Felipe Augusto André Ishiy

Estudo de Marcadores moleculares no processo de diferenciação osteogênica de células-tronco mesenquimais

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2016

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Evaluation of molecular markers in osteogenic differentiation of mesenchymal stem cells

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutor em Ciências, na Área de Biologia/Genética.

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Table of Contents

Abstract

Resumo

Chapter 1 - General Introduction 01

Objectives..21

Chapter 2 - *Increased In Vitro Osteopotential in SHED Associated with Higher IGF2 expression When Compared with hASCS*...

Chapter 3 - *CD105 low expression levels promotes increased osteogenesis in SHED and depends on microRNA regulation*...

Chapter 4 - *Improvement of In Vitro Osteogenic Potential though Differentiation of Induced Pluripotent Stem Cells from Human Exfoliated Dental Tissue towards Mesenchymal-like Stem Cells*..

Chapter 5 - *Neural crest-derived mesenchymal cells in the aetiology of Treacher Collins syndrome*...

Chapter 6 - General Discussion and conclusions...22

Chapter 7 - Appendix: Additional publications and participations in Conferences/Meetings..26

References...30

Abstract

The use of stem cells is a promising therapeutic approach for tissue engineering by their ability to boost tissue regeneration, and to model *in* vitro human genetics disorderssince it provides continuous supplies of cells with differentiation potential. Our study has been focused in the identification of molecules or mechanisms that could contribute to a better osteogenesis in mesenchymal stem cells (MSC). To achieve our goals we have explored the osteopontential differences of stem cells from different sources. In this regard, we have observed that MSCs from human exfoliated deciduous teeth (SHED) presented higher *in vitro* osteogenic differentiation potential (OD) as compared to MSCs derived from human adipose tissue (hASCs). Through microarray analysis and cell sorting, we have shown that IGF2 and CD105 expression levels contribute to these osteopontential cell differences, that is, higher IGF2 expression levels and lower CD105 expression levels were associated with the increased osteogenic potential of SHED as compared to hASCs. The molecular mechanisms associated with the diferent expression levels of IGF2 and CD105 in these cells were also investigated. Despite the advantages of adult MSCs they can exhibit drawbacks such as restricted self-renewal and limited cell amounts. Induced Pluripotent Stem Cells (iPSC) technology has emerged as an alternative cell source, as they provide more homogeneous cellular populations with prolonged self-renewal and higher plasticity. We verified that the OD of MSC-like iPSC differs from MSCs and it depends on the iPSCs originating cellular source. Comparative *in vitro* osteogenesis analysis showed higher osteogenic potential in MSC-like cells derived from iPS-SHED when compared with MSC-like cells from iPS-FIB and SHED. iPSCs can be also used as a tool to model genetic disorders. We have thus proposed to verify if it could be possible to *in vitro*model Treacher-Collins syndrome, a condition with deficient craniofacial bone development. We have compared the effects of pathogenic mutations in *TCOF1* gene in cell proliferation, differentiation potential between MSCs, dermal fibroblasts, neural-crest like and MSC-like cells differentiated from iPSCs.TCS cells showed changes in cell properties anddysregulated expression of chondrogenesis markers during osteogenic and chondrogenic differentiation. In summary, the comparative analysis of stem cells of different sources allow us to identify markers that may facilitate osteogenesis and that it is possible to establish an *in vitro* model to Treacher-Collins syndrome.

Resumo

O uso de células-tronco trata-se de uma abordagem terapêutica promissora para a engenharia de tecidos, devido à sua capacidade na regeneração de tecidos, e para modelamento *in vitro* de distúrbios genéticos humanos, uma vez que fornece um abastecimento contínuo de células com potencial de diferenciação. Nosso estudo se propos a identificar moléculas e mecanismos que contribuem na otimização da osteogênese de células-tronco mesenquimais (MSCs). Para atingir nossos objetivos exploramos as diferenças no potencial osteogênico (PO) de MSCs de diferentes fontes. Observamos que MSCs de polpa de dente decíduo humano (SHED) apresentaram maior PO em comparação com as MSC derivadas de tecido adiposo humano (hASCs). Através de análise de microarray de expressão e *cell sorting,* demonstramos que os níveis de expressão de IGF2 e CD105 contribuem para as diferenças do PO, onde a maior expressão de IGF2 e menor expressão de CD105 estão associadas a maior PO em SHED quando comparado as hASCs. Também investigamos os mecanismos moleculares associados aos diferentes níveis de expressão de IGF2 E CD105 em ambas as fontes celulares. Apesar das vantagens, as MSCs podem apresentar pontos negativos como restrita auto-renovação e menor quantidade de células. Células-tronco pluripotentes induzidas (iPSC) surgem como uma fonte celular alternativa, proporcionando populações celulares homogêneas com auto-renovação prolongada e maior plasticidade. O PO de *MSC-like* iPSC difere de MSCs, e este potencial é dependente da fonte celular em que as iPSCs são obtidas. Análise comparativa de PO *in* vitro demonstrou maior osteogênse em células *MSC-like* derivadas de iPS-SHED quando comparada as células *MSC-like* de iPSCs-fibroblastos e SHED. iPSCs também podem ser utilizadas como ferramenta para investigar doenças genéticas humanas. Propomos a modelagem *in* vitro da síndrome de Treacher-Collins (TSC), doença que acomete as estruturas craniofaciais durante o desenvolvimento ósseo. Comparamos os efeitos de mutações patogênicas no gene TCOF1 na proliferação celular, potencial de diferenciação entre MSCs, fibroblastos dérmicos, *neural-crest like* e células *MSC-like* diferenciadas de iPSCs. Células de pacientes TCS exibiram alterações em propriedades celulares e na expressão de marcadores osteogênicos e condrogênicos. Em resumo, a análise comparativa de células-tronco de diferentes fontes permitiu a identificação de marcadores e mecanismos que podem facilitar a osteogênese e tambem demonstramos que é possível modelar *in vitro* a síndrome de Treacher-Collins.

CHAPTER I

Introduction

1- Introduction

1. Tissue Engineering and Regenerative Medicine

Bone tissue is the supporting structure of our organism responsible for blood cell production and it is an important source of minerals (Freyschmidt, 1993). Due to the importance of this tissue for our organism the development of new therapies in bone tissue engineering and regenerative medicine rises from the necessity to obtain more efficient and satisfactory results in bone reconstruction procedures, particularly in situations that involve large regions to be reconstructed. The current golden standard in bone tissue engineering is the transplantation of autologous bone graft to repair bone loss due to disease, malformation or trauma, but this approach is associated with critical shortcomings, such as pain and morbidity in the graft site, and limited tissue supply (Amini et al., 2012; Bose et al., 2012; Grayson et al., 2015; Healy et al., 2007; Holzwarth & Ma, 2011; Huang et al., 2015).

Tissue engineering can be described as a multidisciplinary area, including knowledge from the fields of engineering, biology and medicine. Langer and Vacanti (1993) proposed that three main pillars conceptually support tissue engineering: progenitor cells, biomaterials/scaffolds and factors/signaling molecules (Casser-Bette et al., 1990; Langer & Vacanti, 1993; Glotzbach et al. 2011). It is essential to understand and characterize each one of these pillars to optimize and enhance bone reconstruction and regenerative processes. Below we describe the importance of

progenitor cells to the establishment of a functional bone with compatibility and integration to the bone tissue environment (Badylak & Nerem, 2010).

1.1 Stem Cells

Stem cells have emerged as a promising tool for regenerative medicine and tissue engineering mainly because of their ability to replicate themselves and originate the same non-specialized cell type over long periods (self-renewal) and due to their differentiation potential capacity (Bianco et al, 2013; He et al, 2009).

Stem cells can be classified depending on their differentiation plasticity (**Figure 1**) that can be divided in two broad types: pluripotent stem cells (human embryonic stem cells - hESCs and induced pluripotent stem cells - iPSCs) that harbor the capacity of differentiation in all three germ layers of the developing embryo and in all adult cell types, and the adult multipotent stem cells (hematopoietic stem cells - HSCs, mesenchymal stem cells - MSCs) that generate specific lineages or tissues and can be found in different adult tissues (Bazley *et al,* 2015; Jung, *et al.* 2012; Phinney and Prockop, 2007; Takahashi and Yamanaka, 2006).

Despite the potential of differentiation in all cell types of the adult body, pluripotent stem cells (hESCs and iPSCs) face considerable obstacles to their use in regenerative medicine and tissue engineering due to the intrinsic capacity of teratoma formation when delivered *in vivo,* and the ethical issues related to the use of human embryos to obtain hESCs (Miura et al, 2009; Okita et al, 2007; Takahashi and Yamanaka, 2006; Takahashi et al, 2007). iPSCs have been shown to develop teratoma more efficiently andmore aggresively *in vivo* when compared to hESCs (Gutierrez-

Aranda et al, 2010). There is still no effective method to circunvent and eliminate the possibility of teratoma formation when using hESCs and iPSCs (Mohseni et al 2014).

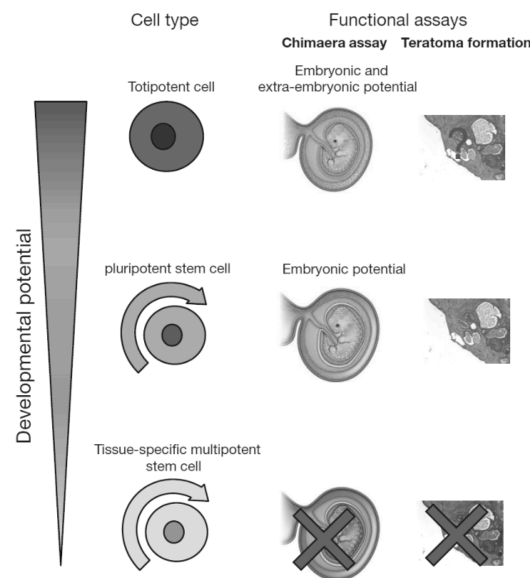

Figure 1. Classification of stem cells depending on their developmental potency (Adapted from Angeles *et al,* Nature, 2015).

Mesenchymal Stem Cells - MSCs (Caplan, 1991) offer several advantages in autologous cell therapy and tissue engineering field due to lower risk of tumorigenicity (when compared with hESCs or iPSCs), immunomodulatory properties and easier access and their easy obtainment when compared with ESCs (Bara et al 2014; Caplan and Dennis, 2006).

MSCs can act in different and essential steps of bone maintenance (Bielby et al, 2007) and during bone regeneration after trauma. During the first moment (hours to days after injury) MSCs contribute to the local immunomodulation process through production of immunosuppressive factors (Grayson et al, 2015; Najar et al, 2016; Nauta, et al, 2007). After days or weeks of injury, MSCs can supply osteo-

chondroprogenitor cells to repair the bone tissue due to their capacity of multipotent differentiation (Bruder et al, 1994; Chamberlain et al, 2007; Guan et al, 2012).

These multipotent, plastic-adherent and colony-forming cell populations can be characterized through an immunophenotype marker panel (positive staining for CD73, CD90 and CD105, and negative for CD11B or CD14, CD19 or CD79α, CD34, CD45 and HLA-DR), whichwas first characterized in bone marrow-derived stem cells-BMSCs (Dominic et al, 2006; Friedenstein et al 1966, 1970; Pittenger et al, 1999).

