Runt-Related Transcription Factor 2 Induction During Differentiation of Wharton's Jelly Mesenchymal Stem Cells to Osteoblasts Is Regulated by Jumonji AT-Rich Interactive Domain 1B Histone Demethylase

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RUNX2 induction during differentiation of Wharton's Jelly Mesenchymal Stem Cells to osteoblasts is regulated by JARID1B histone demethylase.

Running title: JARID1B modulates RUNX2 in WJ-MSC osteogenesis

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AUTHOR CONTRIBUTION

F.B. and H.S.: conception and design; collection and/or assembly of data; data analysis and interpretation; manuscript writing. C.P. and M.C.: collection and/or assembly of data; data analysis and interpretation. L.D. and J.P.: collection and/or assembly of data. J.L.: provision of study material from patients. M.M. and V.P.: conception and design; data analysis and interpretation; manuscript writing.

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ABSTRACT

Novel bone regeneration approaches aim to obtain immature osteoblasts from somatic stem cells. Umbilical cord Wharton's jelly mesenchymal stem cells (WJ-MSC) are an ideal source for cell therapy. Hence, the study of mechanisms involved in WJ-MSC osteoblastic differentiation is crucial to exploit their developmental capacity. Here, we have assessed epigenetic control of the RUNX2 osteogenic master regulator gene in WJ-MSC. We present evidence indicating that modulation of RUNX2 expression through preventing JARID1B histone demethylase activity is relevant to enhance WJ-MSC osteoblastic potential. Hence, JARID1B loss of function in WJ-MSC results in increased *RUNX2/p57* expression. Our data highlights JARID1B activity as a novel target to modulate WJ-MSC osteoblastic differentiation with potential applications in bone tissue engineering.

INTRODUCTION

Bone defects involving massive loss of bone tissue are frequent in pathological conditions as trauma due to common or osteoporosis linked accidents, congenital malformations or tumor resection { ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1615/CritRevBiomedEng.v40.i5.10", "ISBN" : "0278-940X (Print)\\r0278-940X (Linking)", "ISSN" : "0278-940X", "PMID" : "23339648", "abstract" : "The worldwide incidence of bone disorders and conditions has trended steeply upward and is expected to double by 2020, especially in populations where aging is coupled with increased obesity and poor physical activity. Engineered bone tissue has been viewed as a potential alternative to the conventional use of bone grafts, due to their limitless supply and no disease transmission. However, bone tissue engineering practices have not proceeded to clinical practice due to several limitations or challenges. Bone tissue engineering aims to induce new functional bone regeneration via the synergistic combination of biomaterials, cells, and factor therapy. In this review, we discuss the fundamentals of bone tissue engineering, highlighting the current state of this field. Further, we review the recent advances of biomaterial and cell-based research, as well as approaches used to enhance bone regeneration. Specifically, we discuss widely investigated biomaterial scaffolds, micro- and nano-structural properties of these scaffolds, and the incorporation of biomimetic properties and/or growth factors. In addition, we examine various cellular approaches, including the use of mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), adult stem cells, induced pluripotent stem cells (iPSCs), and platelet-rich plasma (PRP), and their clinical application strengths and limitations. We conclude by overviewing the challenges that face the bone tissue engineering field, such as the lack of sufficient

vascularization at the defect site, and the research aimed at functional bone tissue engineering. These challenges will drive future research in the field.", "author" : [{ "dropping-particle" : "", "family" : "Amini", "given" : "Ami R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Laurencin", "given" : "Cato T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nukavarapu", "given" : "Syam P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Critical reviews in biomedical engineering", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["2012"]] }, "page" : "363-408", "title" : "Bone tissue engineering: recent advances and challenges.", "type" : "article-journal", "volume" : "40" }, "uris" : ["http://www.mendeley.com/documents/?uuid=fa0a362e-ff92-444b-9157-0f21289fe843"] }], "mendeley" : { "formattedCitation" : "[1]", "plainTextFormattedCitation" : "[1]", "previouslyFormattedCitation" : "[1]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Current standard methods to replace missing bone tissue including bone grafts obtained from the patient itself (autograft) or from a different individual (allograft) result in several difficulties for patients including expensive and invasive procedures, immunoreactivity and infection **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "ISSN" : "1090-3941", "PMID" : "12384890", "abstract" : "Bone grafts are used in musculoskeletal surgery to restore structural integrity and enhance osteogenic potential. The demand for bone graft for skeletal reconstruction in bone tumor, revision arthroplasty, and trauma surgery, couple with recent advances in understanding and application of the biology of bone transplantation, has resulted in an exponential increase in the number of bone-grafting procedures performed over the last decade. It is estimated that 1.5 million

bone-grafting procedures are currently performed worldwide each year, compared to a fraction of that number 20 years ago. Major developments also have resulted in the harvesting, storage, and use of bone grafts and production of graft derivatives, substitutes, and bone-inducing agents.", "author" : [{ "dropping-particle" : "", "family" : "Hubble", "given" : "Matthew J W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Surgical technology international", "id" : "ITEM-1", "issued" : { "date-parts" : [["2002", "9"]] }, "page" : "261-5", "title" : "Bone grafts.", "type" : "article-journal", "volume" : "10" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9bb3cea3-0fcc-41f8-8390-2fcb2975ef33"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.cpm.2014.09.011", "ISSN" : "1558-2302", "PMID" : "25440415", "abstract" : "Rapid bone graft incorporation for structural rigidity is essential. Early range of motion, exercise, and weight-bearing are keys to rehabilitation. Structural and nonstructural bone grafts add length, height, and volume to alter alignment, function, and appearance. Bone graft types include: corticocancellous autograft, allograft, xenograft, and synthetic graft. Autogenic grafts are harvested from the patient, less likely to be rejected, and more likely to be incorporated; however, harvesting adds a procedure and donor site complication is common. Allografts, xenografts, and synthetic grafts eliminate secondary procedures and donor site complications; however, rejection and slower incorporation can occur.", "author" : [{ "dropping-particle" : "", "family" : "Shibuya", "given" : "Naohiro", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jupiter", "given" : "Daniel C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Clinics in podiatric medicine and surgery", "id" : "ITEM-2", "issue" : "1", "issued" : { "date-parts" : [["2015", "1"]] }, "page" : "21-34", "title" : "Bone graft substitute: allograft

and xenograft.", "type" : "article-journal", "volume" : "32" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6e6fac13-64ef-4274-850d-cea5d18c6ee7"] }], "mendeley" : { "formattedCitation" : "[2, 3]", "plainTextFormattedCitation" : "[2, 3]", "previouslyFormattedCitation" : "[2, 3]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Bone tissue engineering including mixing osteogenic cells, growth factors and a suitable biomaterial scaffold appears as a new alternative, for which it is necessary to optimize the osteoblastic potential of cell sources **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "ISSN" : "0954-4119", "PMID" : "21287823", "abstract" : "As the population ages, the number of operations performed on bone is expected to increase. Diseases such as arthritis, tumours, and trauma can lead to defects in the skeleton requiring an operation to replace or restore the lost bone. Surgeons can use autografts, allografts, and/or bone graft substitutes to restore areas of bone loss. Surgical implants are also used in addition or in isolation to replace the diseased bone. This review considers the application of available bone grafts in different clinical settings. It also discusses recently introduced bioactive biomaterials and highlights the clinical difficulties and technological deficiencies that exist in our current surgical practice.", "author" : [{ "dropping-particle" : "", "family" : "Brydone", "given" : "A S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Meek", "given" : "D", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Maclaine", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Proceedings of the Institution of Mechanical Engineers. Part H, Journal of engineering in medicine", "id" : "ITEM-1", "issue" : "12", "issued" : { "date-parts" : [["2010", "12"]] }, "page" : "1329-43", "title" : "Bone grafting, orthopaedic biomaterials,

and the clinical need for bone engineering.", "type" : "article-journal", "volume" : "224" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c4efe6b3-561e-4138-bfb6-d0bf07cce282"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1186/scrt10", "ISSN" : "1757-6512", "PMID" : "20637059", "abstract" : "Treatment of extensive bone defects requires autologous bone grafting or implantation of bone substitute materials. An attractive alternative has been to engineer fully viable, biological bone grafts in vitro by culturing osteogenic cells within three-dimensional scaffolds, under conditions supporting bone formation. Such grafts could be used for implantation, but also as physiologically relevant models in basic and translational studies of bone development, disease and drug discovery. A source of human cells that can be derived in large numbers from a small initial harvest and predictably differentiated into bone forming cells is critically important for engineering human bone grafts. We discuss the characteristics and limitations of various types of human embryonic and adult stem cells, and their utility for bone tissue engineering.", "author" : [{ "dropping-particle" : "", "family" : "Marolt", "given" : "Darja", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Knezevic", "given" : "Miomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Novakovic", "given" : "Gordana Vunjak", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research & therapy", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2010"]] }, "page" : "10", "title" : "Bone tissue engineering with human stem cells.", "type" : "article-journal", "volume" : "1" }, "uris" : ["http://www.mendeley.com/documents/?uuid=fa253c75-5aca-4c1f-8996-61e0664fa847"] }], "mendeley" : { "formattedCitation" : "[4, 5]", "plainTextFormattedCitation" : "[4, 5]",

"previouslyFormattedCitation" : "[4, 5]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

