (E6-associated protein) and presents a tissue-specific imprinting; maternal predominant transcription is observed in the brain, but UBE3A is expressed from both alleles in most other tissues. A RNA antisense is considered responsible for this tissue-specific imprinting, a large transcript that extends to UBE3A from the small nuclear ribonucleoprotein N (SNURF/SNRPN) promoter region that contains an imprinting center. Most of AS cases (70%) result from a de novo maternal deletion of 15q11-q13; other mechanisms include: maternal UBE3A gene mutations, imprinting center defects, paternal uniparental disomy. AS is characterized by developmental delay, severe mental retardation, movement disorder, absent speech, seizures, ataxia, typical happy disposition with outbursts of laughter, hyperactivity, microcephaly, macrostomia and prognathism. Recently, the derivation of stem cells lines from patients with different genetic abnormalities has provided new research models to understanding pathological mechanisms responsible for these conditions. The identification of the underlying molecular process might open new avenues to prevent the disease progression. The aim of the present study is to analyze stem cells from human exfoliated deciduous teeth (SHED) differentiation into neurons-like cells comparing SHED obtained from AS patients and normal controls.
We have established 3 lineages of SHED from AS patients, two with maternal deletions and one with UBE3A gene de novo mutation. All lineages were characterized as stem cells: they showed a self-

renewal capacity, expression of mesenchymal stem cells markers and were able to differentiate into osteocytes, adipocytes and chondrocytes. We used a neuronal differentiation protocol, which has already been described, to compare the behavior of the cells during the neuronal induction. No morphological differences were observed between patients and control cells. Firstly, immunocytochemistry was done with neurons derived from SHED obtained from control donors, and the cells were positive to MAP-2 and TUJ1, which are neurons-specific proteins. Moreover, these cells expressed MAP-2 and nestin (protein present in immature neurons) through RT-PCR. Expression and functional data are currently under investigation. The ability to differentiate mesenchymal stem cells from AS patients into cells positive for neuronal markers might represents an important resource for studying the genetic mechanisms responsible for Angelman syndrome etiology.

PRECLINICAL STUDY OF MESENCHYMAL STEM CELL HUMAN UMBILICAL CORD TRANSPLANTATION IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE. Pereira, Márcia Cristina L.[1], Secco, Mariane[2], Janjoppi, Luciana[1], Oliveira, Carolina Rodini[1], Suzuki, Daniela Emi[1], Gonçalves, Ariádne Sanches[1], Zatz, Mayana[2], Okamoto, Oswaldo Keith[1]. [1]Department of Neurology and Neurosurgery, Federal University of São Paulo, São Paulo (SP), Brazil, [2]Human Genome Research Center, Department of Genetic and Evolutive Biology, University of São Paulo, São Paulo (SP), Brazil.

Preclinical evaluation of stem cell-based therapies for Parkinson´s Disease (PD) is an important step to address fundamental questions such as alternative types of cells for transplantation, definition of dose regimen, infusion routes, and safety issues. Here, we investigated the therapeutic potential of mesenchymal stem cells (MSC) isolated from human umbilical cord, a rich and easily accessible source of MSC, in an experimental model of PD induced by the neurotoxin 1-metyl, 4-phenyl, 1, 2,3, 6-tetrahydropyridine (MPTP). When infused in the right cerebral ventricle of rats, MSC could be detected in small amounts mainly in the ipsilateral striatum, one week after the cell injection, as revealed by immunofluorescence with antibody specific to human DNA. This distribution pattern was observed in either control or MPTP-treated rats. In contrast, when injected directly in the right striatum, a greater proportion of MSC could be detected mainly in the striatum and in the substantia nigra (SN) at both ispilateral and contralateral sides relative to the cell infusion site. MSC could be detected 30 days after injection either by histological analysis or magnetic resonance imaging. Animals exposed to MPTP displayed motor deficits and bilateral degeneration of dopaminergic neurons in the SN, verified by the Nissl staining and by immunohistochemistry for tyrosine hydroxylase-positive neurons. Intraestriatal infusion of MSC preserved dopaminergic neurons in the SN and significantly improved motor deficits. Similar effects were not found in control animals (sham) or in rats treated with human fibroblasts. These findings support the potential application of human umbilical cord MSC in experimental PD treatment.