In the last decades, MSCs have been isolated from several human adult tissues such as peripheral blood (Zvaifler *et al*., 2000), umbilical cord blood (Erices *et al.*, 2000), fetal tissues (Campagnoli *et al.*, 2001), adipose tissue (Zuk *et al.,* 2001; Zuk *et al.*, 2002), amniotic liquid (In'tanker *et al*., 2003), umbilical *cord* (Sarugaser *et al.,* 2005), dental pulp (Gronthos *et al.,* 2000; Miura et al., 2003), from *orbicularis oris* muscle (Bueno et al., 2009), among others.

Despite the discovery of new sources, markers and use of stem cells, Bone Marrow Mesenchymal Stem Cells (BMSCs) remained the most studied adult stem cells (Friedenstein et al., 1966; Kadiyala et al., 1997; Nakagawa et al, 2016; Rada et al, 2011; Seong et al., 2010). BMSCs and other stem cell sources have been used in clinical trials specifically to skeletal regeneration and bone tissue engineering as exemplified in **Figure 2,** demonstrating the importance and potential use of these cell sources for regenerative medicine. In stem cell research field there are multiple avenues now open including: stem cells as tools for modeling human diseases mechanisms, identification of bioactive factors and regenerative medicine (Bianco et al, 2013).

After bone injury the organism initiates a cascade of key regenerative steps including action of proinflammatory cytokines, homing of osteogenic progenitor cells

and immune cells. Thus, the introduction of progenitor cells/stem cells during these events could be essential to achieve optimal osteogenesis (Dimitriou et al, 2005; Grayson et al, 2015).

Clinical trials in which stromal cells were used for skeletal regeneration			
Indication	**Cell source**	**Cell processing and delivery**	**Clinical trial**
MSC			
Non-union of bone	Autologous	Direct injection	NCT00512434, NCT01788059
		Implantation with carrier	NCT00250302, NCT01626625, NCT01958502
ONFH	Autologous	Direct injection	NCT02065167,
		Implantation with carrier	NCT01605383
Other (spine fusion, osteoarthritis)	Autologous	Direct injection	NCT01210950
		Implantation with carrier	NCT01552707,
	Allogeneic	Direct injection	NCT01603836,
		Implantation with carrier	NCT00001391,
ASC			
Non-union of bone	Autologous	Implantation with carrier	NCT01532076
	Allogeneic	Direct injection	NCT02140528
ONFH	Autologous	Direct injection	NCT01643655
Other (spine fusion, osteoarthritis)	Autologous	Direct injection	NCT01501461, NCT01885819, NCT02142842,
		Implantation with carrier	NCT01633892,

Figure 2. Examples of Clinical trials in bone tissue engineering using BMSCs and hASCs as an alternative source of mesenchymal stem cells for skeletal regeneration (Abbreviations: ASC, adipose-tissue derived stromal cell, MSC, bone marrow-derived stromal cell; ONFH: osteonecrosis of the femoral head, NCT- clinical trial number) (Adapted from Grayson *et al,* Nature, 2015).

1.2 Alternative MSC sources

Recently efforts to search alternative sources of adult MSCs for bone tissue engineering and regenerative medicine arise due to some limitations presented by BMSCs such as pain, morbidity, possibility of infection during the invasive extraction

process, and low quantity of mesenchymal stem cells obtained (Caplan, 2009; Derubeis and Cancedda, 2004).

Two promissing MSC types are stem cells from human dental pulp cells (Grontos et al., 2001; Miura et al, 2003) and human adipose tissue-derived stem cells-hASCs (Zuk et al., 2002). These two cell sources have shown important characteristics for regenerative medicine/bone tissue engineering such as: accessible source without morbidiy and pain (**Figure 3**), multipotential differentiation and higher proliferation capacity when compared with BMSCs (Gronthos et al., 2000; Kerkis et al., 2006; Laino et al., 2006; Mizuno et al, 2010; Nakamura et al, 2009). Regarding human dental pulp cells, we will focus on those obtained from exfoliated teeh (SHED, Miura et al., 2003) instead of from adult human teeth (DPSC- Gronthos et al., 2001), as this represents one the cell sources here studied.

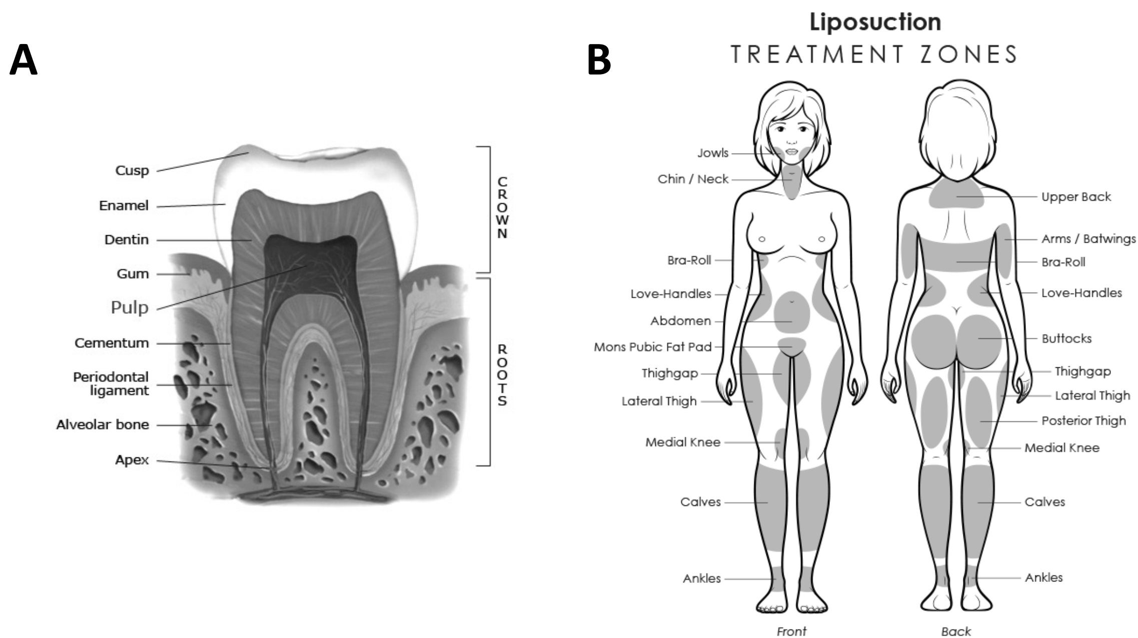

Figure 3.A- Scheme demonstrating the pulp localization in the teeth structure (Adapted from http://www.studiodentaire.com/en/glossary/pulp.php); **B**- Localization of adipose tissue in which liposuction can be performed to obtain ASCs (Adapted from http://drmathewplant.com/liposuction-toronto/). As the cells are obtained from the discarded tissue, this procedure does not cause any additional morbidity or pain to the donor individuals.

SHED exhibits important characteristics to achieve promising results in bone regeneration such as maintenance of cellular plasticity and immunophenotype after freeze-thaw cycles (d'Aquino et al. 2009; Laino et al., 2005; Laino et al., 2006; Huang et al., 2009), *in vivo* bone formation in animal models (Alongi et al, 2010, de Mendonca Costa et al, 2008), and this cell type was used in human clinical applications (D´Aquino et al, 2009; Giuliani et al, 2013).

The international Fat Applied Technology Society adopted the term adipose-derived stem cell (ASCs) to identify the alternative source of MSCs that were firstly described by Zuk et al (2002) (Baer et al, 2012). The material extracted from human subjects under local anesthesia (Mizuno et al, 2012) in which these cells can be obtained is routinely discarded after aesthetics liposuction procedures, and it was demonstrate that obesity incidence has increased substantially in the past years, thus facilitating the obtainment of the necessary material to isolate ASCs (Bourin et al, 2013; Gimble et al., 2007; Witkowska-Zimny & Walenko, 2011).

The autologous use of ASCs facilitates the use of this source in clinical trials and treatments of different kind of diseases as: peripheral vascular/cardiovacular diseases (number clinical trial-NCT01211028), soft tissue augmentation of craniofacial structure (Yoshimura et al, 2008), articular cartilage lesion (NCT01399749) and craniofacial bone reconstruction (Lendeckel et al, 2004; Mesimaki et al, 2009), but despite the easiness obtainemnt ASCs present lower osteogenic potential when compared with SHED (Fanganiello et al., 2015).

Although SHED and hASCs show promising characteristics for regenerative medicine, which is the best cellular source to bone tissue engineering remains to be established. Stem cells from different sources cells might possess differences in their *in*

vitro differentiation potential towards osteoblastic cells (Al-Nbaheen et al 2013). These differences are not completely understood, and they could be partly related to their tissue of origin (Baksh et al., 2007; Huang et al., 2009; Kern et al., 2006). The selection of the best cellular source, or the best subpopulation could be performed through the dissection of possible markers or mechanisms related to enhanced osteogenesis.

The efficacy of treatments using MSCs are still unsatisfactory due to cellular heterogeneity presented by the MSC populations leading to experimental variability, compromising the proliferation and differentiation capacity. It is not possible to morphologically distinguish MSCs from fibroblasts, the *ex-vivo* MSC cultures contain phenotypically distinct cellular types in different commitment stages, and there is no unanimity of intrinsic and specific surface molecules to distinguish MSCs and to differentiate these cells from other cell types (Lv *et al.,* 2014; Mizuno *et al*., 2012).

1.3 Different approaches to understand the osteogenic potential of MSCs

In order to find the best cellular source for bone tissue engineering and regenerative medicine, different approaches are being used such as: Cell reprogramming technology (Takahashi and Yamanaka, 2006), mesenchymal stem cells differentiated from pluripotent stem cells (Barberi et al, 2005) and the study of specific molecular markers and transcriptional pathways, and methodologies to obtain a more homogeneous cellular population to understand the osteogenic potential differences between MSCs (Chung et al, 2013; Levi et al, 2010).

The main methodology employed to this purpose is the use of surface markers to isolate, characterize and compare different subpopulations of MSCs. Using different

surface markers, CD49 and STRO-1, Rada and collaborators (2011) isolated subpopulations of rat adipose derived stem cells and observed differences in osteogenic potential and mesenchymal stem cells markers. Other groups have isolated from dental pulp stem cells a subpopulation called SBP-DPSC (Stromal bone producing-dental pulp stem cell) positive for c-Kit, CD34 and negative for CD45, and this cell population exhibited *in vitro* and *in vivo* osteogenesis and were highly clonogenic (Laino et al, 2005; Papaccio et al 2006).

MSCs are expanded in monolayer plastic flasks and during this expansion process a certain population of cells are selected leading to changes in their phenotype (Bara et al, 2014). A significant immunophenotypic change in hASCs has been shown during serial cellular passage with temporal alteration of mesenchymal stem cell markers as: CD29 (Cluster of differentiation- Adhesion marker), CD34 (Hematopoietic), CD73, 90 and 105 (Mesenchymal) (Mitchell et al. 2006, McIntosh et al. 2006).

CD90 (THY-1) is a mesenchymal stem cell marker associated with osteoblastogenic lineages (Hosoya et al, 2012). Sorted subpopulations of hASCs with higher expression of CD90 exhibited higher *in vitro* and *in vivo* osteogenic differentiation when compared with subpopulations with lower CD90 expression and the heterogeneous (unsorted) populations (Chung et al, 2013).