Embryonic stem cells (ESCs), perinatal and adult mesenchymal stem cells (MSCs) have been proposed as sources of cells for differentiating to osteoblasts **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcp.21200", "ISSN" : "0021-9541", "PMID" : "17620285", "abstract" : "Adult mesenchymal stem cells (MSCs) can be isolated from bone marrow or marrow aspirates and because they are culture-dish adherent, they can be expanded in culture while maintaining their multipotency. The MSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues. These tissue-engineered materials show considerable promise for use in rebuilding damaged or diseased mesenchymal tissues. Unanticipated is the realization that the MSCs secrete a large spectrum of bioactive molecules. These molecules are immunosuppressive, especially for T-cells and, thus, allogeneic MSCs can be considered for therapeutic use. In this context, the secreted bioactive molecules provide a regenerative microenvironment for a variety of injured adult tissues to limit the area of damage and to mount a self-regulated regenerative response. This regenerative microenvironment is referred to as trophic activity and, therefore, MSCs appear to be valuable mediators for tissue repair and regeneration. The natural titers of MSCs that are drawn to sites of tissue injury can be augmented by allogeneic MSCs delivered via the bloodstream. Indeed, human clinical trials are now under way to use allogeneic MSCs for treatment of myocardial infarcts, graft-versus-host disease, Crohn's Disease, cartilage and meniscus repair, stroke, and spinal cord injury. This review summarizes the biological basis for the in vivo functioning of MSCs through development

and aging.", "author" : [{ "dropping-particle" : "", "family" : "Caplan", "given" : "Arnold I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["2007", "11"]] }, "page" : "341-7", "title" : "Adult mesenchymal stem cells for tissue engineering versus regenerative medicine.", "type" : "article-journal", "volume" : "213" }, "uris" : ["http://www.mendeley.com/documents/?uuid=3c256b4c-12a9-4bec-b974-23a09619fff5"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1186/scrt10", "ISSN" : "1757-6512", "PMID" : "20637059", "abstract" : "Treatment of extensive bone defects requires autologous bone grafting or implantation of bone substitute materials. An attractive alternative has been to engineer fully viable, biological bone grafts in vitro by culturing osteogenic cells within three-dimensional scaffolds, under conditions supporting bone formation. Such grafts could be used for implantation, but also as physiologically relevant models in basic and translational studies of bone development, disease and drug discovery. A source of human cells that can be derived in large numbers from a small initial harvest and predictably differentiated into bone forming cells is critically important for engineering human bone grafts. We discuss the characteristics and limitations of various types of human embryonic and adult stem cells, and their utility for bone tissue engineering.", "author" : [{ "dropping-particle" : "", "family" : "Marolt", "given" : "Darja", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Knezevic", "given" : "Miomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Novakovic", "given" : "Gordana Vunjak", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research & therapy", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2010"]] }, "page" : "10", "title" : "Bone tissue engineering with human stem cells.", "type" :

"article-journal", "volume" : "1" }, "uris" : ["http://www.mendeley.com/documents/?uuid=fa253c75-5aca-4c1f-8996-61e0664fa847"] }], "mendeley" : { "formattedCitation" : "[5, 6]", "plainTextFormattedCitation" : "[5, 6]", "previouslyFormattedCitation" : "[5, 6]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. MSCs show a specific surface marker profile and a high capacity to engage adipogenic, chondrogenic and osteogenic lineage commitment, reflecting an important therapeutic potential **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1080/14653240600855905", "ISSN" : "1465-3249", "PMID" : "16923606", "abstract" : "The considerable therapeutic potential of human multipotent mesenchymal stromal cells (MSC) has generated markedly increasing interest in a wide variety of biomedical disciplines. However, investigators report studies of MSC using different methods of isolation and expansion, and different approaches to characterizing the cells. Thus it is increasingly difficult to compare and contrast study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. While these criteria will probably require modification as new knowledge unfolds, we believe this minimal set of standard criteria will foster a more uniform characterization of MSC and facilitate the exchange of data among investigators.", "author" : [{ "dropping-particle" : "", "family" : "Dominici", "given" : "M", "non-dropping-particle" : "", "parse-

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tendon, fat, and other connective tissues. These tissue-engineered materials show considerable promise for use in rebuilding damaged or diseased mesenchymal tissues. Unanticipated is the realization that the MSCs secrete a large spectrum of bioactive molecules. These molecules are immunosuppressive, especially for T-cells and, thus, allogeneic MSCs can be considered for therapeutic use. In this context, the secreted bioactive molecules provide a regenerative microenvironment for a variety of injured adult tissues to limit the area of damage and to mount a self-regulated regenerative response. This regenerative microenvironment is referred to as trophic activity and, therefore, MSCs appear to be valuable mediators for tissue repair and regeneration. The natural titers of MSCs that are drawn to sites of tissue injury can be augmented by allogeneic MSCs delivered via the bloodstream. Indeed, human clinical trials are now under way to use allogeneic MSCs for treatment of myocardial infarcts, graft-versus-host disease, Crohn's Disease, cartilage and meniscus repair, stroke, and spinal cord injury. This review summarizes the biological basis for the in vivo functioning of MSCs through development and aging.", "author" : [{ "dropping-particle" : "", "family" : "Caplan", "given" : "Arnold I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2007", "11"]] }, "page" : "341-7", "title" : "Adult mesenchymal stem cells for tissue engineering versus regenerative medicine.", "type" : "article-journal", "volume" : "213" }, "uris" : ["http://www.mendeley.com/documents/?uuid=3c256b4c-12a9-4bec-b974-23a09619fff5"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1126/science.284.5411.143", "ISBN" : "10.1126/science.284.5411.143", "ISSN" : "00368075", "PMID" : "10102814", "abstract" : "Human mesenchymal stem cells are thought to be multipotent cells, which are present in adult marrow, that can replicate as

undifferentiated cells and that have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma. Cells that have the characteristics of human mesenchymal stem cells were isolated from marrow aspirates of volunteer donors. These cells displayed a stable phenotype and remained as a monolayer in vitro. These adult stem cells could be induced to differentiate exclusively into the adipocytic, chondrocytic, or osteocytic lineages. Individual stem cells were identified that, when expanded to colonies, retained their multilineage potential.", "author" : [{ "dropping-particle" : "", "family" : "Pittenger", "given" : "Mark F.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mackay", "given" : "Alastair M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Beck", "given" : "Stephen C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jaiswal", "given" : "Rama K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Douglas", "given" : "Robin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mosca", "given" : "Joseph D", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Moorman", "given" : "Mark A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Simonetti", "given" : "Donald W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Craig", "given" : "Stewart", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Marshak", "given" : "Daniel R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science", "id" : "ITEM-3", "issue" : "1999", "issued" : { "date-parts" : [["2011"]] }, "page" : "143-148", "title" : "Multilineage

Potential of Adult Human Mesenchymal Stem Cells", "type" : "article-journal", "volume" : "143" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a6e08d2f-c24b-48e1-b26c-158f3986f835"] }], "mendeley" : { "formattedCitation" : "[6\u20138]", "plainTextFormattedCitation" : "[6\u20138]", "previouslyFormattedCitation" : "[6\u20138]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. In particular, the umbilical cord-derived Wharton's jelly MSCs (WJ-MSC) that are perinatal stem cells located in the stromal tissue between vasculature and amniotic epithelium of the umbilical cord **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1371/journal.pone.0006498", "ISBN" : "1932-6203 (Electronic)\\n1932-6203 (Linking)", "ISSN" : "1932-6203", "PMID" : "19652709", "abstract" : "BACKGROUND: Mesenchymal progenitor cells (MPCs) have been isolated from a variety of connective tissues, and are commonly called \"mesenchymal stem cells\" (MSCs). A stem cell is defined as having robust clonal self-renewal and multilineage differentiation potential. Accordingly, the term \"MSC\" has been criticised, as there is little data demonstrating self-renewal of definitive single-cell-derived (SCD) clonal populations from a mesenchymal cell source. METHODOLOGY/PRINCIPAL FINDINGS: Here we show that a tractable MPC population, human umbilical cord perivascular cells (HUCPVCs), was capable of multilineage differentiation in vitro and, more importantly, contributed to rapid connective tissue healing in vivo by producing bone, cartilage and fibrous stroma. Furthermore, HUCPVCs exhibit a high clonogenic frequency, allowing us to isolate definitive SCD parent and daughter clones from mixed gender suspensions as determined by Y-chromosome fluorescent in situ hybridization. CONCLUSIONS/SIGNIFICANCE: Analysis of the multilineage differentiation capacity of SCD parent clones and daughter

clones enabled us to formulate a new hierarchical schema for MSC self-renewal and differentiation in which a self-renewing multipotent MSC gives rise to more restricted self-renewing progenitors that gradually lose differentiation potential until a state of complete restriction to the fibroblast is reached.", "author" : [{ "dropping-particle" : "", "family" : "Sarugaser", "given" : "Rahul", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hanoun", "given" : "Lorraine", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Keating", "given" : "Armand", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stanford", "given" : "William L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Davies", "given" : "John E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "PloS one", "id" : "ITEM-1", "issue" : "8", "issued" : { "date-parts" : [["2009"]] }, "page" : "e6498", "title" : "Human mesenchymal stem cells self-renew and differentiate according to a deterministic hierarchy.", "type" : "article-journal", "volume" : "4" }, "uris" : ["http://www.mendeley.com/documents/?uuid=be3c51d7-7037-4d30-84f4-97d00152d205"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.bpobgyn.2015.07.006", "ISSN" : "15216934", "PMID" : "26482184", "abstract" : "The discovery of mesenchymal stem cells (MSCs) in perinatal sources, such as the amniotic fluid (AF) and the umbilical connective tissue, the so-called Wharton's jelly (WJ), has transformed them into promising stem cell grafts for the application in regenerative medicine. The advantages of AF-MSCs and WJ-MSCs over adult MSCs, such as bone marrow-derived mesenchymal stem cells (BM-MSCs), include their minimally invasive isolation procedure, their more primitive cell character without being tumourigenic, their low immunogenicity and their potential