DO FACTORS RELEASED FROM DYSTROPHIC MUSCLE ENHANCE MYOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD TISSUE? Secco, Mariane[1], Vieira, Natassia M.[1], Jazedje, Tatiana[1], Bueno Junior, Carlos R.[1], Valadares, Marcos[1], Okamoto, Oswaldo K.[2], Zatz, Mayana[11]Human Genome Research Ctr Biosciences Inst - University of Sao Paulo, São Paulo, Brazil, [2]Department of Neurology and Neurocirurgy, Federal University of São Paulo, São Paulo, Brazil

Progressive muscular dystrophies are a group of disorders characterized by progressive and irreversible muscle degeneration for which there is no therapy. Human umbilical cord tissue (HUCT) has been considered as an important source of mesenchymal stem cells (MSC) with the ability to differentiate into distinct cell types. However, there is limited information concerning the most favorable conditions to induce differentiation of MSC into muscle cells. It has been proposed that factors released

from injured muscle provide the signals that contribute to the establishment of a favorable microenvironment to initiate the regeneration process. However, it is not known whether local signals released after damage are specific for muscle progenitor cells or whether they also promote homing as well as the myogenic commitment and differentiation of human MSC. Here we investigated, for the first time, if dystrophic muscle releases factor(s) capable of inducing the myogenic differentiation of MSC from HUCT *in vitro*. For this purpose, a conditioned medium from *mdx* muscle, the murine model of Duchenne muscular dystrophy, was prepared and its myogenic effect on MSC was evaluated after incubating cells in culture medium containing either 0,5% FBS alone (negative control) or supplemented with 0,5 mg/mL of conditioned medium. A medium supplemented with 3% of horse serum was used as a positive control. We observed that the incubation of MSC isolated from HUCT in the conditioned medium prepared from *mdx* muscle, was much more efficient in the differentiation of MSC in muscle cells and fusion into multinucleated myotubes (with a transient increase of MyoD and myogenin expression), as compared to cells incubated under control conditions. These results suggest that inflammatory and growth factors with myogenic effects, present in this conditioned medium, may be involved in such MSC differentiation. In summary, our experiments showed that the signals released from *mdx* dystrophic muscle were capable to enhance the myogenic differentiation of MSC from umbilical cord tissue. These results may have important applications for future therapies in patients with different forms of muscular dystrophies.

DO MESENCHYMAL STEM-CELLS FROM DIFFERENT SOURCES HAVE THE SAME POTENTIAL TO ORIGINATE MUSCLE CELLS WHEN INJECTED INTO THE DYSTROPHIC SJL MICE? **Vieira, Natássia[1], Zucconi, Eder[1], Bueno Junior, Carlos[1], Secco, Mariane[1], Brandalise, Vanessa[1], Suzuki, Miriam F.[1], Bartolini, Paoloi[2], Vainzof, Mariz[1], Zatz, Mayana[1]** [1]Human Genome Research Ctr Biosciences Inst - Univ of Sao Paulo, São Paulo / SP, Brazil, [2]National Nuclear Energy Commission-IPEN-CNEN, São Paulo, São Paulo / SP, Brazil

Limb-girdle muscular dystrophies (LGMD) are a group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The murine model of LGMD2B, *SJL* mice, carries a deletion in the dysferlin gene that causes a reduction in the protein levels to 15% of normal. The mice show muscle weakness that begins at 4-6 weeks and is complete by 8 months of age. The possibility to restore the defective muscle protein and improve muscular performance by cell therapy is a promising approach for the treatment of LGMD or other forms of progressive muscular dystrophies. Umbilical cord tissue and adipose tissue are rich sources of mesenchymal stem cells, which have been widely investigated for cell-based therapy studies. We have recently shown that human adipose-derived stem cells (hASCs) can differentiate into skeletal muscle when in contact with dystrophic muscle *in vitro* and *in vivo*. Here we injected human umbilical cord mesenchymal stem cells into the *SJL* mice with the same protocol used for hASCs, aiming to analyze their potential to differentiate into muscle cells as compared with the previous results obtained with hASCs. We evaluated their ability to: engraft into recipient dystrophic muscle; express human muscle proteins in the dystrophic host and improve muscular performance. The main objective of this study is to evaluate if mesenchymal stem-cells from different sources have the same potential to reach and originate muscle cells *in vivo* or if this capability is influenced by the niche from where they were obtained.

- **Congresso da Sociedade Americana de Genetica Humana, Washington, 2-6 de Novembro, 2010.**

How different FGFs contribute to Apert Syndrome phenotype. ***E. Yeh, R. D. Fanganiello, D. Y. Sunaga, M. R. Passos-Bueno*****.**