Another interesting surface marker that has been used to isolate subpopulations of MSCs is the mesenchymal stem cell surface marker: endoglin (CD105), a transmembrane co-receptor of TGFB1 that regulates the proliferation, differentiation potential, immune response and angiogenesis as described in **Figure 4** (Nassiri *et al.,* 2011*;* Whitman, and Raftery 2005; Sanz-Rodriguez *et al.*, 2004; Warrington *et al*, 2005).

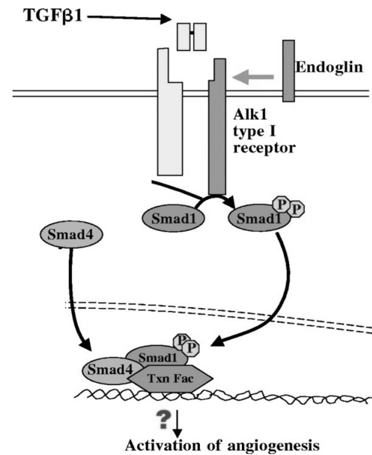

Figure 4. Role of Endoglin acting as a co-receptor of TGFB1 in the regulation of angiogenesis (Adapted from Whitman, and Raftery 2005)

Inhibition of TGFB1 by the receptor kinase inhibitor SB431542 (**Figure 5**) leads to an acceleration of BMP signaling and consequently the maturation of osteoblastic mesenchymal cells, with higher alkaline phosphatase activity and extracellular matrix mineralization (Maeda et al., 2004). In a complementary assay, recombinant TGFB1 treatment repressed cellular proliferation and *in vitro* osteogenic differentiation in adipose derived stem cells from humans and mice (Levi et al, 2010).

Subpopulations negative for the mesenchymal surface marker CD105 (Endoglin) of murine bone marrow stem cells showed higher *in vitro* osteogenesis, adipogenesis and capacity of immunomodulation when compared with positive CD105 subpopulation and heterogeneous (unsorted) population (Anderson *et al,* 2013).

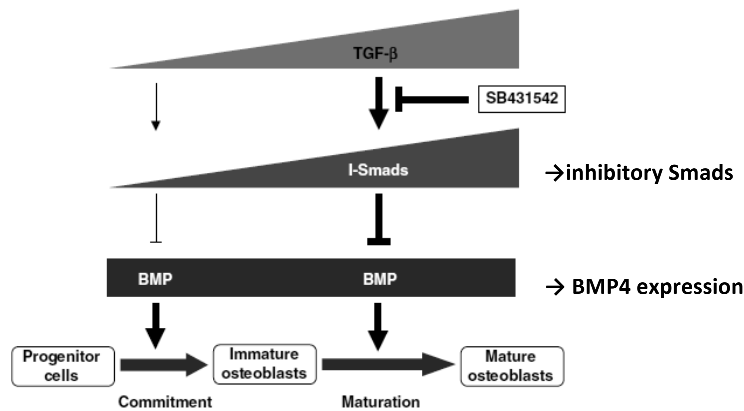

Figure 5. Scheme showing the BMP, SMADs and TGFB1 cross talk during osteogenesis. BMP induces the osteogenic differentiation acting in the early and late maturation of osteoblasts. Due to TGFB signaling, inhibitory SMADs suppress BMP and osteogenic differentiation. The presence of the TGFB1 receptor kinase inhibitor **SB431542,** suppress the expression of TGFB1 leading to an acceleration of BMP signaling and enhancement of osteogenesis (Adapted from Maeda *et al*, 2004).

Single-cell transcriptional analysis of hASCs, has demonstrated that different expression levels of CD105 were correlated with osteogenic markers expression and osteogenesis. Subpopulation of hASCs sorted for lower CD105 expression showed higher *in vitro* and *in vivo* osteogenic potential when compared with higher CD105 expression subpopulation and with the heterogeneous-unsorted population (Levi et al., 2011). Despite the important correlation between CD105 and osteogenesis, this study did not explore which molecules are involved in the regulation of CD105 expression. It is still unexplored if this correlation can be observed in MSCs obtained from other tissue sources.

Beyond the heterogeneity issue, MSCs differentiation potential can diverge among tissue of origin (Robey, 2011), present cellular replicative senescence (after long *in vitro* expansion somatic cells presents a restricted ability of self-renewal)

(Campis and Fagnagna, 2007; Ksiasek, 2009), and lower quantity of colony forming units-fibroblastic (CFU-f) per tissue during aging as observed at **Figure 6** (Caplan, 2007).

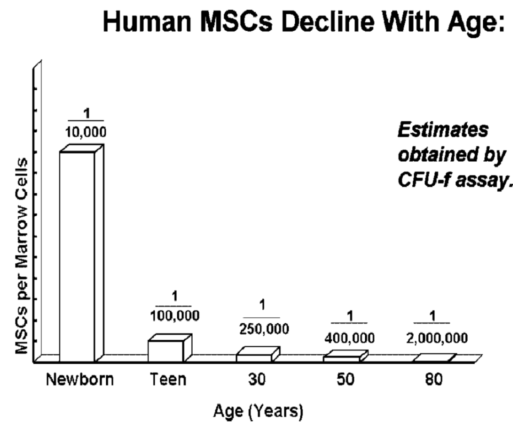

Figure 6. The number of colony forming units-fibroblastic (CFU-f) decline with aging, and the quantity of MSCs in bone marrow is reduced in adult and elderly subjects when compared to teens or newborn. (Adapted from Caplan, 2007).

1.4 iPSCs: an innovative technology

Cell reprogramming technology is an interesting way to bypass the lower expansion capacity of MSCs that limit their use in regenerative medicine (Katsara et al, 2011; Yoshida and Yamanaka, 2010). For example, iPSCs allow the derivation of countless cells with self-renewal capacity and pluripotential (Yu et al, 2007; Chin et al, 2009). In 2006 Takahashi and Yamanaka demonstrate that mature somatic cells could be reprogrammed through a retrovirus-mediated transduction method using four defined transcription factors (*OCT4, SOX2, KLF4*, and *C-MYC*). After transduction cells acquire a “pluripotency state” with properties similar to ESCs, such as capacity of differentiation into any cell type, gene expression profile of ESCs, high proliferation rate and self-renewal (Aasen et al, 2008; Puri and Nagy, 2012).

The main advantage using iPSCs approach is the simplicity and reproducibility of the technique that establish new avenues in regenerative medicine, basic research and disease modeling (Haraguchi et al, 2012; Tabar and Studer, 2014; Yamanaka, 2012). IPSCs can be directly derived from somatic tissues of adult subjects, avoiding the considerable concerns of ESCs use, such as ethical issues related to destruction of human embryos (ESCs are derived from the inner cell mass of mammalian blastocysts) and the probability of immunological rejections due to allograft ESCs use, and since the demonstration of iPSCs technology the number of publications in this area has considerably increased as illustrated in **Figure 7** (Cahan and Daley, 2013; Stadtfeld and Hochedlinger, 2010; Yamanaka, 2009). IPSCs can be obtained in a wide range of age, from newborns up to 74 years old, (Hossini et al, 2015), differently from MSCs that demonstrated limitation related to patient-age obtainment (Caplan, 2007).

The first strategy for cellular reprogramming was the use of retroviral and lentiviral transduction of "Yamanaka factors" (*OCT4, SOX2, KLF4*, and *C-MYC*), but these transgene insertions could disrupt the sequence of native genes leading to genetic modifications (Yoshida and Yamanaka, 2010).

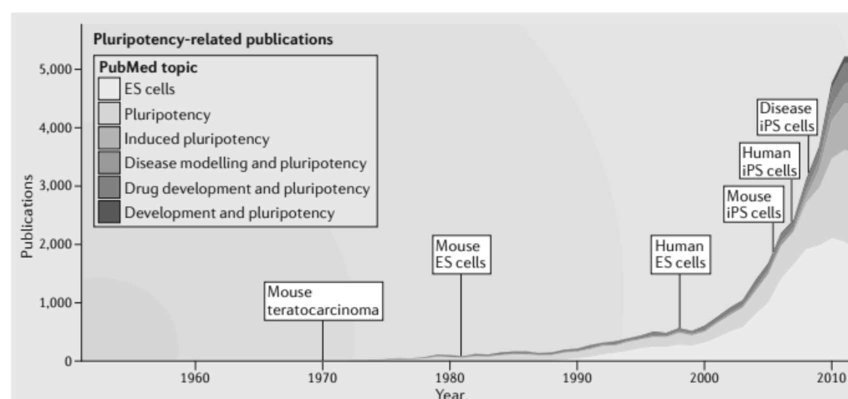

Figure 7. Somatic cells reprogramming to iPSCs in mouse 2006 (Takahashi and Yamanaka, 2006) and human dermal fibroblasts in 2007 (Yamanaka et al, 2007) increased and expanded the use of pluripotent stem cells for

regenerative medicine, drug screening, and disease modeling (Park et al, 2008). The publications in iPSCs area increased and the ESCs studies diminished (Adapted from Cahan and Daley, 2013).

Thus, laborious efforts and new methodologies have been developed to increase the reprogramming efficiency and reduce the needs of genetic modifications such as adenovirus, plasmid vector, removable transposon and episomal vector systems (Kaji et al, 2009; Okita et al, 2008 Okita et al, 2011; Stadtfeld et al, 2008; Woltjen et al, 2009; Yusa et al, 2009). Different human cell types derived from several somatic tissues can be used to obtain iPSCs. Human dermal fibroblasts were the first cell type used to obtain iPSCs (Takahashi et al., 2007) and thereafter several other somatic cell types as keratinocytes (Aasen et al, 2008), primary fetal tissues and adult MSCs (Park et al, 2008), fresh peripheral blood (Loh et al, 2009 and 2010), SHED (Yan et al, 2010), BMSCs (Megges et al, 2015) and hASCs (Zapata-Linares et al, 2016) have been successfully used. Mesenchymal stem cells derived from iPSC, called MSC-like cells, have been investigated as a good alternative cell source for bone regeneration. It is possible that these cells can better recapitulate bone development and being an intermediate cell-type between iPSCs and a fully specialized and differentiated lineage limit the drawbacks related to teratoma formation and open the possibilities to clinical use (Barberi et al, 2005; Jung et al, 2012; Guzzo et al, 2012).

There are diverse methods to obtain mesenchymal-like cells from pluripotent stem cells such as: isolating cells that migrate from embryoid bodies (EBs) formed via suspension culture (Hwang et al, 2008), monolayer differentiation through epithelial-mesenchymal transition that derives mesenchymal progenitor cells (Boyd et al, 2009), single-cell plating of iPSCs in gelatin-coated plates with mesenchymal induction media

(Nakagawa et al, 2009), culturing iPSCs on thin fibrillar type-I collagen coating (Liu et al, 2012) and the use of a small molecule inhibitor - transforming growth factor pathway inhibitor SB431542 (Chen et al, 2012).

MSC-like cells show the same characteristics of MSCs extracted from adult tissues without displaying *in vivo* tumor formation (Hematti et al, 2011; Gruenloh et al, 2011; Villa-diaz et al, 2012). MSCs differentiated from iPSCs exhibited greater regenerative potential, higher survival rate, telomerase activity and less senescence when compared with BMSCs (Diederichs and Tuan, 2014), and showed *in vitro* immunomodulatory properties (Giuliani et al 2011).