autologous application in congenital disorders and when cryopreserved in adulthood. This chapter gives an overview of the biology of AF-MSCs and WJ-MSCs, and their regenerative potential based on the results of recent preclinical and clinical studies. In the end, open questions concerning the use of WJ-MSCs and AF-MSCs in regenerative medicine will be emphasized.", "author" : [{ "dropping-particle" : "", "family" : "Joerger-Messerli", "given" : "Marianne S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Marx", "given" : "Caterina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Oppliger", "given" : "Byron", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mueller", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "V.", "family" : "Surbek", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schoeberlein", "given" : "Andreina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Best Practice & Research Clinical Obstetrics & Gynaecology", "id" : "ITEM-2", "issued" : { "date-parts" : [["2016", "2"]] }, "page" : "30-44", "title" : "Mesenchymal Stem Cells from Wharton's Jelly and Amniotic Fluid", "type" : "article-journal", "volume" : "31" }, "uris" : ["http://www.mendeley.com/documents/?uuid=33af9228-9d45-3220-84f9-8c97e52ef1ae"] }], "mendeley" : { "formattedCitation" : "[9, 10]", "plainTextFormattedCitation" : "[9, 10]", "previouslyFormattedCitation" : "[9, 10]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** and hence ideal candidates for bone tissue engineering **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.3390/ijms140611692", "ISBN" : "1169211712", "ISSN" :

"1422-0067", "author" : [{ "dropping-particle" : "", "family" : "Kim", "given" : "Dae-Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Staples", "given" : "Meaghan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shinozuka", "given" : "Kazutaka", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pantcheva", "given" : "Paolina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kang", "given" : "Sung-Don", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Borlongan", "given" : "Cesar", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "International Journal of Molecular Sciences", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["2013"]] }, "page" : "11692-11712", "title" : "Wharton\u2019s Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications", "type" : "article-journal", "volume" : "14" }, "uris" : ["http://www.mendeley.com/documents/?uuid=83b46155-71ec-4eb8-a3ee-3c999a5ccfe9"] }], "mendeley" : { "formattedCitation" : "[11]", "plainTextFormattedCitation" : "[11]", "previouslyFormattedCitation" : "[11]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . WJ-MSCs have several relevant features that bring significant attention from the biomedical community: WJ-MSCs are increasingly accessible, easily isolated and expanded *in vitro* and their harvesting involves minor ethical issues, as umbilical cords are usually considered as medical waste **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1634/stemcells.2004-0013", "ISSN" : "10665099", "author" : [{ "dropping-particle" : "", "family" : "Wang", "given" : "Hwai-Shi", "non-dropping-particle"

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Moreover, the number of WJ-MSCs in the umbilical cord samples is high in comparison to other sources of adult MSC including bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) that are isolated at poor cell yields **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.acthis.2015.02.005", "ISBN" : "1618-

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0372 (Electronic)\\r0065-1281 (Linking)", "ISSN" : "00651281", "PMID" : "25747736", "abstract" : "Multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSC), can be isolated from bone marrow or other tissues, including fat, muscle and umbilical cord. It has been shown that MSC behave in vitro as stem cells: they self-renew and are able to differentiate into mature cells typical of several mesenchymal tissues. Moreover, the differentiation toward non-mesenchymal cell lineages (e.g. neurons) has been reported as well. The clinical relevance of these cells is mainly related to their ability to spontaneously migrate to the site of inflammation/damage, to their safety profile thanks to their low immunogenicity and to their immunomodulation capacities. To date, MSCs isolated from the post-natal bone marrow have represented the most extensively studied population of adult MSCs, in view of their possible use in various therapeutical applications. However, the bone marrow-derived MSCs exhibit a series of limitations, mainly related to their problematic isolation, culturing and use. In recent years, umbilical cord (UC) matrix (i.e. Wharton's jelly, WJ) stromal cells have therefore emerged as a more suitable alternative source of MSCs, thanks to their primitive nature and the easy isolation without relevant ethical concerns. This review seeks to provide an overview of the main biological properties of WJ-derived MSCs. Moreover, the potential application of these cells for the treatment of some known dysfunctions in the central and peripheral nervous system will also be discussed.", "author" : [{ "dropping-particle" : "", "family" : "Frausin", "given" : "Stefano", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Viventi", "given" : "Serena", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Verga Falzacappa", "given" : "Lucia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Quattromani", "given" : "Miriana Jlenia",

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(FuGENE). Gene array analysis showed that HUCPVCs also expressed Wnt signaling pathway genes that have been implicated in the regulation of MSCs. The similar characteristics between HUCPVCs and MSCs support the applicability of HUCPVCs for cell-based therapies. Disclosure of potential conflicts of interest is found at the end of this article.", "author" : [{ "dropping-particle" : "", "family" : "Baksh", "given" : "Dolores", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yao", "given" : "Raphael", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tuan", "given" : "Rocky S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cells (Dayton, Ohio)", "id" : "ITEM-2", "issue" : "6", "issued" : { "date-parts" : [["2007", "6"]] }, "page" : "1384-92", "title" : "Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow.", "type" : "article-journal", "volume" : "25" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4af7b5fe-2ed2-4ad6-8dd7-e8e9d84fd7fe"] }], "mendeley" : { "formattedCitation" : "[13, 14]", "plainTextFormattedCitation" : "[13, 14]", "previouslyFormattedCitation" : "[13, 14]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }. WJ-MSC also show high proliferation rates and differentiation potential, representing an intermediate state between ESCs and adult MSCs { ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1007/s12015-010-9166-x", "ISBN" : "1201501091", "ISSN" : "15508943", "PMID" : "20602182", "abstract" : "The human umbilical cord that originates from the embryo is an extra-embryonic membrane and the Wharton's jelly within it is a rich source of stem cells (hWJSCs). It is not definitely known whether these cells behave as human embryonic stem cells (hESCs), human

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mesenchymal stem cells (hMSC) or both. They have the unique properties of high proliferation rates, wide multipotency, hypoimmunogenicity, do not induce teratomas and have anticancer properties. These advantages are important considerations for their use in cell based therapies and treatment of cancers. In a search for properties that confer these advantages we compared a detailed transcriptome profiling of hWJSCs using DNA microarrays with that of a panel of known hESCs, hMSCs and stromal cells. hWJSCs expressed low levels of the pluripotent embryonic stem cell markers including POUF1, NANOG, SOX2 and LIN28, thus explaining why they do not produce teratomas. Several cytokines were significantly upregulated in hWJSCs including IL12A which is associated with the induction of apoptosis, thus explaining their anticancer properties. When GO Biological Process analysis was compared between the various stem cell types, hWJSCs showed an increased expression of genes associated with the immune system, chemotaxis and cell death. The ability to modulate immune responses makes hWJSCs an important compatible stem cell source for transplantation therapy in allogeneic settings without immunorejection. The data in the present study which is the first detailed report on hWJSC transcriptomes provide a foundation for future functional studies where the exact mechanisms of these unique properties of hWJSCs can be confirmed.", "author" : [{ "dropping-particle" : "", "family" : "Fong", "given" : "Chui Yee", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chak", "given" : "Li Ling", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Biswas", "given" : "Arijit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tan", "given" : "Jee Hian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gauthaman", "given" : "Kalamegam", "non-dropping-

particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chan", "given" : "Woon Khiong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bongso", "given" : "Ariff", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem Cell Reviews and Reports", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2011"]] }, "page" : "1-16", "title" : "Human Wharton's Jelly Stem Cells Have Unique Transcriptome Profiles Compared to Human Embryonic Stem Cells and Other Mesenchymal Stem Cells", "type" : "article-journal", "volume" : "7" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e56e56e1-c705-44e6-bc3e-ab29f75b6bc0"] }], "mendeley" : { "formattedCitation" : "[15]", "plainTextFormattedCitation" : "[15]", "previouslyFormattedCitation" : "[15]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . In addition, WJ-MSC have the ability to moderate the immune response, making them suitable for allogeneic transplants **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.2174/1574888X11308010012", "ISSN" : "1574888X", "author" : [{ "dropping-particle" : "", "family" : "Rocca", "given" : "Giampiero", "non-dropping-particle" : "La", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Iacono", "given" : "Melania", "non-dropping-particle" : "Lo", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Corsello", "given" : "Tiziana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Corrao", "given" : "Simona", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Farina", "given" : "Felicia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Anzalone", "given" : "Rita", "non-dropping-particle" :

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Derived Mesenchymal Stem Cells: Future of Regenerative Medicine? Recent Findings and Clinical Significance", "type" : "article-journal", "volume" : "2015" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b05cc19b-11a7-41d8-88c3-b2d808863eb0"] }], "mendeley" : { "formattedCitation" : "[16, 17]", "plainTextFormattedCitation" : "[16, 17]", "previouslyFormattedCitation" : "[16, 17]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }}.