Apert syndrome (AS) is characterized by craniosynostosis and limb abnormalities and is primarily caused by p.S252W mutations in FGFR2, which lead to prolonged ligand-receptor engagement and violation of FGFR2 ligand binding specificity. This mutation compromises the patient even after birth, particularly by a high rate of post-surgical cranial resynostosis. Despite a large literature on FGF-FGFR signaling, we still have unanswered questions concerning the pathways activated by each FGF, the effect of this mutation on cellular function and how the mutation ultimately cause the phenotype. By evaluation of cellular behavior in periosteal fibroblastoid cells from the coronal suture of AS p.S252W patients (n=3) and from matched controls (n=3) we observed that p.S252W mutation is associated with excessive proliferation and enhanced migration in the presence of FGF2 and FGF10. These cells also have increased osteogenic potential, which is inhibited by FGF2 and FGF10. Furthermore, we show that FGFs 2 and 10 trigger different downstream signaling pathways in AS cells and the most relevant pathways are associated with CNS development and inflammatory diseases, respectively. Since appropriate inflammation is involved in wound healing in adult humans and given that the recurrence of suture fusion after surgical repair in AS patients implies a rapid wound healing, this analysis indicates the role of FGF10 in $FGFR2^{+/S252W}$ periosteal cells as a starter and all the altered cell behavior we observed in response to FGF2 and FGF10 are very likely involved in the resynostosis process, as these ligands are expressed in the sutures even after birth. Overall, the new correlations between cellular functions and transcriptome alterations in the presence of the p.S252W mutation can partly explain the high rate of cranial resynostosis after surgical intervention.

- **The American Society of Bone and Mineral Research Meeting / Toronto, ON / 15-19 de outubro de 2010**

Enhanced healing of rat calvarial critical size defects with beta-tricalcium phosphate (beta-TCP) discs associated with dental pulp and adipose-derived stem cells R.D.Fanganiello1, F.A.A. Ishy1, C. Ribeiro2, D.F. Bueno1, A.H.A. Bressiani2, M.R. Passos-Bueno1

Development of biomaterials to replace bone implants is fundamentally important in the field of tissue engineering and an ideal scaffold to support bone regeneration must have both osteoconductive and osteoinductive properties. The osteoconductive characteristics can be finetuned controlling the macro-structure and the micro-architecture of the biomaterial to be used as a scaffold. On the other hand the osteoinductive properties might be enhanced associating the scaffold with cells harboring osteogenic potential. In this study we screened for micro-macro configurations of 5 different biomaterials to heal rat calvarial critical size defects when associates with adult stem cells of 2 origins. Stem cell populations were isolated from dental pulp of human deciduous teeth (DPSCs) and of human adipose tissue (ASCs) and cell culture was established. These cell populations were mainly positively marked for mesenchymal stem cell antigens (CD29, CD90, CD105, SH3 and SH4) while negative for hematopoietic cell markers (CD14, CD34, CD45, CD117) and for endothelial cell marker (CD31). After in vitro induction these populations were capable to undergo osteogenic differentiation, as evidenced by alkaline phosphatase and alyzarin red staining. Both populations were independently associated (10e5) to 4.5 mm discs of different types of biomaterials: equine trabecular bone (Bioteck), processed porcine bone (BioOSS), powdered hidroxiapatite (HA) (OsteoSynt), biphasic synthetic beta-TCP / HA and monophasic beta-TCP. In vivo ossification was accessed using a rat (Wistar) model of paired critical

size defects. Control groups were performed transplanting the cell free biomaterials. Through histological hematoxylin / eosin staining we compared the in vivo bone formation induced by the different kinds of biomaterials paired with their control groups and we
attested an enhanced ossification of the monophasic beta-TCP when associated with DPSCs or with ASCs as early as 1 month after the surgical intervention, with new bone formation and osteoblast linings surrounding several pores of the scaffold. In this work we showed that this screening and the successful association of adult stem cell to monophasic beta-TCP are solid starting points in order to test both the *in vivo* potential of stem cells from other origins and the clinical applications of these associations with monophasic beta-TCP.

- **III International Symposium of Stem Cells, Gramado, October 2010, Brasil**

Stem cells from dental pulp of neonatal tooth: characterization and expression of neural crest markers Bueno, Daniela F[1]; Almada, Bruno V P[1]; Kobayashi, Gerson Shigeru[1]; Amaral, Cassio E R[2]; Aguena, Meire[1]; Jazedje,Tatiana[1]; Passos-Bueno, Maria Rita[1]