MSC-like cells also showed important properties in animal models such as: survival and commitment when differentiated to the chondrogenic lineage (Hwang et al 2008), potential utility in periodontal regeneration due to a considerable increment of mineralized tissue and bone regeneration in a periodontal defect animal model (Hynes et al 2013), enhancement of the vascular and muscle regeneration ameliorating severe limb ischemia (Lian et al 2010) and new bone formation process in calvarial defects in immunocompromised mice (Villa-diaz et al, 2012). However, it is still unclear if the osteopotential properties vary depending on the cell source used for cell reprogrammation by the pluripotency factors.

1.5 iPSCs as tool for *in vitro* disease modeling

Most of the knowledge on the mechanisms of human genetic diseases has been based on mouse models. This biological system approach have been considered the golden standard for modeling *in vivo* human disease, but the species-specific

differences between mice and humans related to physiological, biochemical, molecular and anatomical aspects have prompt the search for new methods to model human diseases (Tiscornia et al. 2011).

iPSCs have recently arisen as a new promising option to model human diseases *in vitro* and there are currently several successful examples such as studies of mechanisms related to Alzheimer´s disease (Israel et al, 2012; Yagi et al, 2011), Cardiotoxicity (Cohen et al, 2011), Down syndrome (Shi et al, 2012), Fragile X syndrome (Sheridan et al, 2011), Parkinson´s disease (Byers et al, 2011; Devine et al, 2011; Sanchez-Danes et al, 2012), Diabetes, Types 1 and 2 (Maehr et al, 2009; Ohmine et al, 2012), Multiple sclerosis (Song et al, 2012), Rett syndrome (Ananiev et al, 2011), autism spectrum disorders (Griesi-Oliveira et al., 2013; Machado et al., 2016) and many others. It would be also invaluable to model craniofacial disorders, as cranial human development is quite peculiar and might involve species-specific signaling.

One main advantage of cell reprogramming is that it enables the study and analysis of a specific disease (**Figure 8**), including rare disorders and syndromes in which the involvement of the individual's genome containing disease-specific alterations is mandatory (Trounson et al, 2012). Further, iPSCs can be differentiated towards cell types affected by the disease, under optimal conditions to observe relevant phenotypes. For example Miller and collaborators (2013) developed a strategy to induce aging features in iPSCs to model *in vitro* Parkinson's disease. Thus, the advent of iPSCs from patients paved the way to obtain different cell types from the same subject, which was previously not possible, revealing disease-relevant cellular pathology and enlightening human disease at the molecular and cellular level (Grskovic et al 2011).

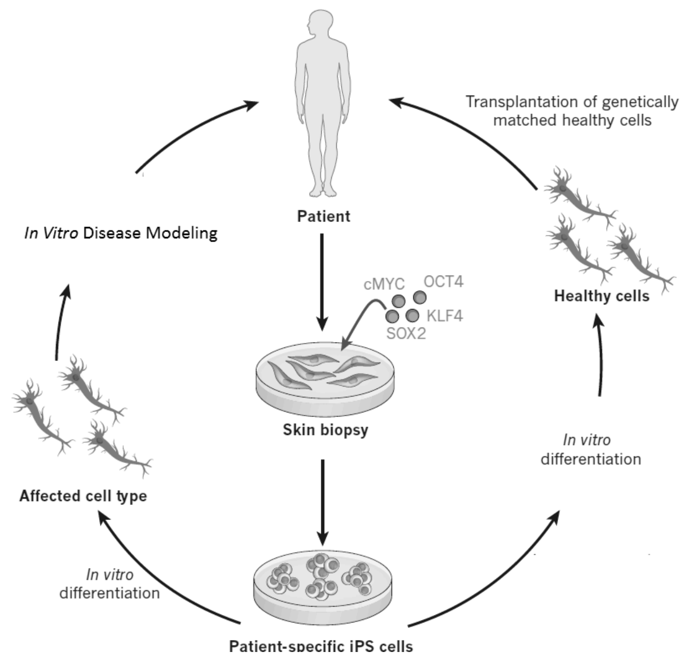

Figure 8. Applications of iPSCs. Cellular reprogramming technology allows the use of patient-specific cells from skin biopsy for example, and through the generation of iPSCs they can be used in the treatment of diseases (tissue engineering/Regenerative medicine) or to model and study some specific disease (Adapted from Robinton and Daley, Nature, 2012).

Here we describe the use of iPSC technology to model human developmental disease in which the pathogenic mutation is associated with craniofacial bone malformation. We selected the Treacher-Collins syndrome, an autosomal dominant rare mandibulofacial dysostosis that affects the craniofacial structures during human development. The main objective of this approach is to contribute to the comprehension and elucidation of new pathways and important mechanisms to bone neoformation and differentiation, more specifically the craniofacial bone formation. Despite the great advance in the field of stem cells in bone regeneration, as listed in **Figure 2**, the number of clinical trials in this area is still limited. Therefore, we expect that the *in vitro* recapitulation of craniofacial bone development can fill some missing lacuna in this field. This knowledge will possibly contribute to new molecules and

pathways associated to this process, which in turn allow the development of better protocols in human bone reconstruction and therapies.

1.6 Treacher-Collins Syndrome

Treacher-Collins syndrome (TCS) is a rare autosomal dominant mandibulofacialdysostosis present in 1:50,000 live births (Online Mendelian inheritance in Man (OMIM) TCS1, OMIM #154500; TCS2, OMIM#613717; TCS3, OMIM #248390) affecting facial morphogenesis (Phelps et al, 1981; Jones et al, 2008). The classic findings of this syndrome present variable expressivity such as antimongoloid slanting of the palpebral fissures, colobomas of the lower eyelids, hypoplasia of the facial bones, alteration of the external and middle ear ossicles resulting in some cases in conductive hearing loss, and cleft palate (Edwards et al, 1997; Mittmadn and Rodman, 1992; Trainor et al., 2009). Most patients require distraction osteogenesis during their rehabilitation in order to correct facial bone hypoplasia, but such interventions often do not provide good, long-lasting results (Kobus & Wójcicki, 2006; McCarthy & Hopper, 2002; Plomp et al 2016). Therefore, both craniofacial development and regeneration are compromised in these patients. Understanding the role of TCOF1 in these process possibly will shed new light on bone reconstruction.

TCS results from genetic alterations in TCOF1, POLR1C and POLR1D, that lead to nonsense-mediated mRNA decay or truncations, and the vast majority of TCS cases are caused by loss-of-function mutations in TCOF1 (Splendore et al., 2000). The gene TCOF1 encodes the TREACLE protein, a 144 kDa nucleolar phosphoprotein associated with ribosome biogenesis, (Gonzales et al., 2005), being essential for proper cell

growth and proliferation (Sakai et al, 2016). On the other hand, mutations in POLR1C and POLR1D are associated with deterioration of RNA polymerase I/III also leading to deficiency in ribosome biogenesis (Dauwerse et al., 2011; Trainor and Merrill, 2014).

This deficiency in ribosome biogenesis leads to lower cell proliferation, higher neuroepithelial apoptosis and insufficient formation of migrating cranial neural crest cells, that prompt the hypoplasia of craniofacial elements such as bone, cartilage and connective tissue, therefore the most affected cellular type in TCS is the neural crest cells, and the severe TCS cases needs major reconstructive surgeries that are rarely fully corrective (Jones et al 2008).

Understanding embryonic development is important to unravel the mechanisms that lead to the phenotype. NCCs emerge from the neuroepithelium and migrate into the pharyngeal arches, and then in the next step they proliferate and differentiate in important craniofacial structures such as cartilage, facial bones and connective tissue (Bhatt et al., 2013; Gong et al., 2014; Twigg & Wilkie, 2015).

In animal models for TCS, TCOF1 knockout leads to impairment of ribosome biogenesis resulting in higher apoptosis rate and deficit in the NCCs proliferation, culminating in cartilage and facial bone hypoplasia (Dixon et al., 1997; Jones et al., 1999; Dixon et al., 2006; Jones et al., 2008; Weiner et al., 2012). The use of animal models does not always reveal all mechanisms and cellular types affected in human TCS subjects, thus the use of iPSC technology with the capacity of differentiation in a wide range of cell types is essential to study human pathogenic mutations in *TCOF1*.

The possibility to recapitulate human embryogenesis through the use of iPSC technology is a promising strategy to study human craniofacial diseases, including TCS. Since NCCs are the most affected cellular type in TCS animal models, the

differentiation of iPSCs in iPSC-derived NCCs (iNCCs) is essential to observe if cells derived from TCS subjects present alterations related to important cellular properties like cell proliferation, apoptosis and differentiation potential (Menendez et al., 2013; Sakai et al., 2016).

Therefore the generation of iNCCs from iPSCs from TCS and control subjects will be essential to compare and observe alteration of cellular properties, and complement data acquired from animal models. The *in vitro* modeling and derivation of human iPSCs, iNCCs and MSC-like cells could further elucidate the underlying pathogenetic mechanism of TCS, especially in regards to the osteodifferentiation of craniofacial bones affected by the syndrome. This knowledge may provide foundation for the improvement of bone therapeutic strategies in the future.

Objectives

The main objectives of this work are:

- To compare the osteogenic potential of mesenchymal stem cells derived from exfoliated deciduous teeth and human adipose tissue and dissect the factors that lead to the differences related to*in vitro* osteogenesis in these mesenchymal stem cells;

- To verify if the osteogenic differentiation potential of mesenchymal cells derived from induced pluripotent stem cells depends on the somatic cell source.

- To verify if iPSCs can be used to model Treacher-Collins Syndrome.

CHAPTER VI

General Discussion and Conclusions

6 - General Discussion and Conclusions

In this work, through the use of different approaches such as microarray expression analysis and molecular surface sorting, we have shown that mesenchymal stem cells, most particularly those derived from dental pulp of human exfoliated teeth (SHED), present an efficient *in vitro* osteogenesis and that at least two factors seem to contribute to the regulation of this process. We also have shown the advantages of the use of iPSCs with the derivation of MSC-like cells that exhibited higher osteogenesis *in vitro* as compared to adult stem mesenchymal cells, and their use in the genetic field through the obtainment of iPSCs to model Treacher Collins syndrome.

We first described that SHED present an increased osteogenic potential as compared to hASCs. In order to answer which factors could be regulating these differences, we performed microarray expression analysis to investigate the osteogenesis in adult MSCs. We observed that *IGF2* were upregulated in SHED when compared to hASCs. We hypothesized that this marker could be directly associated with the higher osteogenic potential in SHED, and through a treatment/supplementation with exogenous *IGF2* and *IGF2* inhibitor (chromeceptin) we observed a positive correlation of this marker with *in vitro* osteogenesis, supporting our hypothesis. We also have shown that imprinting is involved in the regulation of IGF2 expression in SHED. Thus we propose *IGF2* as an osteogenic biomarker in MSCs, which can be used to pre-select cells to be used in bone tissue engineering or alternatively, the use of IGF2 to induce better osteogenesis could be explored.