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Growing WJ-MSC in an osteogenic media containing dexamethasone, ascorbate and β-glycerolphosphate can induce the acquisition of some hallmarks of osteoblastic cells recapitulating specific regulatory events of the developmental pathway that leads to the osteoblast phenotype {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Mosna", "given" : "Federico", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sensebe", "given" : "Luc", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "STEM CELLS AND DEVELOPMENT", "id" : "ITEM-1", "issue" : "10", "issued" : { "date-parts" : [["2010"]] }, "page" : "1-53", "title" : "Human Bone Marrow and Adipose Tissue Mesenchymal Stem Cells : A User \u2019 s Guide", "type" : "article-journal", "volume" : "19" }, "uris" : ["http://www.mendeley.com/documents/?uuid=47a3f484-a43a-48e9-a87f-853cb335ba51"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1186/scrt328", "ISBN" : "1757-6512 (Electronic)\\r1757-6512 (Linking)", "ISSN" : "1757-6512", "PMID" : "24073831", "abstract" : "The standard procedure for the osteogenic differentiation of multipotent stem cells is treatment of a confluent monolayer with a cocktail of dexamethasone (Dex),

ascorbic acid (Asc) and \u03b2-glycerophosphate (\u03b2-Gly). This review describes the effects of these substances on intracellular signaling cascades that lead to osteogenic differentiation of bone marrow stroma-derived stem cells. We conclude that Dex induces Runx2 expression by FHL2/\u03b2-catenin-mediated transcriptional activation and that Dex enhances Runx2 activity by upregulation of TAZ and MKP1. Asc leads to the increased secretion of collagen type I (Col1), which in turn leads to increased Col1/\u03b12\u03b21 integrin-mediated intracellular signaling. The phosphate from \u03b2-Gly serves as a source for the phosphate in hydroxylapatite and in addition influences intracellular signaling molecules. In this context we give special attention to the differences between dystrophic and bone-specific mineralization.", "author" : [{ "dropping-particle" : "", "family" : "Langenbach", "given" : "Fabian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Handschel", "given" : "J\u00f6rg", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research & therapy", "id" : "ITEM-2", "issue" : "5", "issued" : { "date-parts" : [["2013"]] }, "page" : "117", "title" : "Effects of dexamethasone, ascorbic acid and \u03b2-glycerophosphate on the osteogenic differentiation of stem cells in vitro.", "type" : "article-journal", "volume" : "4" }, "uris" : ["http://www.mendeley.com/documents/?uuid=74c91794-7d2a-4d2c-bca0-99fc1bd0d0f3"] }], "mendeley" : { "formattedCitation" : "[18, 19]", "plainTextFormattedCitation" : "[18, 19]", "previouslyFormattedCitation" : "[18, 19]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. However, we and others have recently shown that osteogenic stimulation of WJ-MSCs results in a limited osteoblast differentiation (OD) program restricted at least partially, through repressive epigenetic mechanisms **{**ADDIN CSL_CITATION { "citationItems" : [

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"", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bustos", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aedo", "given" : "S\u00f3crates", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Veronica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "9", "30"]] }, "title" : "Epigenetic Signatures at the RUNX2-P1 and Sp7 Gene Promoters Control Osteogenic Lineage Commitment of Umbilical Cord-Derived Mesenchymal Stem Cells.", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4a544d50-d112-39c5-892a-27c2d48f44cd"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.3892/ijmm.2017.2872", "ISSN" : "1107-3756", "author" : [{ "dropping-particle" : "", "family" : "Marupanthorn", "given" : "Kulisara", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tantrawatpan", "given" : "Chairat", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kheolamai", "given" : "Pakpoom", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE", "id" :

"ITEM-2", "issue" : "39", "issued" : { "date-parts" : [["2017"]] }, "page" : "654-662", "title" : "Bone morphogenetic protein-2 enhances the osteogenic differentiation capacity of mesenchymal stromal cells derived from human bone marrow and umbilical cord", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=5c7e6251-4061-4c2a-9b71-9ab0fc98e7b6"] }], "mendeley" : { "formattedCitation" : "[20, 21]", "plainTextFormattedCitation" : "[20, 21]", "previouslyFormattedCitation" : "[20, 21]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Therefore, there is need for a deeper understanding of the key molecular mechanisms that modulate WJ-MSC osteogenic commitment to provide insights for their potential use in a therapeutic context.

Runt-related transcription factor 2 (RUNX2) is the master regulator of the OD program **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "ISSN" : "1381-6128", "PMID" : "14529540", "abstract" : "Runx2/Cbfa/AML3 is a member of the runt homology domain family of transcription factors, essential for osteoblast differentiation and bone formation. Defining the molecular mechanisms by which Runx2 can function as a master regulatory gene for activating the program of osteoblastogenesis has provided novel insights for transcriptional regulation of tissue-specific genes. Regulation of Runx expression has the potential to serve as a basis for the design of novel therapeutic strategies for promoting bone formation. Here we review the unique properties of Runx2 that mediate several key functions necessary for regulating skeletogenesis, controlling osteoblast growth and differentiation, and integrating the complex pathways required for bone formation and turnover.", "author" : [{ "dropping-particle" : "", "family" : "Lian", "given" : "J B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" },

{ "dropping-particle" : "", "family" : "Stein", "given" : "G S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Current pharmaceutical design", "id" : "ITEM-1", "issue" : "32", "issued" : { "date-parts" : [["2003", "1"]] }, "page" : "2677-85", "title" : "Runx2/Cbfa1: a multifunctional regulator of bone formation.", "type" : "article-journal", "volume" : "9" }, "uris" : ["http://www.mendeley.com/documents/?uuid=291531fe-7488-4b05-bb44-ee1a59633cb4"] }], "mendeley" : { "formattedCitation" : "[22]", "plainTextFormattedCitation" : "[22]", "previouslyFormattedCitation" : "[22]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Key roles of RUNX2 were evidenced by the complete lack of bone formation in *Runx2* null mice **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/S0092-8674(00)80258-5", "ISBN" : "0092-8674", "ISSN" : "00928674", "PMID" : "9182763", "abstract" : "A transcription factor, Cbfa1, which belongs to the runt-domain gene family, is expressed restrictively in fetal development. To elucidate the function of Cbfa1, we generated mice with a mutated Cbfa1 locus. Mice with a homozygous mutation in Cbfa1 died just after birth without breathing. Examination of their skeletal systems showed a complete lack of ossification. Although immature osteoblasts, which expressed alkaline phophatase weakly but not Osteopontin and Osteocalcin, and a few immature osteoclasts appeared at the perichondrial region, neither vascular nor mesenchymal cell invasion was observed in the cartilage. Therefore, our data suggest that both intramembranous and endochondral ossification were completely blocked, owing to the maturational arrest of osteoblasts in the mutant mice, and demonstrate that Cbfa1 plays an essential role in osteogenesis.", "author" : [{ "dropping-particle" : "", "family" : "Komori", "given" : "T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" },

{ "dropping-particle" : "", "family" : "Yagi", "given" : "H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nomura", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yamaguchi", "given" : "A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sasaki", "given" : "K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Deguchi", "given" : "K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shimizu", "given" : "Y", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bronson", "given" : "R.T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gao", "given" : "Y.-H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Inada", "given" : "M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sato", "given" : "M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Okamoto", "given" : "R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kitamura", "given" : "Y", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yoshiki", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kishimoto", "given" : "T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Cell", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["1997"]] }, "page" : "755-764", "title" : "Targeted Disruption of Cbfa1 Results in a Complete Lack of Bone Formation owing to Maturational Arrest of Osteoblasts", "type" : "article-journal",

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developmental timing and cell type- specific expression of type I and type II Cbfa isoforms, and not necessarily molecular properties or sequences that reside in the N-terminus of Cbfa1, are the principal determinants of the osteogenic activity of Cbfa1.", "author" : [{ "dropping-particle" : "", "family" : "Banerjee", "given" : "C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Javed", "given" : "a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Choi", "given" : "J Y", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Green", "given" : "J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosen", "given" : "V", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "a J", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "J L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "J B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "G S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Endocrinology", "id" : "ITEM-1", "issue" : "9", "issued" : { "date-parts" : [["2001"]] }, "page" : "4026-4039", "title" : "Differential regulation of the two principal Runx2/Cbfa1 N-terminal isoforms in response to bone morphogenetic protein-2 during development of the osteoblast phenotype", "type" : "article-journal", "volume" : "142" }, "uris" : ["http://www.mendeley.com/documents/?uuid=12c24766-d29a-4847-9e73-3cec0a5e53a1"] }], "mendeley" : { "formattedCitation" : "[24]", "plainTextFormattedCitation" : "[24]", "previouslyFormattedCitation" : "[24]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-

language/schema/raw/master/csl-citation.json" }**}** by governing the expression of several bone-related genes including *SP7*, *BGLAP*, Alkaline phosphatase (*ALP*), Collagen 1A (*COL1A*) and Bone sialoprotein (*BSP*) **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/sj.onc.1207676", "ISSN" : "0950-9232", "PMID" : "15156188", "abstract" : "We present an overview of Runx involvement in regulatory mechanisms that are requisite for fidelity of bone cell growth and differentiation, as well as for skeletal homeostasis and the structural and functional integrity of skeletal tissue. Runx-mediated control is addressed from the perspective of support for biological parameters of skeletal gene expression. We review recent findings that are consistent with an active role for Runx proteins as scaffolds for integration, organization and combinatorial assembly of nucleic acids and regulatory factors within the three-dimensional context of nuclear architecture.", "author" : [{ "dropping-particle" : "", "family" : "Stein", "given" : "Gary S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Javed", "given" : "Amjad", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zaidi", "given" : "Sayyed K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Young", "given" : "Daniel W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Choi",