Introduction: Neural crest stem cells (NCSCs) are a multipotent cell population derived from the cranial neural crest, in the embryo. Along with induced pluripotent and embryonic stem cells, NCSCs have the potential to differentiate into neuronal lineages, and thus constitute a promising cell source to be employed in cellular therapy for neurodegenerative diseases. However, obtaining NCSCs from human embryonic tissues is unfeasible. On the other hand, it is known that the dental pulp, a neural crest-derived adult tissue, harbors NSCS subpopulations. In this context, the neonatal tooth, which abnormally erupts shortly after birth, may be more enriched with NCSCs than deciduous teeth.
Purpose: To isolate and characterize stem cells from a neonatal tooth pulp and verify the expression of neural crest cell markers, comparing clonal populations of neonatal tooth- and deciduous tooth pulp-derived stem cells.
Material and methods: The cells were isolated from the pulp of a lateral incisive neonatal tooth from a 1-month-old infant; and from the lateral incisive exfoliated tooth from a 7-year-old child. To establish the primary cell cultures, we used the previously described pre-plating technique (Costa & Bueno *et al*., 2009). The cells were characterized using flow cytometry for stem cell antigens, and their plasticity to differentiate into mesodermal derivatives was tested under appropriate conditions. Four clonal populations were derived from each primary culture, and total RNA was extracted from all 8 clones. Quantitative Real Time-PCR (qRT-PCR) was performed to assess the expression levels of *AMIGO3* and *GABRR3*.
Results: We isolated cells with fibroblast-like morphology from the natal tooth. These cells reacted positively (95% +- 5%) with CD29, CD90, CD105, SH3 and SH4, and negatively with CD45 and CD31. The populations were capable to undergo chondrogenic, adipogenic, osteogenic and myogenic differentiation. Moreover, qRT-PCR assays demonstrated that the mean expression for AMIGO3 and GABRR3 were higher in neonatal tooth-derived clones (0.615353 and 1.285952, respectively) when compared to the deciduous tooth-derived clones (0.282663 for AMIGO3 and 0.434381 for GABRR3).
Conclusion: We showed that cells from this neonatal tooth exhibit typical phenotypic characteristics and differentiation potential similar to mesenchymal stem cells *in vitro*. However, there is a higher expression of neural crest cell markers in clonal populations of the neonatal tooth. These preliminary results suggest that neonatal teeth-derived stem cell cultures may be enriched with a larger NCSC population than dental pulp stem cells. However, further research is necessary to establish the neonatal tooth as a novel source of NCSCs in post-natal tissues.

COMPARABLE PHENOTYPES FOR OTOSPHERES FROM GUINEA PIG AND MOUSE COCHLEA.

OITICICA J; BARBOZA-JUNIOR, LC; BATISSOCO, AC; HADDAD, LA ; MINGRONI-NETTO RC; BENTO RF.

Background: Culturing otospheres from dissociated organ of Corti is an appropriate startingpoint aiming at the development of cell therapy for hair cell loss. Although guinea pigs havebeen widely used as an excellent experimental model for studying the biology of the inner ear,the mouse cochlea has been more suitable for yielding otospheres *in vitro*. The aim of this study was to compare conditions and outcomes of otosphere suspension cultures from dissociated organ of Corti of either mouse or guinea pig at postnatal day three (P3), and to evaluate the guinea pig as a potential cochlea donor for preclinical cell therapy. **Methods:** Organs of Corti were surgically isolated from P3 guinea pig or mouse cochlea, dissociated andcultivated under non-adherent conditions. Cultures were maintained in serum-free DMEM:F12medium, supplemented with epidermal growth factor (EGF) plus either basic fibroblast growth factor (bFGF) or transforming growth factor alpha (TGFα). Immunofluorescence assays wereconducted for phenotype characterization. **Results:** The TGFα group presented a number of spheres significantly higher than the bFGF group. Although mouse cultures yielded more cells per sphere than guinea pig cultures, sox2 and nestin distributed similarly in otosphere cells from both organisms. We present evidence that otospheres retain properties of inner ear progenitor cells such as self-renewal, proliferation, and differentiation into hair cells or supporting cells. **Conclusions:** Dissociated guinea pig cochlea produced otospheres *in vitro*, expressing sox2 and nestin similarly to mouse otospheres. Our data is supporting evidence for the presence of inner ear progenitor cells in the postnatal guinea pig. However, there is limited viability for these cells in neonatal guinea pig cochlea when compared to the differentiationpotential observed for the mouse organ of Corti at the same developmental stage.

- **1st meeting on Stem Cell Research of the Instituto de Química: Perspectives of Stem Cells". 2010, São Paulo-SP, Brazil.**

POSTNATAL MOUSE AND MATURE GUINEA PIG COCHLEA AS POTENTIAL SOURCE OF Sox2 AND NESTIN POSITIVE OTOSPHERES

BATISSOCO, AC; BARBOZA-JUNIOR, LC; LEZIROVTZ K; HADDAD, LA ; MINGRONI-NETTO RC; BENTO RF; OITICICA.