We do not expect that *IGF2* expression it is the only factor contributing to the *in vitro* osteogenesis differences between SHEDand hASCs. In order to address this question, we choose to use other approach to study the differences of *in vitro* osteogenesis in SHED and

hASCs. Using transcriptional analysis and cell sorting we demonstrated that SHED presented lower expression of CD105 when compared to hASCs and that the low levels of CD105 in SHED contribute to its *in vitro* osteogenic potential. Through *in silico* analysis and gain/loss function assays we showed that the microRNA 1287 is a promising candidate in CD105 regulation in SHED and hASCs and that *IGF2* is not apparently involved in CD105 expression levels regulation. These results thus show that inverse correlation of CD105 expression with osteogenic potential is not specific to hASC (Levi et al., 2011) and it is cell-specific regulated.

Based on the above-mentioned findings, we reinforce that the regulation of the osteogenic potential in adult MSCs is a multifactorial process and our work has contributed to the identification of two factors involved in this regulation. Further studies are required to evaluate if each of these factors alone or in combination in cell culture assays would contribute to a more efficient osteogenesis in adult MSC.

Further, we observed that the cell source used to reprogram and derive iPSCs is an important factor to achieve a better *in vitro* osteogenesis, as MSC-like from SHED presented higher osteogenesis compared to MSC-like from fibroblasts and from adult SHED. Our findings thus strength the importance of cell type/source selection to derive iPSCs and depending on the purpose or tissue to be obtained the cell type/source could be a limiting factor and influence the final results. Neither *IGF2* nor CD105 seemed to be involved with the increased osteogenic potential of the MSC-like. Finally, in the last chapter we describe an *in vitro* model to Treacher-Collins syndrome. Using different methods, we have compared derived neural crest cells (iNCCs), neural crest-derived mesenchymal cells (nMSCs) and MSC-like cells from iPSC, which allow us to recapitulate different stages of craniofacial development. We observed alteration of osteogenesis/chondrogenesis in MSC-like cells derived from patients. nMSCs from Treacher-Collins subjects presented a higher apoptosis rate, consistent with the findings in the literature using animal models to Treacher-Collins syndrome. Therefore, through several

analyses, we have shown that we obtained reliable iNCC, nMSCs and iMSCs from patients and controls and these cell types can thus be used to model TCS. Through the use of pluripotent cells of patients with bone genetic disorders, we expect to better understand craniofacial bone development and identify new pathways critical to this process that could recapitulate *in vitro* bone formation. We expect that this approach may also contribute to better bone reconstruction therapies in the future.

CHAPTER VII

Additional publications and participations in Conferences/Meetings

8 -Appendix: Additional publicationsand participations in Conferences/Meetings

PARTICIPATIONS IN CONFERENCES/MEETINGS

Ishiy, FAA; Ornelas, CM; Fanganiello, RD; Griesi-Oliveira, K; Sdrigotti, MA; Calcaterra, N; Passos-Bueno, MR. Induced pluripotent stem cells to model treacher collins syndrome in a dish.13th international society of stem cell research.**2015**. Stockholm, Sweden. **Poster Presentation**

Ishiy, FAA; Fanganiello, RD; Capelo, LP; Kuriki, PS; Morales, AG; Passos-Bueno, Mr. CD105 expression as a marker to explain osteogenic potential differences of mesenchymal stem cells of different sources. 12th international society of stem cell research.**2014**. Vancouver, Canada. **Poster Presentation**

Fanganiello R.D., **Ishiy FAA,** Yumi D., Capelo L.P., Passos-Bueno M.R. Differentially expressed genes associated to higher osteogenic potential of human mesenchymal stem cells. International Society of Stem Cells Research Meeting.**2013**. Boston, Massachussetts, EUA. **Poster Presentation**

FanganielloRD, **Ishiy FAA,** Capelo LP, Aguena M, Bueno DF, Almada, BP, Martins MT, Passos-Bueno MR. Comparison of the osteogenic potential of adult stem cells from different sources. The American Society of Human Genetics.**2012**. San Francisco, California, EUA. **Poster Presentation**

Ishiy FAA, FanganielloRD, Kobayashi GS, Sunaga DY, Capelo LP, Passos-Bueno MR.Gene expression analysis of mesenchymal stem cells during initial osteoblastogenic differentiation.VII Brazilian Congress on Stem Cells and Cell Therapy.**2012**. São Paulo, SP, Brazil. Hospital Sírio-Libanês and FeComércio. **Poster Presentation**

FanganielloRD, Capelo LP, **Ishiy FAA,** , Almada, BP, Aguena M, Bueno DF, Martins MT, Passos-Bueno MR. Comparison of the osteogenic potential of adult stem cells from different sources. American Society of Human Genetics Meeting.**2012.** San Francisco, California, EUA. **Poster presentation**

PUBLICATIONS – ORIGINAL ARTICLES

Modeling Treacher-Collins Syndrome using iPSCs. **Ishiy FAA**, Gerson S. Kobayashi, Camila M. Musso, Luiz C. Caires, Ernesto Goulart, Andressa G. Morales, Karina Griesi-Oliveira, Patrícia Semedo-Kuriki, Fanganiello RD, Maria Rita Passos-Bueno.(*preparation*).

Modelling Richieri-Costa-Pereira syndrome with iPSC-derived neural crest cells.Gerson S. Kobayashi, **Ishiy FAA**, Camila M. Musso, Luiz C. Caires, Ernesto Goulart, Andressa G. Morales, Karina Griesi-Oliveira, Patrícia Semedo-Kuriki, Maria Rita Passos-Bueno. (*preparation*).

hsa-miR-1287 regulates *in vitro* osteogenic potential of SHED through downregulation of CD105.**Ishiy FAA**, Fanganiello RD, Kobayashi GS, Kuriki PS, Passos-Bueno MR. (*preparation*).

Cnbp ameliorates Treacher Collins Syndrome craniofacial anomalies through a pathway that involves redox-responsive genes.de Peralta, M. S. P., Mouguelar, V. S., Sdrigotti, M. A., **Ishiy, FAA**, Fanganiello, R. D., Passos-Bueno, M. R., ... & Calcaterra, N. B. (2016). Cell Death & Disease, 7(10), e2397.**doi: 10.1038/cddis.2016.299**.

Non-specific FGFR2 ligands, FGF19 and FGF10, lead to abnormal cellular behavior in Apert syndrome-derived fibroblast and stem cells. Yeh E, Atique R, Fanganiello RD, Sunaga DY, **Ishiy FAA**, Passos-Bueno MR.**doi: 10.1089/scd.2016.0018. Epub 2016 Jun 23**

Increased in vitro osteopotential in SHED associated with higher IGF2 expression when compared with hASCs. Fanganiello RD, **Ishiy FAA**, Kobayashi GS, Cruz LA, Sunaga DY, Passos-Bueno MR. Stem Cell Reviews and Reports, **2015. DOI 10.1007/s12015-015-9592-x**

Improvement of In Vitro Osteogenic Potential through Differentiation of Induced Pluripotent Stem Cells from Human Exfoliated Dental Tissue towards Mesenchymal-Like Stem Cells.**Ishiy FAA**, Fanganiello RD, Griesi-Oliveira K, Suzuki AM, Kobayashi GS, Morales AG, Capelo LP, Passos-Bueno MR. Stem Cells International, 9 pages, **2015. doi: 10.1155/2015/249098**

FGFR2 Mutation confers a Less Drastic Gain of Function in Mesenchymal Stem Cells Than in Fibroblasts. Yeh E, Atique R, **Ishiy FAA**, Fanganiello RD, Alonso N, Matushita H, da Rocha KM, Passos-Bueno MR. Stem Cells Review and Reports, v.8, p. 685-695, **2012. Doi:10.1007/S12015-011-9327-6**

Optimization of Parameters for a More Efficient Use of Adipose-Derived Stem Cells in Regenerative Medicine Therapies.Aguena M, Fanganiello RD, Tissiani LAL, **Ishiy, FAA**, Atique R, Alonso N, Passos-Bueno MR. Stem Cells International, v. 2012, p. 1-7, **2012. Doi:10.1155/2012/202610**

References

Aasen, T., Raya, A., Barrero, M. J., Garreta, E., Consiglio, A., Gonzalez, F., ... & Edel, M. (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature biotechnology, 26(11), 1276-1284.

Al-Nbaheen, M., Ali, D., Bouslimi, A., Al-Jassir, F., Megges, M., Prigione, A., ... & Aldahmash, A. (2013). Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Stem Cell Reviews and Reports, 9(1), 32-43.

Alongi DJ, Yamaza T, Song Y, et al. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regenerative medicine. 2010;5(4):617-631. doi:10.2217/rme.10.30.

Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng 2012; 40(5): 363–408.

Ananiev, G., Williams, E. C., Li, H., & Chang, Q. (2011). Isogenic pairs of wild type and mutant induced pluripotent stem cell (iPSC) lines from Rett syndrome patients as in vitro disease model. PloS one, 6(9), e25255.

Casser-Bette, M., Murray, A.B., Closs, E.I. et al. Calcif Tissue Int (1990) 46: 46. doi:10.1007/BF02555824

Badylak, S.; Nerem, R. Progress in tissue engineering and regenerative medicine. Proceedings of the National Academy of Sciences of the United States of America, v. 107, n.8, p. 3285-3286, 2010.

Baer PC, Geiger H, “Adipose-Derived Mesenchymal Stromal/Stem Cells: Tissue Localization, Characterization, and Heterogeneity,” Stem Cells International, vol. 2012, Article ID 812693, 11 pages, 2012. doi:10.1155/2012/812693

Bara, J. J., Richards, R. G., Alini, M. and Stoddart, M. J. (2014), Concise Review: Bone Marrow-Derived Mesenchymal Stem Cells Change Phenotype Following In Vitro Culture: Implications for Basic Research and the Clinic. Stem Cells, 32: 1713–1723. doi:10.1002/stem.1649

Barberi T., Willis L. M., Socci N. D., Studer L. (2005). Derivation of multipotent mesenchymal precursors from human embryonic stem cells. PLoS Med. 2:e161. 10.1371/journal.pmed.0020161

Baksh, D., Song, L., & Tuan, R. S. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy.Journal of cellular and molecular medicine. v.8, n.3, p.301-16, 2004. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15491506

Bazley Faith A., Liu Cyndi F., Yuan Xuan, Hao Haiping, All Angelo H., De Los Angeles Alejandro, Zambidis Elias T., Gearhart John D., and Kerr Candace L.. Stem Cells and Development. November 2015, 24(22): 2634-2648. doi:10.1089/scd.2015.0100.

Bhatt, S., Diaz, R., & Trainor, P. A. (2013). Signals and switches in Mammalian neural crest cell differentiation. Cold Spring Harbor perspectives in biology, 5(2), a008326.

Bianco, P. et al. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nat. Med. 19, 35–42 (2013).

Bielby R, Jones E, McGonagle, D. The role of mesenchymal stem cells in maintenance and repair of bone. Injury Volume 38, Issue 1, Supplement, March 2007, Pages S26-S32

Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 2012;30(10):546–554. doi: 10.1016/j.tibtech.2012.07.005.

BOURIN P, BUNNELL BA, CASTEILLA L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics (IFATS) and Science and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15(6):641-648. doi:10.1016/j.jcyt.2013.02.006.

Boyd, N. L., Robbins, K. R., Dhara, S. K., West, F. D., & Stice, S. L. (2009). Human Embryonic Stem Cell–Derived Mesoderm-like Epithelium Transitions to Mesenchymal Progenitor Cells. Tissue Engineering Part A, 15(8), 1897-1907.