"given" : "Je-Yong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pockwinse", "given" : "Shirwin M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Oncogene", "id" : "ITEM-1", "issue" : "24", "issued" : { "date-parts" : [["2004", "5", "24"]] }, "page" : "4315-29", "title" : "Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression.", "type" : "article-journal", "volume" : "23" }, "uris" : ["http://www.mendeley.com/documents/?uuid=da29a8b1-f61a-3d62-a72a-1f9a45dba104"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1146/annurev.genom.9.081307.164437", "ISSN" : "1527-8204", "PMID" : "18767962", "abstract" : "The skeleton contains three specific cell types: chondrocytes in cartilage and osteoblasts and osteoclasts in bone. Our understanding of the transcriptional mechanisms that lead to cell differentiation along these three lineages has increased considerably in the past ten years. In the case of chondrocytes and osteoblasts advances have been made possible largely through the molecular elucidation of human skeletal dysplasias. This review discusses the key transcription factors that regulate skeletogenesis and highlights their function, mode of action, and regulation by other factors, with a special emphasis on how human genetics has contributed to this knowledge.", "author" : [{ "dropping-particle" : "", "family" : "Karsenty", "given" : "Gerard", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Annual Review of Genomics and Human Genetics", "id" : "ITEM-2", "issue" : "1", "issued" : { "date-parts" : [["2008", "9"]] }, "page" : "183-196", "title" : "Transcriptional Control of Skeletogenesis", "type" : "article-journal", "volume" : "9" }, "uris" : ["http://www.mendeley.com/documents/?uuid=835a7404-2362-386f-8664-502cc03651fb"] }], "mendeley" : { "formattedCitation" : "[25, 26]", "plainTextFormattedCitation" : "[25,

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Given this pivotal role of RUNX2 during OD its expression level has been proposed as a convenient target for modulating human MSC differentiation in tissue engineering. RUNX2 overexpression strategies have been tested successfully to enhance human adult MSC OD *in vitro* {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.biomaterials.2010.09.042", "ISBN" : "1878-5905 (Electronic)\\r0142-9612 (Linking)", "ISSN" : "01429612", "PMID" : "20947160", "abstract" : "In the present study, we tested the hypothesis that electroporation-mediated transfer of Runx2, Osterix, or both genes enhances the in vitro and in vivo osteogenesis from adipose stem cells (ASCs). ASCs were transfected with Runx2, Osterix, or both genes using electroporation, and further cultured in monolayer or in PLGA scaffold under osteogenic medium for 14 days, then analyzed for in vitro osteogenic differentiation. Transfected ASC-PLGA scaffold hybrids were also implanted on nude mice to test for in vivo ectopic bone formation. Runx2 and Osterix genes were strongly expressed in ASCs transfected with each gene on day 7, decreasing rapidly on day 14. Runx2 protein was strongly expressed in ASCs transfected with the Runx2 gene, while Osterix protein was strongly expressed in ASCs transfected with either or both Runx2 and Osterix genes. Overexpression of Runx2 and Osterix significantly increased the gene expression of osteogenic differentiation markers (alkaline phosphatase [ALP], osteocalcin [OCN], type I collagen [COL1A1], and bone sialoprotein [BSP]) in ASCs. Transfection of Runx2 and Osterix genes enhanced the protein expression of OCN, type I collagen, and BSP, as demonstrated by Western blot analysis, and ALP

activity as well as enhancing mineralization in the monolayer culture and ASC-PLGA scaffold hybrids. Runx2- or Osterix-transfected ASC-PLGA scaffold hybrids promoted bone formation in nude mice after 6 weeks of in vivo implantation. ?? 2010 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Lee", "given" : "Jai Sun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "Jong Min", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "Il", "family" : "Im", "given" : "Gun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biomaterials", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2011"]] }, "page" : "760-768", "publisher" : "Elsevier Ltd", "title" : "Electroporation-mediated transfer of Runx2 and Osterix genes to enhance osteogenesis of adipose stem cells", "type" : "article-journal", "volume" : "32" }, "uris" : ["http://www.mendeley.com/documents/?uuid=87d20448-41e3-4b1a-af25-e93dc2453dda"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.biomaterials.2010.03.019", "ISBN" : "1878-5905 (Electronic)\\r0142-9612 (Linking)", "ISSN" : "01429612", "PMID" : "20413153", "abstract" : "Adipose tissue contains multipotent mesenchymal stem cells (MSCs) that are able to differentiate into various tissues. Bone morphogenetic protein 2 (BMP2) is known as one of the key osteogenesis induction factors in MSCs. Recently, several new transcription factors that contribute to osteogenic differentiation have been reported, among them Runx2, Osterix, and Dlx5. We hypothesized that adipose-derived stromal cells (ASCs) could be induced to efficiently differentiate into osteocytes by the co-expression of the BMP2 and Runx2 genes. To prove this hypothesis, we constructed a bicistronic vector encoding the BMP2 and Runx2 genes linked to the 'self-cleaving' 2A peptide sequence. BMP2/Runx2-ASCs showed a gradual increase in alkaline phosphatase activity for two weeks. RT-PCR analysis

and alizarin red staining revealed a high expression of osteogenesis-related markers (osteopontin, osteocalcin and collagen type I) and increased mineralization in BMP2/Runx2-ASCs compared to BMP2-ASCs. Six weeks after in vivo transplantation, BMP2/Runx2-ASCs also showed a significant increase in bone formation compared to ASCs and BMP2-ASCs. These findings demonstrate that the co-transfection of two osteogenic lineage-determining genes can enhance osteogenic differentiation of ASCs. ?? 2010 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Lee", "given" : "Suk Jun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kang", "given" : "Sun Woong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Do", "given" : "Hyun Jin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Inbo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shin", "given" : "Dong Ah", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kim", "given" : "Jae Hwan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "Soo Hong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biomaterials", "id" : "ITEM-2", "issue" : "21", "issued" : { "date-parts" : [["2010"]] }, "page" : "5652-5659", "publisher" : "Elsevier Ltd", "title" : "Enhancement of bone regeneration by gene delivery of BMP2/Runx2 bicistronic vector into adipose-derived stromal cells", "type" : "article-journal", "volume" : "31" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b1d7956a-37de-4a3d-87fd-dc0c209bfc34"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1016/j.ymthe.2005.03.009", "ISSN" : "15250016", "author" : [{ "dropping-particle" : "", "family" : "Zhao", "given" : "Z", "non-

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dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Innamorati", "given" : "Giulio", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Valenti", "given" : "Maria Teresa", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem Cell Reviews and Reports", "id" : "ITEM-4", "issued" : { "date-parts" : [["2012"]] }, "page" : "891-897", "title" : "Transcription Factor Runx2 and its Application to Bone Tissue Engineering", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=48dae6bf-e2e4-4dab-be82-9cf0b147c475"] }], "mendeley" : { "formattedCitation" : "[27\u201330]", "plainTextFormattedCitation" : "[27\u201330]", "previouslyFormattedCitation" : "[27\u201330]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. However, it is necessary to also establish biomedical-compatible strategies that modulate the endogenous RUNX2 expression, particularly in WJ-MSCs.

We have recently demonstrated that JARID1B (Jumonji AT-rich interactive domain 1B) histone demethylase represses *Runx2/p57* expression in undifferentiated MSC by removing the H3K4me3 activating histone mark at *Runx2/p57* P1 promoter (Rojas et al. 2015). Accordingly, JARID1B knockdown favors *Runx2/p57* expression and osteogenic commitment of murine MSCs, demonstrating that JARID1B control can serve as a molecular switch of MSC cell fate. Hence, we hypothesize that JARID1B-mediated epigenetic repression of *RUNX2/p57* gene is conserved in human MSC and that this molecular mechanism is relevant to modulate *RUNX2/p57* expression. This mechanism

may then represent a valuable tool for modulating osteoblastic-lineage commitment of WJ-MSC.

Here, we address the modulatory effects of RUNX2 during OD of WJ-MSC. Ectopic RUNX2 expression stimulates WJ-MSC OD. Endogenous *RUNX2/p57* transcription is transiently induced during WJ-MSC differentiation, in a process that is antagonized by JARID1B. Accordingly, JARID1B loss of function via specific small molecule inhibition or shRNA results in increased *RUNX2/p57* transcription and removal of repressive epigenetic marks at the P1 promoter. Together our results place epigenetic modulation of RUNX2/p57 expression as a novel target to enhance the efficacy of WJ-MSC osteoblastic differentiation and as a critical component for future applications in bone tissue engineering.

MATERIALS AND METHODS

Cell culture and ~~osteogenic~~ differentiation analysis

Umbilical cords were collected from healthy donors who underwent caesarean sections after full-term pregnancy at the Hospital Luis Tisné Brousse obstetrics and gynecology service as part of an established collaboration with Dr. Palma's laboratory.