Otospheres obtained *in vitro* from dissociated organ of Corti are a starting point aiming at the development of cell therapy for hair cell loss. Although the guinea pig has been widely used as an excellent experimental model for studying the biology of the inner ear, the mouse cochlea has been more successful in yielding otospheres *in vitro*. The aim of this study was to compare conditions and outcomes of suspension cultures of dissociated organ of Corti from mouse and guinea pig at postnatal day three (P3), and to evaluate the guinea pig as a potential cochlea donor for preclinical cell therapy. Cells from the organ of Corti were surgically isolated from P3 guinea pig or mouse cochlea, and cultivated under non-adherent conditions. Cultures were maintained in serum-free DMEM:F12 medium, supplemented with epidermal growth factor (EGF) plus either basic fibroblast growth factor (β-FGF) or transforming growth factor alpha (TGF-α). Anti-nestin and anti-sox2 antibodies were used in indirect immunofluorescence of otospheres after 15 days *in vitro*. The TGFα group presented a significantly higher number of spheres than the β-FGF group. Although mouse cultures yielded more cells per sphere than guinea pig cultures, sox2 and nestin were expressed and distributed similarly in otosphere cells from both organisms. Dissociated guinea pig cochlea produced otospheres *in vitro*, expressing sox2 and nestin similarly to mouse otospheres. Cells labeled for these two markers is supporting evidence for the presence of inner ear progenitor cells in the postnatal guinea pig, retaining differentiation potential, as observed in the mouse.

- **XXXIX Annual Meeting of The Brazilian Biochemistry and Molecular Biology Society, 18 a 21 de Maio de 2010, Foz do Iguaçu, Paraná.**

EXPRESSION OF E2F1 DURING POST-NATAL BRAIN DEVELOPMENT: A POSSIBLE ROLE IN CEREBELLAR NEUROPROGENITOR CELL PROLIFERATION.

Suzuki, D.E.1, Ariza C.B.2, Porcionatto, M.A.2, Okamoto, O.K.1 1Disciplina de Neurologia Experimental, Departamento de Neurologia e Neurocirurgia, UNIFESP, São Paulo, Brazil; 2Disciplina de Biologia Molecular, Departamento Bioquímica, UNIFESP, São Paulo, Brazil.

In the developing cerebellum, new granule neurons are generated from neuroprogenitor cells which extensively proliferate until maturation of the cerebellar cortex. This process lasts until early post-natal stages and disturbances may contribute to development of medulloblastoma. At the molecular level, neuroprogenitor cell proliferation is regulated through an orchestrated action of the SHH, NOTCH and WNT pathways but the underlying mechanism still need to be dissected. Here, we hypothesized a possible involvement of the E2F1 transcription factor. Granular neuroprogenitor cells were purified from rat cerebellum in different post-natal days (P1, P3, P6, P9, and P12). [3H]-thymidine-incorporation assays revealed cell proliferation activity peaking at P3, with a subsequent continuing decrease in proliferation rates occurring from P6 to P12. This gradual decline in proliferating neuroprogenitors paralleled the extent of cerebellum maturation confirmed by histological analysis with cresyl violet staining and expression profile of SHH, NOTCH, and WNT4 genes by real-time PCR. A time-course analysis of E2F1 expression in neuroprogenitors revealed significantly higher levels at P12, correlating with decreased cell proliferation. Expression of the cell cycle inhibitor p18Ink4, a target of E2F1, was also significantly higher at P12. Altogether, these results suggest that E2F1 may be involved in the inhibition of granular neuroprogenitor cell proliferation during post-natal development.

HOXC9 transcription factor is aberrantly expressed in Medulloblastoma. Rodini, C.O.1, Suzuki, D.E.1, Pereira, M.C.L1, Janjoppi, L.1, Toledo, S.R.C2, Okamoto, O.K.1

1Disciplina de Neurologia Experimental, UNIFESP, São Paulo, Brazil; 2Department of Morphology, Division of Genetics - Instituto de Oncologia Pediátrica, GRAACC/UNIFESP, São Paulo, Brazil.

Medulloblastoma is the leading cause of death related to cancer in early childhood. Generation of this highly malignant primary tumor of the central nervous system is linked to aberrant neurogenesis during brain development. However, the molecular mechanisms involved still need to be clarified and the identification of prognostic markers is of great clinical relevance. A previous study with glioblastoma stem cells found that up-regulation of HOXC9 is correlated with neoplastic transformation. Here, we evaluated whether this transcription factor with critical roles in development was involved in medulloblastoma formation and clinical outcome. Tumors were classified according to clinical parameters into high risk (HR, n=16) and low risk (LR, n=16) groups. Gene expression was assessed by real-time PCR. Both presence and level of expression were considered in the analyses. Non-neoplastic control tissues did not express HOXC9. In tumors, a HOXC9 expression could be detected in 62.5% and 37.5% of HR and LR samples, respectively. Furthermore, mean expression values for HOXC9 were 4-times higher in HR (15.72+34.04) than in LR (3.94+5.87) tumors. Our results indicate that HOXC9 is up-regulated in medulloblastoma with a tendency of higher frequency and level of HOXC9 expression in HR tumors. However, no significant correlations were found regarding development of metastasis or overall survival rates.