Bruder, S. P., Fink, D. J. and Caplan, A. I. (1994), Mesenchymal stem cells in bone development, bone repair, and skeletal regenaration therapy. J. Cell. Biochem., 56: 283–294. doi:10.1002/jcb.240560303

Bueno, D. F., Kerkis, I., Costa, A. M., Martins, M. T., Kobayashi, G. S., Zucconi, E., Fanganiello, R. D., Salles, FT.,Almeida, AB., do Amaral, C.E. R., Alonso, N., Passos-Bueno, MR. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in cleft lip and/or palate patients. Tissue engineering. Part A. v. 15, n.2, p.427-35, 2009. doi:10.1089/ten.tea.2007.0417

Byers, B., Cord, B., Nguyen, H. N., Schüle, B., Fenno, L., Lee, P. C., ... & Palmer, T. D. (2011). SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate α-synuclein and are susceptible to oxidative stress. PloS one, 6(11), e26159.

Cahan, Patrick, and George Q. Daley. "Origins and implications of pluripotent stem cell variability and heterogeneity." Nature reviews Molecular cell biology 14.6 (2013): 357-368.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98(8):2396–2402. doi: 10.1182/blood.V98.8.2396.

Campisi, J., & di Fagagna, F. D. A. (2007). Cellular senescence: when bad things happen to good cells. Nature reviews Molecular cell biology, 8(9), 729-740.

Caplan, A. I. (1991), Mesenchymal stem cells. J. Orthop. Res., 9: 641–650. doi:10.1002/jor.1100090504

Caplan, A. I. and Dennis, J. E. (2006), Mesenchymal stem cells as trophic mediators. J. Cell. Biochem., 98: 1076–1084. doi:10.1002/jcb.20886

Caplan, A. I. (2007). Adult mesenchymal stem cells for tissue engineering versus regenerative medicine.Journal of cellular physiology, 213(2), 341-347.

Caplan, A. I. Why are MSCs therapeutic ? New data : new insight. Journal of Pathology. v. 217, p. 318-324, 2009. doi:10.1002/path

Chamberlain, G., Fox, J., Ashton, B. and Middleton, J. (2007), Concise Review: Mesenchymal Stem Cells: Their Phenotype, Differentiation Capacity, Immunological Features, and Potential for Homing. STEM CELLS, 25: 2739–2749. doi:10.1634/stemcells.2007-0197

Chen, Y. S., Pelekanos, R. A., Ellis, R. L., Horne, R., Wolvetang, E. J., & Fisk, N. M. (2012). Small molecule mesengenic induction of human induced pluripotent stem cells to generate mesenchymal stem/stromal cells. Stem cells translational medicine, 1(2), 83-95.

Chin, M. H., Mason, M. J., Xie, W., Volinia, S., Singer, M., Peterson, C., ... & Khvorostov, I. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell stem cell, 5(1), 111-123.

Chung MT, Liu C, Hyun JS, Lo DD, Montoro DT, Hasegawa M, Li S, Sorkin M, Rennert R, Keeney M, Yang F, Quarto N, Longaker MT, Wan DC. CD90 (Thy-1)-positive selection enhances osteogenic capacity of human adipose-derived stromal cells. Tissue Eng Part A 2013; 19: 989-997 [DOI: 10.1089/ ten.tea.2012.0370]

Cohen, J. D., Babiarz, J. E., Abrams, R. M., Guo, L., Kameoka, S., Chiao, E., ... & Kolaja, K. L. (2011). Use of human stem cell derived cardiomyocytes to examine sunitinib mediated cardiotoxicity and electrophysiological alterations. Toxicology and applied pharmacology, 257(1), 74-83.

D'Aquino, R., Graziano, A., Sampaolesi, M., Laino, G., Pirozzi, G., De Rosa, A., & Papaccio, G. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell death and differentiation. v.14, n.6, p.1162-71, 2007. doi:10.1038/sj.cdd.4402121

D' Aquino, R., Graziano, A., Laino, G., & Papaccio, G. Dental pulp stem cells: a promising tool for bone regeneration. Stem cell reviews. v.4, n.1, p. 21-6, 2008. doi:10.1007/s12015-008-9013-5

D' Aquino, R., De Rosa, A., Lanza, V., Tirino, V., Laino, L., Graziano, A., Desiderio, V., Laino, G., Papaccio, G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. European cells & materials, v.18, p.75-83, 2009. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19908196

Dauwerse, J. G., Dixon, J., Seland, S., Ruivenkamp, C. A., van Haeringen, A., Hoefsloot, L. H., ... & Zweier, C. (2011). Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. Nature genetics, 43(1), 20-22.

Derubeis AR, Cancedda R. Bone marrow stromal cells (BMSCs) in bone engineering: limitations and recent advances. Ann Biomed Eng. 2004;32:160–165.

De Mendonça Costa A, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, Cerruti H, Alonso N, Passos-Bueno MR. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. The journal of Craniofacil Surgery.v.19, n.1, p. 204-10, 2008.

M.J. Devine, M. Ryten, P. Vodicka, A.J. Thomson, T. Burdon, H. Houlden, F. Cavaleri, M. Nagano, N.J. Drummond, J.W. Taanman, et al.

Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. Nat. Commun., 2 (2011), p. 440

Diederichs, S., & Tuan, R. S. (2014). Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor. Stem cells and development, 23(14), 1594-1610.

Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury. 2005;36:1392–1404.

Dixon, J., Edwards, S. J., Anderson, I., Brass, A., Scambler, P. J., & Dixon, M. J. (1997). Identification of the complete coding sequence and genomic organization of the Treacher Collins syndrome gene. Genome research, 7(3), 223-234.

Dixon, J., Jones, N. C., Sandell, L. L., Jayasinghe, S. M., Crane, J., Rey, J. P., ... & Trainor, P. A. (2006). Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. Proceedings of the National Academy of Sciences, 103(36), 13403-13408.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, DJ. and Horwitz, EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.Cytotherapy. 2006.doi:10.1080/14653240600855905

Edwards, S. J., Gladwin, A. J., & Dixon, M. J. (1997). The mutational spectrum in Treacher Collins syndrome reveals a predominance of mutations that create a premature-termination codon. American journal of human genetics, 60(3), 515.

Erices, Conget & Minguell (2000) Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. British Journal of Haematology. 2000;109:235–242. doi: 10.1046/j.1365-2141.2000.01986.x.

Friedenstein, A. J., S. Piatetzky, Ii e K. V. Petrakova. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol, v.16, n.3, Dec, p.381-90. 1966

Freyschmidt J. (1993). Skeletterkrankungen. Springer- Verlag, Berlin, Heidelberg, Germany.

Giuliani A., Manescu A., Langer M., Rustichelli F., Desiderio V., Paino F., et al. . (2013). Three graft vascularization is a critical rate-limiting step in skeletal stem cell-mediated posterolateral spinal fusion. Stem Cells Transl. Med. 2, 316–324. 10.5966/sctm.2012-0136

Gimble, Jeffrey M., Adam J. Katz, and Bruce A. Bunnell. "Adipose-derived stem cells for regenerative medicine." Circulation research 100.9 (2007): 1249-1260.

Giuliani, M., Fleury, M., Vernochet, A., Ketroussi, F., Clay, D., Azzarone, B., ... & Durrbach, A. (2011). Long-lasting inhibitory effects of fetal liver mesenchymal stem cells on T-lymphocyte proliferation. PLoS One, 6(5), e19988.

Glotzbach, J. P., Wong, V. W., Gurtner, G. C., & Longaker, M. T. Regenerative medicine. Current problems in surgery. V.48, n.3, p. 148-212. 2011 doi:10.1067/j.cpsurg.2010.11.002

Gong, S. G. (2014). Cranial neural crest: Migratory cell behavior and regulatory networks. Experimental cell research, 325(2), 90-95.

Grayson WL, Bunnell BA, Martin E et al. Stromal cells and stem cells in clinical bone regeneration. Nat rev Endocrinol 2015;11:140–150.

Gronthos, S., M. Mankani, J. Brahim, P. G. Robey e S. Shi. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A, v.97, n.25, Dec 5, p.13625-30. 2000.

Guan M, Yao W, Liu R, et al. Directing mesenchymal stem cells to bone to augment formation and increase bone mass. Nat Med. 2012;18(3):456–62.

Gutierrez-Aranda I, Ramos-Mejia V, Bueno C, Munoz-Lopez M, Real PJ, Mácia A, Sanchez L, Ligero G, Garcia-Parez JL, Menendez P. Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection. Stem Cells. 2010 Sep;28(9):1568-70. doi: 10.1002/stem.471.

Guzzo, R. M., Scanlon, V., Sanjay, A., Xu, R. H., & Drissi, H. (2014). Establishment of human cell type-specific iPS cells with enhanced chondrogenic potential. Stem Cell Reviews and Reports, 10(6), 820-829.

Griesi ‐ Oliveira, K., Sunaga, D. Y., Alvizi, L., Vadasz, E., & Passos ‐ Bueno, M. R. (2013). Stem cells as a good tool to investigate dysregulated biological systems in autism spectrum disorders. Autism Research, 6(5), 354-361.

Fan He, Xiaodong Chen, and Ming Pei. Tissue Engineering Part A. December 2009, 15(12): 3809-3821. doi:10.1089/ten.tea.2009.0188.

Fanganiello, R. D., Ishiy, F. A. A., Kobayashi, G. S., Alvizi, L., Sunaga, D. Y., & Passos-Bueno, M. R. (2015). Increased In Vitro Osteopotential in SHED Associated with Higher IGF2 Expression When Compared with hASCs. Stem Cell Reviews and Reports, 11(4), 635-644.

Gonzales, B., Henning, D., So, R. B., Dixon, J., Dixon, M. J., & Valdez, B. C. (2005). The Treacher Collins syndrome (TCOF1) gene product is involved in pre-rRNA methylation. Human molecular genetics, 14(14), 2035-2043.

Gruenloh, W., Kambal, A., Sondergaard, C., McGee, J., Nacey, C., Kalomoiris, S., ...& Nolta, J. A. (2011). Characterization and in vivo testing of mesenchymal stem cells derived from human embryonic stem cells. Tissue engineering Part A, 17(11-12), 1517-1525.

Grskovic, M., Javaherian, A., Strulovici, B., & Daley, G. Q. (2011). Induced pluripotent stem cells—opportunities for disease modelling and drug discovery. Nature reviews Drug discovery, 10(12), 915-929.

Haraguchi, Y., Shimizu, T., Yamato, M., & Okano, T. (2012). Concise review: cell therapy and tissue engineering for cardiovascular disease. Stem cells translational medicine, 1(2), 136-141.

Healy KE, Guldberg, RE. Bone tissue engineering. J Musculoskelet Neuronal Interact 2007; 7(4):328-330

Hematti, P. (2011). Human embryonic stem cell-derived mesenchymal progenitors: an overview. Embryonic Stem Cell Therapy for Osteo-Degenerative Diseases: Methods and Protocols, 163-174.

Holzwarth JM, Ma PX. Biomimetic nanofibrous scaffolds for bone tissue engineering.Biomaterials. 2011 Dec; 32(36): 9622–9629.