Donors provided informed consents according to ethical guidelines of Universidad de Chile; favorable ethical approval was given by Servicio de Salud Metropolitano Oriente (SSMO). WJ-MSC were obtained and characterized as described { ADDIN

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Briefly, umbilical cords were cut transversally in 1 cm pieces and longitudinally opened in Petri dishes containing PBS. Umbilical veins and arteries were located and removed. Blood vessel-free umbilical cord explants were then collected and cut in 5 mm pieces and transferred to tissue culture plates positioning WJ in contact to the plate and amnion facing up. Explants were then cultured in DMEM (Thermo Fisher Scientific) supplemented with 10% FBS (Hyclone) at 37ºC in 5% CO_2 for 8 days and then removed to allow adhered WJ-MSC to growth until a stable culture was achieved. Media was changed every 48 h during this period. Because MSC are a heterogeneous population of stem cells, we used the ISCT

(International Society of Cell Therapy) guidelines to characterize them as primary cultures of WJ-MSC. WJ-MSC from 4 different donors were analyzed by flow citometry (see below). The phenotype of WJ-MSC was further defined by the multipotency of these cells in culture according to described methods {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1186/scrt232", "ISBN" : "1757-6512 (Electronic)\\r1757-6512 (Linking)", "ISSN" : "1757-6512", "PMID" : "23845279", "abstract" : "INTRODUCTION: Human Wharton's jelly (WJ) has become a preferred source of mesenchymal stem cells (MSCs) whose clinical applications are limited by the use of adequate xeno-free (XF), in vitro manipulation conditions. Therefore, the objective of our study was to characterize WJ-derived MSCs (WJ-MSCs), isolated by different methods and cultured in a commercially available, MSC XF medium, not least of all by investigating their endothelial differentiation capacity.\\n\\nMETHODS: WJ explants and enzymatically dissociated WJ cells were cultured in a defined, XF medium for MSCs. Adherent cells at passages 2 and 5 were characterized as MSCs by flow cytometry, MTT, real-time quantitative reverse transcription PCR, and functional multipotent differentiation assays. The endothelial differentiation capacity of MSCs isolated and expanded until passage 2 in the MSC XF medium, and then subcultured for five passages in a commercially available endothelial growth medium (group A), was assessed over serial passages, as compared to adherent WJ-derived cells isolated and expanded for five consecutive passages in the endothelial medium (group B).\\n\\nRESULTS: The MSC phenotype of WJ explant- and pellet-derived cells, isolated and expanded in the MSC XF medium, was proven based on the expression of CD44/CD73/CD90/CD105 surface markers and osteo-/adipo-/chondrogenic multipotent differentiation potential, which differed according to the isolation method and/or passage number. Upon exposure to

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endothelial differentiation cues, cells belonging to group A did not exhibit endothelial cell characteristics over serial passages; by contrast, WJ pellet-derived cells belonging to group B expressed endothelial characteristics at gene, protein and functional levels, potentially due to culture conditions favoring the isolation of other stem/progenitor cell types than MSCs, able to give rise to an endothelial progeny.\\n\\nCONCLUSIONS: The use of defined, MSC XF media for isolation and expansion of human WJ-MSCs is a prerequisite for the establishment of their real endothelial differentiation capacity, as candidates for clinical therapy applications. Thus, the standardization of WJ-MSCs isolation and culture expansion techniques in defined, MSC XF media, for their accurate characterization, would be a priority in the stem cell research field.", "author" : [{ "dropping-particle" : "", "family" : "Corotchi", "given" : "Maria Cristina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Popa", "given" : "Mirel Adrian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Remes", "given" : "Anca", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sima", "given" : "Livia Elena", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gussi", "given" : "Ilinca", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lupu Plesu", "given" : "Marilena", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research & therapy", "id" : "ITEM-1", "issue" : "4", "issued" : { "date-parts" : [["2013"]] }, "page" : "81", "publisher" : "Stem Cell Research & Therapy", "title" : "Isolation method and xeno-free culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells.", "type" : "article-journal", "volume" : "4" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=76a2e21c-414a-4a50-bac2-b95eeee03589"] }], "mendeley" : { "formattedCitation" : "[32]", "plainTextFormattedCitation" : "[32]", "previouslyFormattedCitation" : "[32]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }. Partial characterization of our WJ-MSCs is shown as supplemental material (Fig S1).

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Human umbilical vein endothelial cells (HUVEC) were isolated and cultured as described {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1007/s10456-014-9432-7", "ISSN" : "1573-7209", "PMID" : "24728929", "abstract" : "Disorders in skin wound healing are a major health problem that requires the development of innovative treatments. The use of biomaterials as an alternative of skin replacement has become relevant, but its use is still limited due to poor vascularization inside the scaffolds, resulting in insufficient oxygen and growth factors at the wound site. In this study, we have developed a cell-based wound therapy consisting of the application of collagen-based dermal scaffolds containing mesenchymal stem cells from Wharton's jelly (WJ-MSC) in an immunocompetent mouse model of angiogenesis. From our comparative study on the secretion profile between WJ-MSC and adipose tissue-derived MSC, we found a stronger expression of several well-characterized growth factors, such as VEGF-A, angiopoietin-1 and aFGF, which are directly linked to angiogenesis, in the culture supernatant of WJ-MSC, both on monolayer and 3D culture conditions. WJ-MSC proved to be angiogenic both in vitro and in vivo, through tubule formation and CAM assays, respectively. Moreover, WJ-MSC consistently improved the healing response in vivo in a mouse model of human-like dermal repair, by triggering angiogenesis and further providing a suitable

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on the presence of classical angiogenic factors. Recent evidence suggests that axonal guidance proteins and their receptors can also act as angiogenic regulators. Netrin, a family of laminin-like proteins, specifically Netrin-1 and 4, act via DCC/Neogenin-1 and UNC5 class of receptors to promote or inhibit angiogenesis, depending on the physiological context. METHODS Mesenchymal stem cells secrete a broad set of classical angiogenic factors. However, little is known about the expression of non-canonical angiogenic factors such as Netrin-1. The aim was to characterize the possible secretion of Netrin ligands by Wharton's jelly-derived mesenchymal stem cells (WJ-MSC). We evaluated if Netrin-1 presence in the conditioned media from these cells was capable of inducing angiogenesis both in vitro and in vivo, using human umbilical vein endothelial cells (HUVEC) and chicken chorioallantoic membrane (CAM), respectively. In addition, we investigated if the RhoA/ROCK pathway is responsible for the integration of Netrin signaling to control vessel formation. RESULTS The paracrine angiogenic effect of the WJ-MSC-conditioned media is mediated at least in part by Netrin-1 given that pharmacological blockage of Netrin-1 in WJ-MSC resulted in diminished angiogenesis on HUVEC. When HUVEC were stimulated with exogenous Netrin-1 assayed at physiological concentrations (10-200 ng/mL), endothelial vascular migration occurred in a concentration-dependent manner. In line with our determination of Netrin-1 present in WJ-MSC-conditioned media we were able to obtain endothelial tubule formation even in the pg/mL range. Through CAM assays we validated that WJ-MSC-secreted Netrin-1 promotes an increased angiogenesis in vivo. Netrin-1, secreted by WJ-MSC, might mediate its angiogenic effect through specific cell surface receptors on the endothelium, such as UNC5b and/or integrin \u03b16\u03b21, expressed in HUVEC. However, the angiogenic response of Netrin-1 seems not to be mediated through the RhoA/ROCK pathway. CONCLUSIONS Thus, here we show that

stromal production of Netrin-1 is a critical component of the vascular regulatory machinery. This signaling event may have deep implications in the modulation of several processes related to a number of diseases where angiogenesis plays a key role in vascular homeostasis.", "author" : [{ "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ortiz", "given" : "Mar\u00eda Carolina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Villanueva", "given" : "Andrea", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Villarroel", "given" : "Cynthia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edwards", "given" : "Sandra S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Elliott", "given" : "Mat\u00edas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aedo", "given" : "S\u00f3crates", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Meza", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lois", "given" : "Pablo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Ver\u00f3nica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem Cell Research & Therapy", "id" : "ITEM-2", "issue" : "1", "issued" : { "date-parts" : [["2017", "12", "28"]] }, "page" : "43", "title" : "Netrin-1 acts as a non-canonical angiogenic factor produced by human Wharton\u2019s jelly mesenchymal stem cells (WJ-MSC)", "type" : "article-

journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6af1a7c8-e07d-3e44-a756-1d0c3ea07015"] }], "mendeley" : { "formattedCitation" : "[31, 33]", "plainTextFormattedCitation" : "[31, 33]", "previouslyFormattedCitation" : "[31, 33]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }. Briefly, uUmbilical cords were washed in PBS and umbilical vein located and cannulated at both ends. After PBS washing of umbilical vein lumen through cannula, it was incubated with Collagenase dissolved in M199 media (Thermo Fisher Scientific) for 15 min at 37 °C. HUVEC cell purification was then mechanically stimulated by massaging. HUVEC cells were ~~then~~ collected, centrifuged and resuspended in M199 supplemented with 10% FBS (Hyclone) and 10 % Newborn calf serum (Thermo Fisher Scientific) and plated in gelatin coated plates for 48 h. WJ-MSC and SAOS-2 cells (American Type Culture Collection HTB-85) were cultured in DMEM (Thermo Fisher Scientific) supplemented with 10% FBS (Hyclone) at 37ºC in 5% CO2.