- **56º Congresso Brasileiro de Genetica, Guarujá, SP, 14-17 de setembro, 2010.**

Mesenchymal Stem Cells to be used for drug screening for Apert Syndrome. Células tronco mesenquimais como modelo para screening de drogas para o tratamento da síndrome de Apert. Atique RFT, Yeh E, Fanganiello R, Passos-Bueno MR.

Introdução: A síndrome de Apert é uma doença genética autossômica dominante, causada em geral por 2 mutações de troca de aminoácido no gene *FGFR2*, as quais diminuem a especificidade de ligação do receptor, aumentando sua afinidade tanto por seus ligantes quanto por outros FGFs que normalmente não se ligariam. Um dos principais sinais da síndrome é a fusão das suturas coronais ao nascimento, responsável pelas deformações craniofaciais desses pacientes. Atualmente o único tratamento para a síndrome de Apert são as intervenções cirúrgicas, nas quais as suturas fusionadas são reabertas. No entanto as suturas voltam a se fechar, fazendo necessárias várias cirurgias durante a vida do paciente. Acredita-se que o fechamento das suturas coronais na síndrome de Apert ocorre pelo aumento da proliferação e diferenciação osteogênica das células tronco mesenquimais causadas pela mutação. Sendo assim, drogas capazes de reverter esse fenótipo têm grande potencial para auxiliar no tratamento pós-cirúrgico de portadores da síndrome. Metodologia: Duas drogas foram testadas, 5-Hidroxitriptamina (Serotonina) e PD173074, um inibidor do domínio Tirosina-quinase dos FGFRs. Foram verificados os efeitos das drogas sobre a proliferação celular com a contagem total de células durante 4 dias. Foram utilizadas 3 culturas de pacientes com Apert e 3 controles. A diferenciação osteogênica foi verificada indiretamente pela deposição de cálcio na matriz extracelular por coloração com Vermelho de Alizarina. Resultados: A Serotonina não foi capaz de causar diferenças significativas na proliferação celular ou na deposição de cálcio na matriz. O PD173074 foi capaz de diminuir significativamente a proliferação celular tanto em células de pacientes quanto nas controles. Conclusões: As células tronco mesenquimais se mostraram sensíveis a pelo menos uma das drogas, o que demonstra que são capazes de evidenciar diferenças no fenótipo celular que leva à síndrome, no entanto estudos *in vivo* ainda são necessários para corroborar os resultados encontrados *in vitro*.

56º Congresso Brasileiro de Genética

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Estudo comparativo de linhagens celulares do aparelho reprodutor feminino como *Feeder-layers* de células tronco embrionárias humanas

Pelatti, M; Secco, M; Zatz, M; Jazedje, T

Laboratório de Células Tronco, Centro de Estudos do Genoma Humano, Instituto de Biociências, Universidade de São Paulo.
mayrapelatti@yahoo.com.br

Palavras-chave: *Feeder-layers*, MEF, Células tronco embrionárias

Células Tronco Embrionárias Humanas (CTEH) apresentam um grande potencial de auto-renovação e diferenciação, podendo originar qualquer um dos 216 tecidos do corpo humano. O cultivo das CTEH necessita de uma camada de células que auxiliam no seu desenvolvimento, as chamadas *feeder-layers*. Dentre as várias possibilidades de *feeder-layers* disponíveis, o fibroblasto de embrião de camundongo (MEF – *murine embryonic fibroblast*) é o mais utilizado. Visando mimetizar ao máximo o ambiente de desenvolvimento do embrião humano, o objetivo do nosso trabalho é testar três diferentes linhagens de células do aparelho reprodutor feminino humano como *feeder-layers* de CTEH: células tronco de trompa, de sangue menstrual e de endométrio. Fazendo uma análise comparativa entre estas três linhagens celulares e as linhagens controle (MEF e fibroblasto de pele humana) estamos avaliando quais destas células conferem um melhor suporte para o crescimento das CTEH, qualitativa (através da morfologia, diâmetro e quantidade colônias) e quantitativamente (dosando os fatores liberados pelas *feeder-layers*). A capacidade tumorigênica de todas as linhagens celulares utilizadas neste estudo foram avaliadas *in vivo*, no modelo de camundongo imunossuprimido (*nude*). Neste momento, verificamos que: (1) nenhuma das *feeder-layers* propostas causou tumores nos animais *nude*; (2) as células tronco de trompa apresentaram melhores resultados, sendo inclusive melhores do que a MEF em relação ao número de colônias e número total de células, e melhores do que o fibroblasto de pele humana, em relação ao diâmetro e morfologia das colônias. Avaliaremos, ainda, a expressão dos fatores: bFGF (basic Fibroblast Growth Factor), LIF (Leukemia Inhibitor Factor), TGF-β (Transforming Growth Factor), WINTs 2, 4 e 8b, através de Elisa e Western Blot, e os marcadores específicos: Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 e SOX2, através de RT-PCR e *Realtime*. Com estes resultados, esperamos contribuir para o melhoramento não apenas do cultivo de CTEH em ambiente livre de contaminantes de origem animal, como também propor uma nova alternativa para o cultivo de embriões humanos.
Apoio financeiro: FAPESP/CEPID, INCT, CNPq