Hosoya, A., Hiraga, T., Ninomiya, T., Yukita, A., Yoshiba, K., Yoshiba, N., ...& Nakamura, H. (2012). Thy-1-positive cells in the subodontoblastic layer possess high potential to differentiate into hard tissue-forming cells. Histochemistry and cell biology, 137(6), 733-742.

Hossini, A. M., Megges, M., Prigione, A., Lichtner, B., Toliat, M. R., Wruck, W., ... & Zoubouliss, C. C. (2015). Induced pluripotent stem cell-derived neuronal cells from a sporadic Alzheimer's disease donor as a model for investigating AD-associated gene regulatory networks. BMC genomics, 16(1), 1.

Huang, G. T.-J., Gronthos, S., & Shi, S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. Journal of dental research. v.88, n.9, p.792-806, 2009. doi:10.1177/0022034509340867

Huang GX, Arany, PR, Mooney DJ. Modeling and Validation of Multilayer Poly(Lactide-Co-Glycolide) Scaffolds for In Vitro Directed Differentiation of Juxtaposed Cartilage and Bone Tissue Eng Part A. 2015 Aug 1; 21(15-16): 2228–2240

Hwang, N. S., Varghese, S., & Elisseeff, J. (2008). Controlled differentiation of stem cells. *Advanced drug delivery reviews*, *60*(2), 199-214.

Hynes, K., Menicanin, D., Han, J., Marino, V., Mrozik, K., Gronthos, S., & Bartold, P. M. (2013). Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. Journal of dental research, 0022034513498258.

Israel, M. A., Yuan, S. H., Bardy, C., Reyna, S. M., Mu, Y., Herrera, C., ... & Carson, C. T. (2012). Probing sporadic and familial Alzheimer/'s disease using induced pluripotent stem cells. Nature, 482(7384), 216-220.

in ‘tAnker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., Noort, W. A., Claas, F. H. J., Willemze, R., et al (2003). Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood, 102, 1548–1549.

Jones, N. C., Lynn, M. L., Gaudenz, K., Sakai, D., Aoto, K., Rey, J. P., ... & Dixon, M. J. (2008). Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nature medicine, 14(2), 125-133.

Jones, N. C., Lynn, M. L., Gaudenz, K., Sakai, D., Aoto, K., Rey, J. P., ... & Dixon, M. J. (2008). Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nature medicine, 14(2), 125-133.

Jones, N. C., Lynn, M. L., Gaudenz, K., Sakai, D., Aoto, K., Rey, J. P., ... & Dixon, M. J. (2008). Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nature medicine, 14(2), 125-133.

Jung, Y., Bauer, G. and Nolta, J. A. (2012), Concise Review: Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: Progress Toward Safe Clinical Products. STEM CELLS, 30: 42–47. doi:10.1002/stem.727

Jung, Y., Bauer, G., & Nolta, J. A. (2012). Concise review: induced pluripotent stem cell ‑ derived mesenchymal stem cells: progress toward safe clinical products. Stem cells, 30(1), 42-47.

Kadiyala S, Young RG, Thiede MA, Brude, SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplantation Volume 6, Issue 2, March–April 1997, Pages 125-134

Kim, D., Kim, C. H., Moon, J. I., Chung, Y. G., Chang, M. Y., Han, B. S., ... & Kim, K. S. (2009). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell stem cell, 4(6), 472.

Katsara, O., Mahaira, L. G., Iliopoulou, E. G., Moustaki, A., Antsaklis, A., Loutradis, D., ... & Perez, S. A. (2011). Effects of donor age, gender, and in vitro cellular aging on the phenotypic, functional, and molecular characteristics of mouse bone marrow-derived mesenchymal stem cells. Stem cells and development, 20(9), 1549-1561.

Kerkis, I., Kerkis, A., Dozortsev, D., Stukart-Parsons, G. C., Gomes Massironi, S. M., Pereira, L. V., Caplan, A. I., Cerruti, HF. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells, tissues, organs, v.184, n.3-4, p.105-16, 2006. doi:10.1159/000099617

Kern, S., Eichler, H., Stoeve, J., Klüter, H., & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem cells, 24(5), 1294-1301.

Ksiazek, K. (2009). A comprehensive review on mesenchymal stem cell growth and senescence. Rejuvenation research, 12(2), 105-116.

Kobus, K., & Wójcicki, P. (2006). Surgical treatment of Treacher Collins syndrome. Annals of plastic surgery, 56(5), 549-554.

Laino, G., D'Aquino, R., Graziano, A., Lanza, V., Carinci, F., Naro, F., Pirozzi, G., et al. (2005). A New Population of Human Adult Dental Pulp Stem Cells: A Useful Source of Living Autologous Fibrous Bone Tissue (LAB). Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research, 20(8), 1454-61. doi:10.1359/JBMR.050325

Laino, Gregorio, Carinci, F., Graziano, A., Aquino, R., Lanza, V., Rosa, A. D., Naro, F., Vivarelli, E., Papaccio, G. Scientific Studies In Vitro Bone Production Using Stem Cells Derived From Human Dental Pulp. Journal of Craniofacial Surgery, p.511-515, 2006.

Langer, R., & Vacanti, J. P. ARTICLES Tissue Engineering. Science. v. 260, 14 maio 1993

Lian, Q., Zhang, Y., Zhang, J., Zhang, H. K., Wu, X., Zhang, Y., ... & Au, K. W. (2010). Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation, 121(9), 1113-1123.

Liu, Y., Goldberg, A. J., Dennis, J. E., Gronowicz, G. A., & Kuhn, L. T. (2012). One-step derivation of mesenchymal stem cell (MSC)-like cells from human pluripotent stem cells on a fibrillar collagen coating. PloS one, 7(3), e33225.

Lendeckel, Stefan, et al. "Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report." Journal of Cranio-Maxillofacial Surgery 32.6 (2004): 370-373.

Levi B, James AW, Nelson ER, Vistnes D, Wu B, Lee M, et al. Human adipose derived stromal cells heal critical size mouse calvarial defects. 2010. PLoS One.5(6):e11177.

Levi, B., Wan, D. C., Glotzbach, J. P., Hyun, J., Januszyk, M., Montoro, D., Sorkin, M., et al. (2011). CD105 protein depletion enhances human adipose-derived stromal cell osteogenesis through reduction of transforming growth factor β1 (TGF-β1) signaling. The Journal of biological chemistry, 286(45), 39497-509. doi:10.1074/jbc.M111.256529

Loh, Y. H., Agarwal, S., Park, I. H., Urbach, A., Huo, H., Heffner, G. C., ... & Daley, G. Q. (2009). Generation of induced pluripotent stem cells from human blood. Blood, 113(22), 5476-5479.

Loh, Y. H., Hartung, O., Li, H., Guo, C., Sahalie, J. M., Manos, P. D., ... & Lensch, M. W. (2010). Reprogramming of T cells from human peripheral blood. Cell stem cell, 7(1), 15.

Lv, F. J., Tuan, R. S., Cheung, K., & Leung, V. Y. (2014). Concise review: the surface markers and identity of human mesenchymal stem cells. Stem cells, 32(6), 1408-1419.

Machado, C. O. F., Griesi-Oliveira, K., Rosenberg, C., Kok, F., Martins, S., Passos-Bueno, M. R., & Sertie, A. L. (2016). Collybistin binds and inhibits mTORC1 signaling: a potential novel mechanism contributing to intellectual disability and autism. European Journal of Human Genetics, 24(1), 59-65.

McCarthy, J. G., & Hopper, R. A. (2002). Distraction osteogenesis of zygomatic bone grafts in a patient with Treacher Collins syndrome: a case report. Journal of Craniofacial Surgery, 13(2), 279-283.

Maeda S., Hayashi M., Komiya S., Imamura T., Miyazono K. (2004) EMBO J. 23, 552–56

Maehr, R., Chen, S., Snitow, M., Ludwig, T., Yagasaki, L., Goland, R., ...& Melton, D. A. (2009). Generation of pluripotent stem cells from patients with type 1 diabetes. Proceedings of the National Academy of Sciences, 106(37), 15768-15773.

Megges, M., Geissler, S., Duda, G. N., & Adjaye, J. (2015). Generation of an iPS cell line from bone marrow derived mesenchymal stromal cells from an elderly patient. Stem cell research, 15(3), 565-568.

Menendez, L., Kulik, M. J., Page, A. T., Park, S. S., Lauderdale, J. D., Cunningham, M. L., & Dalton, S. (2013). Directed differentiation of human pluripotent cells to neural crest stem cells. Nature protocols, 8(1), 203-212.

Mesimäki, Karri, et al. "Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells." International journal of oral and maxillofacial surgery 38.3 (2009): 201-209.

McIntosh, K., Zvonic, S., Garrett, S., Mitchell, J. B., Floyd, Z. E., Hammill, L., ... & Goh, B. (2006). The immunogenicity of human adipose ‐ derived cells: temporal changes in vitro. Stem cells, 24(5), 1246-1253.

Miller, J. D., Ganat, Y. M., Kishinevsky, S., Bowman, R. L., Liu, B., Tu, E. Y., ... & Taldone, T. (2013). Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell stem cell, 13(6), 691-705.

Mitchell, J. B., McIntosh, K., Zvonic, S., Garrett, S., Floyd, Z. E., Kloster, A., ... & Wu, X. (2006). Immunophenotype of Human Adipose ‐ Derived Cells: Temporal Changes in Stromal ‐ Associated and Stem Cell–Associated Markers. Stem cells, 24(2), 376-385.

Mittman, D. L., & Rodman, O. G. (1992). Mandibulofacial dysostosis (Treacher Collins syndrome): a case report. Journal of the national medical association, 84(12), 1051.

Miura, M., Gronthos, S., Zhao, M., Lu, B., & Fisher, L. w. SHED: Stem cells from human exfoliated deciduous teeth. PNAS. V.100, n.10, p.598-605, 2003. doi:10.3109/14653249.2010.542462

Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009 Aug;27(8):743-5. doi: 10.1038/nbt.1554. Epub 2009 Jul 9.

Mizuno D, Agata K, Furue H, Kimura S, Narita Y, Watanabe N, Ishii Y, Ueda M, Tojo A, Kagami S. Limited but heterogeneous osteogenic response of human bone marrow mesenchymal stem cells to bone morphogenetic protein-2 and serum. Journal Growth Factors Volume 28, 2010 - Issue 1

Mizuno, H., Tobita, M. and Uysal, A. C. (2012), Concise Review: Adipose-Derived Stem Cells as a Novel Tool for Future Regenerative Medicine. STEM CELLS, 30: 804–810. doi:10.1002/stem.1076

Mohseni R, Hamidieh AA, Verdi J, Hassani AS (2014) Safe Transplantation of Pluripotent Stem Cell by Preventing Teratoma Formation. J Stem Cell Res Ther 4:212. doi:10.4172/2157-7633.1000212

Najar M, Raicevic G, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L. Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. Cytotherapy Volume 18, Issue 2, February 2016, Pages 160–171

Nakagawa, T., Lee, S. Y., & Reddi, A. H. (2009). Induction of chondrogenesis from human embryonic stem cells without embryoid body formation by bone morphogenetic protein 7 and transforming growth factor β1. Arthritis & Rheumatism, 60(12), 3686-3692.