Osteogenic differentiation was performed following previously described protocols {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Mosna", "given" : "Federico", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sensebe", "given" : "Luc", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "STEM CELLS AND DEVELOPMENT", "id" : "ITEM-1", "issue" : "10", "issued" : { "date-parts" : [["2010"]] }, "page" : "1-53", "title" : "Human Bone Marrow and Adipose Tissue Mesenchymal Stem Cells : A User \u2019 s Guide", "type" : "article-journal", "volume" : "19" }, "uris" : [

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"http://www.mendeley.com/documents/?uuid=47a3f484-a43a-48e9-a87f-853cb335ba51"] }], "mendeley" : { "formattedCitation" : "[18]", "plainTextFormattedCitation" : "[18]", "previouslyFormattedCitation" : "[18]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** incubating the cells in an osteoblastic media (OB) containing DMEM 10%FBS supplemented with 10 mM β-glycerolphosphate (Merck), 50 µg/µl ascorbic acid and 0.1 µM dexamethasone (both from Sigma-Aldrich). Culture media was replaced every 48 h. In experiments with WJ-MSCs, triplicates correspond to cells obtained from different umbilical cords, randomly selected. For adipogenic differentiation, WJ-MSCs were cultured in DMEM 10% FBS medium containing 1 µM dexamethasone, 500 µM 3-isobutyl-L-methylxanthine (IBMX), 60 µM indomethacin and 10 µg/mL insulin (all from Sigma Aldrich) at 37°C. Increased expression of phenotypic markers in WJ-MSC induced to adipogenesis was assed by RT-qPCR analysis. For lipid droplet staining, WJ-MSC were fixed in 4% PFA and stained with a 0.2% Oil red O solution in isopropanol (Sigma Aldrich) for 30 min at RT with gentle shaking. Cells were then washed with distilled water prior to imaging.

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Analysis of surface marker expression by flow cytometry

~~**Flow cytometry**~~

~~**Oil red staining**~~

For RUNX2 overexpression, lentiviral particles were produced by transfection of HEK293FT cells with the pCDH-CMV-mRUNX2-EF1-copGFP (LV-RUNX2) vector coding for *RUNX2/p57* or the pCDH-CMV-EF1-copGFP (LV-Ctrl) vector used as control

(System Bio) { ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "98440501", "ISBN" : "0022-538X (Print)\\r0022-538X (Linking)", "ISSN" : "0022-538X", "PMID" : "9765382", "abstract" : "Vectors derived from human immunodeficiency virus (HIV) are highly efficient vehicles for in vivo gene delivery. However, their biosafety is of major concern. Here we exploit the complexity of the HIV genome to provide lentivirus vectors with novel biosafety features. In addition to the structural genes, HIV contains two regulatory genes, tat and rev, that are essential for HIV replication, and four accessory genes that encode critical virulence factors. We previously reported that the HIV type 1 accessory open reading frames are dispensable for efficient gene transduction by a lentivirus vector. We now demonstrate that the requirement for the tat gene can be offset by placing constitutive promoters upstream of the vector transcript. Vectors generated from constructs containing such a chimeric long terminal repeat (LTR) transduced neurons in vivo at very high efficiency, whether or not they were produced in the presence of Tat. When the rev gene was also deleted from the packaging construct, expression of gag and pol was strictly dependent on Rev complementation in trans. By the combined use of a separate nonoverlapping Rev expression plasmid and a 5' LTR chimeric transfer construct, we achieved optimal yields of vector of high transducing efficiency (up to 10(7) transducing units [TU]/ml and 10(4) TU/ng of p24). This third-generation lentivirus vector uses only a fractional set of HIV genes: gag, pol, and rev. Moreover, the HIV-derived constructs, and any recombinant between them, are contingent on upstream elements and trans complementation for expression and thus are nonfunctional outside of the vector producer cells. This split-genome, conditional packaging system is based on existing viral sequences and acts as a built-in device against the generation of productive recombinants. While the actual biosafety of the vector will ultimately be proven in vivo, the

improved design presented here should facilitate testing of lentivirus vectors.", "author" : [{ "dropping-particle" : "", "family" : "Dull", "given" : "T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zufferey", "given" : "R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kelly", "given" : "M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mandel", "given" : "R J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nguyen", "given" : "M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Trono", "given" : "D", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Naldini", "given" : "L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of virology", "id" : "ITEM-1", "issue" : "11", "issued" : { "date-parts" : [["1998"]] }, "page" : "8463-71", "title" : "A third-generation lentivirus vector with a conditional packaging system.", "type" : "article-journal", "volume" : "72" }, "uris" : ["http://www.mendeley.com/documents/?uuid=64ac85a5-9415-4739-99ec-92130b835103"] }], "mendeley" : { "formattedCitation" : "[34]", "plainTextFormattedCitation" : "[34]", "previouslyFormattedCitation" : "[34]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Viral titer was determined as described **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1186/1472-6750-6-34", "ISBN" : "1472-6750 (Electronic)\\r1472-6750 (Linking)", "ISSN" : "1472-6750", "PMID" : "16836756", "abstract" : "BACKGROUND: Lentiviral vectors are efficient vehicles for stable gene transfer in dividing and non-dividing cells. Several improvements in vector design to

increase biosafety and transgene expression, have led to the approval of these vectors for use in clinical studies. Methods are required to analyze the quality of lentiviral vector production, the efficiency of gene transfer and the extent of therapeutic gene expression. RESULTS: We compared lentiviral vector titration methods that measure pg p24/ml, RNA equivalents/ml, transducing units (TU/ml) or mRNA equivalents. The amount of genomic RNA in vector particles proves to be reliable to assess the production quality of vectors encoding non-fluorescent proteins. However, the RNA and p24 titers of concentrated vectors are rather poor in predicting transduction efficiency, due to the high variability of vector production based on transient transfection. Moreover, we demonstrate that transgenic mRNA levels correlate well with TU and can be used for functional titration of non-fluorescent transgenes. CONCLUSION: The different titration methods have specific advantages and disadvantages. Depending on the experimental set-up one titration method should be preferred over the others.", "author" : [{ "dropping-particle" : "", "family" : "Geraerts", "given" : "Martine", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Willems", "given" : "Sofie", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Baekelandt", "given" : "Veerle", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Debyser", "given" : "Zeger", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gijsbers", "given" : "Rik", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "BMC biotechnology", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2006"]] }, "page" : "34", "title" : "Comparison of lentiviral vector titration methods.", "type" : "article-journal", "volume" : "6" }, "uris" : ["http://www.mendeley.com/documents/?uuid=8c30ed96-1bd8-4729-a1bd-bc41ef224896"]

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For knockdown assays, 10^5 WJ-MSCs cultured for 24 h at 37ºC were transduced with lentiviral particles containing shRNA against JARID1B mRNA (shJARID1B) or an unspecific sequence in presence of 8 µg/ml Polybrene **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1634/stemcells.2003-0106", "ISBN" : "1066-5099", "ISSN" : "1066-5099", "PMID" : "16339997", "abstract" : "Human bone marrow (BM) mesenchymal stem/progenitor cells are potentially attractive targets for ex vivo gene therapy. The potential of lentiviral vectors for transducing BM mesenchymal cells was examined using a self-inactivating vector that expressed the green fluorescent protein (GFP) from an internal cytomegalovirus (CMV) promoter. This vector was compared with oncoretroviral vectors expressing GFP from the CMV promoter or a

modified long-terminal repeat that had been optimized for long-term expression in stem cells. The percentage of GFP-positive cells was consistently higher following lentiviral versus oncoretroviral transduction, consistent with increased GFP mRNA levels and increased gene transfer efficiency measured by polymerase chain reaction and Southern blot analysis. In vitro GFP and FVIII expression lasted for several months post-transduction, although expression slowly declined. The transduced cells retained their stem/progenitor cell properties since they were still capable of differentiating along adipogenic and osteogenic lineages in vitro while maintaining high GFP and FVIII expression levels. Implantation of lentivirally transduced human BM mesenchymal cells using collagen scaffolds into immunodeficient mice resulted in efficient engraftment of gene-engineered cells and long-term transgene expression in vivo. These biocompatible BM mesenchymal implants represent a reversible, safe, and versatile protein delivery approach because they can be retrieved in the event of an unexpected adverse reaction or when expression of the protein of interest is no longer required. In conclusion, efficient gene delivery with lentiviral vectors in conjunction with the use of bioengineered reversible scaffolds improves the therapeutic prospects of this novel approach for gene therapy, protein delivery, or tissue engineering.", "author" : [{ "dropping-particle" : "", "family" : "Damme", "given" : "An", "non-dropping-particle" : "Van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thorrez", "given" : "Lieven", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ma", "given" : "Ling", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vandenburgh", "given" : "Herman", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Eyckmans", "given" : "Jeroen", "non-dropping-particle" : "", "parse-names" : false,

"suffix" : "" }, { "dropping-particle" : "", "family" : "Dell'Accio", "given" : "Francesco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bari", "given" : "Cosimo", "non-dropping-particle" : "De", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Luyten", "given" : "Frank", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lillicrap", "given" : "David", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Collen", "given" : "D\u00e9sir\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "VandenDriessche", "given" : "Thierry", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chuah", "given" : "Marinee K L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cells", "id" : "ITEM-1", "issue" : "4", "issued" : { "date-parts" : [["2006"]] }, "page" : "896-907", "title" : "Efficient lentiviral transduction and improved engraftment of human bone marrow mesenchymal cells.", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f566dd72-7663-4258-a30e-594b5c60dfe7"] }], "mendeley" : { "formattedCitation" : "[36]", "plainTextFormattedCitation" : "[36]", "previouslyFormattedCitation" : "[36]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . The RNA consortium (TRC) codes of each shRNA-containing lentivirus are shown in Table S1. Infected cells were incubated at 32°C during 48 h and then moved to 37ºC for the different experimental approaches.