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Diferenciação neuronal de células-tronco derivadas de dentes deciduos de pacientes com a síndrome de Angelman

Cruvinel, EM[1]; Secco, M[1]; Almeida, CAN[1]; Zatz, M[1]; Cassola, AC[2]; Koiffmann, CP[1]

[1]Departmento de Genética e Biologia Evolutiva, Centro de Estudos do Genoma Humano, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil
[2]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil
estela.cruvinel@usp.br

Palavras-chave: Células-tronco, Síndrome de angelman, Diferenciação neuronal, Polpa de dente, UBE3A

A síndrome de Angelman (AS) é uma doença neurocomportamental caracterizada por atraso do desenvolvimento neuropsicomotor, retardamento mental grave, deficiência na fala, epilepsia, ataxia, aspecto feliz com crises de riso facilmente motivadas, microcefalia, braquicefalia, macrostomia e prognatismo. A AS é causada pela perda de função do gene *UBE3A* localizado no cromossomo 15 materno. *UBE3A* codifica uma ubiquitina ligase (E6-AP) e apresenta *imprinting* tecido-específico: transcrito predominantemente materno é encontrado em regiões do cérebro, mas nos demais tecidos a expressão é bialélica. Um RNA antisenso paterno que é expresso no cérebro é considerado o responsável pelo *imprinting*. Quatro mecanismos diferentes podem resultar na AS: aproximadamente 70-75% dos pacientes apresentam deleção materna do segmento 15q11-q13, 2-3% UPD(15) paterna, cerca de 5% têm defeito no (centro de *imprinting* (IC) do cromossomo 15 materno e, finalmente, 8% apresentam mutação no gene *UBE3A*. Recentemente, células-tronco de pacientes com diferentes doenças genéticas têm sido investigadas visando a compreensão dos mecanismos que levam às patologias e ao desenvolvimento de terapias. O objetivo deste trabalho é analisar e comparar neurônios derivados de células-tronco de dente decíduos (SHEDs) de pacientes com AS e controles normais. Foram estabelecidas 3 linhagens de SHEDs de pacientes AS com deleção. Todas as linhagens foram caracterizadas como células-tronco: mostram capacidade de se auto-renovar, expressam proteínas de membrana de células mesenquimais e se diferenciam em adipócitos, condrócitos e osteoblastos. As SHEDs já expressam nestina, que é um marcador neuronal imaturo, e algumas linhagens expressam MAP2, que é uma proteína de neurônio maduro. As SHEDS foram submetidas a um protocolo já descrito de diferenciação neuronal. As células diferenciadas continuaram expressando nestina e algumas linhagens tiveram aumento da expressão de MAP2. A imunocitoquímica mostrou que SHEDs diferenciadas de controles normais apresentaram aumento da expressão de MAP2 e TUJ1, enquanto que linhagens de pacientes não apresentaram aumento da expressão protéica de MAP2 e em 2 linhagens não houve aumento da expressão de TUJ1. Resultados de medidas eletrofisiológicas (técnica de "patch-clamp". configuração "*whole-cell" voltage clamp*) mostraram que SHEDs diferenciadas em neurônios apresentam correntes de sódio e potássio passando por canais dependentes de voltagem, característicos de células excitáveis. Mesmo, células não diferenciadas também apresentavam tais correntes. O fato das SHEDs expressarem constitutivamente algumas proteínas neuronais, incluídas as dos canais pelos quais passam estas correntes, sugerem que são células que já possuem alguma característica de diferenciação neuronal. Além disso, nossos resultados indicam que as células dos pacientes respondem diferentemente ao protocolo de diferenciação neuronal.
Apoio financeiro: CEPID-FAPESP, CNPq

- **XXXIV Congresso Anual da Sociedade Brasileira de Neurociências e Comportamento - SBNeC 2010, 8 a 11 de setembro de 2010, Caxambu, MG.**

TERAPIA CELULAR EM DOENÇA DE PARKINSON: UMA AVALIAÇÃO PRÉ-CLÍNICA DO TRANSPLANTE DE CÉLULAS-TRONCO MESENQUIMAIS DE CORDÃO UMBILICAL HUMANO

Pereira, M. C. L.[1]; Secco, M.[2]; Suzuki, D. E.[1]; Janjoppi, L.[1]; Rodini, C. O.[1]; Zatz, M.[2]; Okamoto, O. K.[1]. [1]Disciplina de Neurologia Experimental, Departamento de Neurologia e Neurocirurgia, Universidade Federal de São Paulo. [2]Centro de Estudos do Genoma Humano, Universidade de São Paulo.