Nakagawa, M., Karagiannis, P. and Yamanaka, S. (2016), When Myc's asleep, embryonic stem cells are dormant. EMBO J, 35: 801–802. doi:10.15252/embj.201694095

Nakamura, S., Yamada, Y., Katagiri, W., Sugito, T., Ito, K., & Ueda, M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. Journal of endodontics. v.35, n.11, p.1536-42, 2009. Elsevier Ltd. doi:10.1016/j.joen.2009.07.024

Nassiri, F., Cusimano, M. D., Scheithauer, B. W., Rotondo, F., Fazio, A., Yousef, G. M., ... & Lloyd, R. V. (2011). Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. Anticancer research, 31(6), 2283-2290.

Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood 2007 110:3499-3506; doi:10.1182/blood-2007-02-069716

Ohmine, S., Squillace, K. A., Hartjes, K. A., Deeds, M. C., Armstrong, A. S., Thatava, T., ... & Ikeda, Y. (2012). Reprogrammed keratinocytes from elderly type 2 diabetes patients suppress senescence genes to acquire induced pluripotency. Aging (Albany NY), 4(1), 60-73.

Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007 Jul 19;448(7151):313-7. Epub 2007 Jun 6.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., & Yamanaka, S. (2008). Generation of mouse induced pluripotent stem cells without viral vectors. Science, 322(5903), 949-953.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., ... & Shibata, T. (2011). A more efficient method to generate integration-free human iPS cells. Nature methods, 8(5), 409-412.

Park, I. H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., ...& Daley, G. Q. (2008). Disease-specific induced pluripotent stem cells. cell, 134(5), 877-886.

Phelps, P. D., Lloyd, G. A., & Poswillo, D. E. (1983). The ear deformities in craniofacial microsomia and oculo-auriculo-vertebral dysplasia.The Journal of Laryngology & Otology, 97(11), 995-1005.

Phinney, D. G. and Prockop, D. J. (2007), Concise Review: Mesenchymal Stem/Multipotent Stromal Cells: The State of Transdifferentiation and Modes of Tissue Repair—Current Views. STEM CELLS, 25: 2896–2902. doi:10.1634/stemcells.2007-0637

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7. doi: 10.1126/science.284.5411.143.

Plomp, R. G., van Lieshout, M. J., Joosten, K. F., Wolvius, E. B., van der Schroeff, M. P., Versnel, S. L., ... & Mathijssen, I. M. (2016). Treacher collins syndrome: A systematic review of evidence-based treatment and recommendations. Plastic and reconstructive surgery, 137(1), 191-204.

Puri, M. C., & Nagy, A. (2012). Concise review: embryonic stem cells versus induced pluripotent stem cells: the game is on. Stem Cells, 30(1), 10-14.

Rada T, Reis RL, Gomes ME. Distinct stem cells subpopulations isolated from human adipose tissue exhibit different chondrogenic and osteogenic differentiation potential. Stem Cell Rev. 2011;7:64–76.

Robey, PG. Cell sources for bone regeneration: the good, the bad, and the ugly (but promising). Tissue engineering. Part B, Reviews. v.17, n.6, p. 423-30, 2011. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3223013&tool=pmcentrez&rendertype=abstract.

Robinton, D. A., & Daley, G. Q. (2012). The promise of induced pluripotent stem cells in research and therapy. Nature, 481(7381), 295-305.

Sánchez‐Danés, A., Richaud‐Patin, Y., Carballo‐Carbajal, I., Jiménez‐Delgado, S., Caig, C., Mora, S., ... & Canals, J. M. (2012). Disease‐specific phenotypes in dopamine neurons from human iPS‐based models of genetic and sporadic Parkinson's disease. EMBO molecular medicine, 4(5), 380-395.

Sakai, D., & Trainor, P. A. (2009). Treacher Collins syndrome: unmasking the role of Tcof1/treacle. The international journal of biochemistry & cell biology, 41(6), 1229-1232.

Sakai, D., Dixon, J., Achilleos, A., Dixon, M., & Trainor, P. A. (2016). Prevention of Treacher Collins syndrome craniofacial anomalies in mouse models via maternal antioxidant supplementation. Nature communications, 7.

Sarugaser, R., Lickorish, D., Baksh, D., Hosseini, M. M. and Davies, J. E. (2005), Human Umbilical Cord Perivascular (HUCPV) Cells: A Source of Mesenchymal Progenitors. STEM CELLS, 23: 220–229. doi:10.1634/stemcells.2004-0166

Sanz-Rodriguez, F., Guerrero-Esteo, M., Botella, L. M., Banville, D., Vary, C. P., & Bernabéu, C. (2004). Endoglin regulates cytoskeletal organization through binding to ZRP-1, a member of the Lim family of proteins. Journal of Biological Chemistry, 279(31), 32858-32868.

Seong, J. M., Kim, B.-C., Park, J.-H., Kwon, I. K., Mantalaris, A., & Hwang, Y.-S. Stem cells in bone tissue engineering. Biomedical materials (Bristol, England). v.5, n.6, 2010. 062001. doi:10.1088/1748-6041/5/6/062001

Sheridan, S. D., Theriault, K. M., Reis, S. A., Zhou, F., Madison, J. M., Daheron, L., ... & Haggarty, S. J. (2011). Epigenetic characterization of the FMR1 gene and aberrant neurodevelopment in human induced pluripotent stem cell models of fragile X syndrome. PloS one, 6(10), e26203.

Shi, Y., Kirwan, P., & Livesey, F. J. (2012). Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. Nature protocols, 7(10), 1836-1846.

Song, B., Sun, G., Herszfeld, D., Sylvain, A., Campanale, N. V., Hirst, C. E., ... & Short, M. (2012). Neural differentiation of patient specific iPS cells as a novel approach to study the pathophysiology of multiple sclerosis. Stem cell research, 8(2), 259-273.

Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., & Hochedlinger, K. (2008). Induced pluripotent stem cells generated without viral integration. Science, 322(5903), 945-949.

Stadtfeld, M., & Hochedlinger, K. (2010). Induced pluripotency: history, mechanisms, and applications. Genes & development, 24(20), 2239-2263.

Splendore, A., Silva, E. O., Alonso, L. G., Richieri‐Costa, A., Alonso, N., Rosa, A., ... & Passos‐Bueno, M. R. (2000). High mutation detection rate in TCOF1 among Treacher Collins syndrome patients reveals clustering of mutations and 16 novel pathogenic changes. Human mutation, 16(4), 315-322.

Tabar, V., & Studer, L. (2014). Pluripotent stem cells in regenerative medicine: challenges and recent progress. Nature Reviews Genetics, 15(2), 82-92.

Takahashi, Kazutoshi et al. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell , 2006 Volume 126 , Issue 4 , 663 - 676

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007 Nov 30;131(5):861-72.

Tiscornia, G., Vivas, E. L., & Belmonte, J. C. I. (2011). Diseases in a dish: modeling human genetic disorders using induced pluripotent cells. Nature medicine, 17(12), 1570-1576.

Trainor, P. A., Dixon, J., & Dixon, M. J. (2009). Treacher Collins syndrome: etiology, pathogenesis and prevention. European Journal of human genetics, 17(3), 275-283.

Trainor, P. A., & Merrill, A. E. (2014). Ribosome biogenesis in skeletal development and the pathogenesis of skeletal disorders. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1842(6), 769-778.

Trounson, A., Shepard, K. A., & DeWitt, N. D. (2012). Human disease modeling with induced pluripotent stem cells. Current opinion in genetics & development, 22(5), 509-516.

Twigg, S. R., & Wilkie, A. O. (2015). A genetic-pathophysiological framework for craniosynostosis. The American Journal of Human Genetics, 97(3), 359-377.

Villa‐Diaz, L. G., Brown, S. E., Liu, Y., Ross, A. M., Lahann, J., Parent, J. M., & Krebsbach, P. H. (2012). Derivation of mesenchymal stem cells from human induced pluripotent stem cells cultured on synthetic substrates. Stem Cells, 30(6), 1174-1181.

Zvaifler NJ, Marinova-Mutafchieva L, Adams G, et al. Mesenchymal precursor cells in the blood of normal individuals. Arthritis Research. 2000;2(6):477-488.

Zuk, P. A., M. Zhu, H. Mizuno, J. Huang, J. W. Futrell, A. J. Katz, P. Benhaim, H. P. Lorenz e M. H. Hedrick. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Engineering, v.7, n.2, Apr, p.211-28. 2001.

Zuk, P. A., M. Zhu, P. Ashjian, D. A. De Ugarte, J. I. Huang, H. Mizuno, Z. C. Alfonso, J. K. Fraser, P. Benhaim e M. H. Hedrick. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell, v.13, n.12, Dec, p.4279-95. 2002.

WARRINGTON, K., HILLARBY, M. C., LI, C., LETARTE, M., & KUMAR, S. (2005). Functional role of CD105 in TGF-β1 signalling in murine and human endothelial cells. Anticancer research, 25(3B), 1851-1864.

Weiner, A. M., Scampoli, N. L., & Calcaterra, N. B. (2012). Fishing the molecular bases of Treacher Collins syndrome. PloS one, 7(1), e29574.

Whitman, M., & Raftery, L. (2005). TGFβ signaling at the summit. Development, 132(19), 4205-4210.

Witkowska-Zimny, M., & Walenko, K. Stem cells from adipose tissue. Cellular & molecular biology letters. v.16, n.2, p.236-57, 2011. doi:10.2478/s11658-011-0005-0

Woltjen, K., Michael, I. P., Mohseni, P., Desai, R., Mileikovsky, M., Hämäläinen, R., ... & Kaji, K. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature, 458(7239), 766-770.

Yamanaka, S. (2009). A fresh look at iPS cells. cell, 137(1), 13-17.

Yamanaka, S. (2012). Induced pluripotent stem cells: past, present, and future. Cell stem cell, 10(6), 678-684.

Yoshimura, Kotaro, et al. "Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells." Aesthetic plastic surgery 32.1 (2008): 48-55.

Yagi, T., Ito, D., Okada, Y., Akamatsu, W., Nihei, Y., Yoshizaki, T., ... & Suzuki, N. (2011). Modeling familial Alzheimer's disease with induced pluripotent stem cells. Human molecular genetics, 20(23), 4530-4539.

Yan, X., Qin, H., Qu, C., Tuan, R. S., Shi, S., & Huang, G. T. J. (2010). iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem cells and development, 19(4), 469-480.

Yoshida, Y., & Yamanaka, S. (2010). Recent stem cell advances: induced pluripotent stem cells for disease modeling and stem cell–based regeneration. Circulation, 122(1), 80-87.

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., ... & Slukvin, I. I. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science, 318(5858), 1917-1920.

Yusa, K., Rad, R., Takeda, J., & Bradley, A. (2009). Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. Nature methods, 6(5), 363-369.

Zhang, J., Lian, Q., Zhu, G., Zhou, F., Sui, L., Tan, C., ... & Stewart, C. L. (2011). A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell stem cell, 8(1), 31-45.

Zapata-Linares, N., Rodriguez, S., Mazo, M., Abizanda, G., Andreu, E. J., Barajas, M., ... & Rodriguez-Madoz, J. R. (2016). Generation and characterization of human iPSC line generated from mesenchymal stem cells derived from adipose tissue. Stem Cell Research, 16(1), 20-23.