Dentre as novas estratégias terapêuticas atualmente em consideração para a Doença de Parkinson (DP), destacam-se os processos neuro-restauradores promovidos pela terapia celular. Embora estudos clínicos iniciais com transplante alogênico de células mesencefálicas fetais tenham demonstrado aumento de síntese de dopamina e atenuação de rigidez e bradicinesia em pacientes transplantados, estudos clínicos randomizados, duplo-cegos e placebo controlados subseqüentes não encontraram melhora clínica significativa, além de reportarem efeitos adversos como distonias e discinesias. Assim, questões fundamentais como tipos alternativos de células para transplante, dose e vias de administração, bem como efeitos terapêuticos e riscos específicos envolvidos, ainda persistem e devem ser avaliadas em uma fase pré-clínica. No presente estudo, investigamos o potencial terapêutico de células-tronco mesenquimais (CTM) obtidas de cordão umbilical humano, uma fonte biológica rica em CTM e de fácil acesso, em um modelo experimental de DP induzido pela neurotoxina 1-metil, 4-fenil, 1, 2, 3,6-tetrahidropiradina (MPTP). Em particular, avaliamos os efeitos da administração intracerebral de CTM sobre a população de neurônios dopaminérgicos e sobre a atividade motora. Ratos Wistar machos adultos foram tratados com 0,2 mg de MPTP (n=10) ou salina (grupo controle, n=10) e, após sete dias, submetidos à infusão intraestriatal de CTM, fibroblastos humanos ou veículo (*sham*), por meio de cirurgia estereotáxica. Após 23 dias do transplante celular, os animais foram inicialmente submetidos a testes comportamentais para avaliação de atividade locomotora, força muscular, acinesia e catalepsia. Posteriormente, os animais foram sacrificados para avaliação de formação de enxerto celular e preservação de neurônios dopaminérgicos. Análises por imunofluorescência com anticorpo específico para DNA humano revelaram a presença de CTM no cérebro de ratos, principalmente no estriado e na substância negra (SN), tanto no lado ipsilateral, quanto no lado contralateral ao sítio de infusão de células. Animais tratados com MPTP apresentaram déficits motores e degeneração bilateral de neurônios dopaminérgicos na SN, verificado tanto pela coloração de neurônios pelo método de Nissl, quanto pela marcação imunohistoquímica para tirosina hidroxilase. Entretanto, animais tratados com MPTP, mas que receberam CTM, apresentaram maior preservação de neurônios dopaminérgicos na SN e melhora significativa de déficits motores ($p < 0,05$ pelo Teste de Bonferroni). O mesmo efeito não foi observado nos animais controles (*sham*), tampouco em animais transplantados com fibroblastos humanos. Esses resultados suportam o potencial uso de CTM de cordão umbilical humano no tratamento da DP e reforçam a continuidade dos estudos para entendimento dos mecanismos envolvidos e avaliação de eventuais efeitos adversos.

Awards

- Winner, IV *Prêmio SAÚDE!* 2009. Fonte: http://saude.abril.com.br/premiosaude/2009-novo/vencedor-mulher.shtml

Trompas de falópio: uma nova fonte de células-tronco multipotentes descartadas em cirurgias **Autores:**Tatiana Jazedje da Costa Silva; Perin, PM; Czeresnia, CE; Maluf, M; Halpern, S ; Secco, M ; Bueno, DF; Vieira, NM; Zucconi, e Zatz, M.
Instituição: Centro de Estudos do Genoma Humano da Universidade de São Paulo; Centro Especializado em Reprodução Humana, em São Paulo e Clínica Celula Mater, em São Paulo

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Perspectiva de um futuro tratamento para osteoporose ou outras doenças ósseas com base em células tronco. Tatiana Jazedje da Costa Silva, Daniela Franco Bueno, Carlos Eduardo Czeresnia, Paulo Marcelo Perin, Mariangela Maluf, Silvio Halpern, Maria Rita Passos Bueno e Mayana Zatz. Centro de Estudos do Genoma Humano do Instituto de Biociências da Universidade de São Paulo (USP).