Pharmacological JARID1B inhibition

After 1 day of culture, 10^5 WJ-MSC were treated with 1 µM PBIT inhibitor (2-(methylphenyl)-1,2-benzisothiazol-3(2H)-one; #16272, Cayman Chemical) **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M112.419861", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Sayegh", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cao", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zou", "given" : "M. R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morales", "given" : "A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Blair", "given" : "L. P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Norcia", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hoyer", "given" : "D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tackett", "given" : "a. J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Merkel", "given" : "J. S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yan", "given" : "Q.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "13", "issued" : { "date-parts" : [["2013"]] }, "page" : "9408-9417", "title" : "Identification of Small Molecule Inhibitors of Jumonji AT-rich Interactive Domain 1B (JARID1B) Histone Demethylase by a Sensitive High Throughput Screen", "type" : "article-journal", "volume" : "288" }, "uris" : ["http://www.mendeley.com/documents/?uuid=1114722f-bf90-481b-bdb5-095d7ccbf4fb"]

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RNA isolation and quantitative PCR

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific) followed by a phenol chloroform extraction. RNA was then resuspended in nuclease free water and its concentration quantified using a Tecan Infinite 200 PRO NanoQuant microplate reader. For cDNA synthesis, 1 µg of mRNA was mixed with 0,25 µg oligo(dT)12-18 (Thermo Fisher Scientific) and denatured for 5 minutes at 72ºC. Denatured mRNA was then incubated with 100 units MMLV reverse transcriptase (Promega), 20 units RNAse inhibitor (New England Biolabs) and 0.5 mM dNTPs (Life Technologies) for 1 h at 37ºC. The cDNA was then diluted five times with nuclease free water and subjected to 20 µl qPCR reactions. PCR quantification was performed using Brilliant II SYBR Green QPCR Master Mix in an Mx3000P thermocycler (Both from Agilent technologies). Gene expression analyses were performed using the ΔΔCt method. GAPDH mRNA expression was used as normalizer. Primer sequences for RT-qPCR assays are listed in supplementary table S2.

Chromatin immunoprecipitation (ChIP)

ChIP assays were performed following previously described protocols **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1126/science.1068356", "ISSN" : "00368075", "PMID" : "11884757", "abstract" : "We

analyzed the ordered recruitment of factors to the human alpha1 antitrypsin promoter around the initial activation of the gene during enterocyte differentiation. We found that a complete preinitiation complex, including phosphorylated RNA pol II, was assembled at the promoter long before transcriptional activation. The histone acetyltransferases CBP and P/CAF were recruited subsequently, but local histone hyperacetylation was delayed. After transient recruitment of the human Brahma homolog hBrm, remodeling of the neighboring nucleosome coincided with transcription initiation. The results suggest that, at this promoter, chromatin reconfiguration is a defining step of the initiation process, acting after the assembly of the Pol II machinery.", "author" : [{ "dropping-particle" : "", "family" : "Soutoglou", "given" : "E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Talianidis", "given" : "Iannis", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science", "id" : "ITEM-1", "issue" : "5561", "issued" : { "date-parts" : [["2002", "3", "8"]] }, "page" : "1901-1904", "title" : "Coordination of PIC Assembly and Chromatin Remodeling During Differentiation-Induced Gene Activation", "type" : "article-journal", "volume" : "295" }, "uris" : ["http://www.mendeley.com/documents/?uuid=1458b82f-2f66-35c9-bad1-c7477db54f00"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1128/MCB.05096-11", "ISSN" : "1098-5549", "PMID" : "21606199", "abstract" : "The Ric-8 gene encodes a guanine exchange factor (GEF) that modulates G protein-mediated signaling, exhibiting a relevant role during regulation of cell division. In mammals, two Ric-8 homologues have been reported (Ric-8A and Ric-8B), and recent studies indicate equivalent roles for each protein. Here, we show that the Ric-8B gene is negatively regulated during osteoblast differentiation by the transcription factor C/EBP\u03b2. Only the larger C/EBP\u03b2 isoform (C/EBP\u03b2-LAP*) downregulates Ric-8B gene promoter activity in

osteoblastic cells. Accordingly, knockdown of C/EBP\u03b2 expression by small intefering RNA in osteoblastic cells results in a significant increase of Ric-8B gene expression. Transient overexpression of Brg1 or Brm, the catalytic subunits of the SWI/SNF chromatin-remodeling complex, inhibits Ric-8B promoter activity. Also, the presence of inactive SWI/SNF complexes in osteoblastic cells results in increased endogenous Ric-8B transcription, indicating that SWI/SNF activity negatively regulates Ric-8B expression. During osteoblast differentiation, Ric-8B gene repression is accompanied by changes in nucleosome placement at the proximal Ric-8B gene promoter and reduced accessibility to regulatory sequences.", "author" : [{ "dropping-particle" : "", "family" : "Grandy", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sepulveda", "given" : "Hugo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pihan", "given" : "Philippe", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez", "given" : "Berta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Olate", "given" : "Juan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular and cellular biology", "id" : "ITEM-2", "issue" : "14", "issued" : { "date-parts" : [["2011", "7", "15"]] }, "page" : "2997-3008", "title" : "The Ric-8B gene is highly expressed in proliferating preosteoblastic cells and downregulated during osteoblast differentiation in a SWI/SNF- and C/EBPbeta-mediated manner.", "type" : "article-journal", "volume" : "31" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=c1edac69-d68f-311d-9367-80e55e68af1e"] }], "mendeley" : { "formattedCitation" : "[38, 39]", "plainTextFormattedCitation" : "[38, 39]", "previouslyFormattedCitation" : "[38, 39]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Cells were first crosslinked using formaldehyde 1% and then sonicated to obtain chromatin fragments around 300 pb. 25µg of these samples were immunoprecipitated respectively, with each specific antibody analyzed or with an unspecific normal IgG as control and collected using protein A-agarose beads. Immunocomplexes were heated at 65°C to reverse the crosslinking and treated with RNase (10µg/ml) and Proteinase K (100µg/ml). DNA was recovered by Phenol/Chloroform extraction and precipitated by standard methods. Quantitative PCR (qPCR) was used to determine the enrichment levels relative to input DNA. Lists of primer sequences and antibodies used in ChIP analysis are indicated in supplementary tables S2 and S3, respectively.

Nuclear protein isolation and Western blot

Total proteins were extracted from cells in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and a protease inhibitor cocktail). Nuclear proteins were obtained as described **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1093/nar/11.5.1475", "ISBN" : "0305-1048 (Print)\\r0305-1048 (Linking)", "ISSN" : "03051048", "PMID" : "6828386", "abstract" : "We have developed a procedure for preparing extracts from nuclei of human tissue culture cells that directs accurate transcription initiation in vitro from class II promoters. Conditions of extraction and assay have been optimized for maximum activity using the major late promoter of adenovirus 2. The extract also directs accurate transcription

initiation from other adenovirus promoters and cellular promoters. The extract also directs accurate transcription initiation from class III promoters (tRNA and Ad 2 VA).", "author" : [{ "dropping-particle" : "", "family" : "Dignani", "given" : "John David", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lebovitz", "given" : "Russell M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roeder", "given" : "Robert G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nucleic Acids Research", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["1983"]] }, "page" : "1475-1489", "title" : "Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei", "type" : "article-journal", "volume" : "11" }, "uris" : ["http://www.mendeley.com/documents/?uuid=bdd8cab0-2aaf-4bd0-8bca-71b8e664fbe7"] }], "mendeley" : { "formattedCitation" : "[40]", "plainTextFormattedCitation" : "[40]", "previouslyFormattedCitation" : "[40]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Briefly, cells were incubated in buffer A (10 mM HEPES pH 7.8, 10 mM KCl, 0.2 mM EDTA, 1 mM DTT and a protease inhibitor cocktail) on ice for 10 minutes, homogeneized using a tissue grinder and centrifuged (10 minutes at 4000 rpm). The isolated nuclei were washed five times with buffer A, centrifuged again and finally resuspended in RIPA buffer. Protein quantification was performed using the DC protein assay (Bio-Rad). Proteins were analyzed by western blot using SDS-polyacrylamide gels and nitrocellulose membranes. Antibodies used for immunodetection are listed in supplementary table S3.

Indirect immunofluorescence

Cells were seeded in glass coverslips and grown as indicated above. After fixation, cells were permeabilized by incubation during 5 minutes in a solution containing 0.1% Triton X-100 PBS and then blocked with 3% BSA for 30 minutes. Cells were incubated with antibodies as listed in supplementary table S3 and stained with DAPI. Cells were mounted using Fluoromount medium (Sigma Aldrich). Images were obtained using a LSM510 Meta confocal microscope through Zeiss LSM Image Browser software (Zeiss) or a CKX415F microscope (Olympus).

Calcium quantification

Matrix mineralization was determined via extracellular calcium quantification using a Calcium Colorimetric Assay Kit (Biovision) according to manufacturer's instructions. WJ-MSCs were washed with PBS, incubated with 2 N HCl (20 hours, room temperature) with gentle shaking to finally collect the supernatants. A calcium standard curve (0-40 µg/ml) was prepared in 2 N HCl and 50 µl of samples were loaded in a 96 well plate. Chromogenic reagent (90 µl) and calcium assay buffer (60 µl) were added and incubated for 5 minutes with gentle shaking protected from light. OD at 575 nm was measured in a Tecan Infinite 200 PRO microplate reader and sample absorbance values were interpolated in the standard curve to determine the calcium concentrations.

Alizarin Red S (ARS) staining

To visualize the mineralized matrix WJ-MSCs were first fixed (9:1 ethanol: 30% formaldehyde; 30 seconds) and then stained with an ARS solution as described **{ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.270.16.9420", "ISBN" : "0021-9258 (Print)\\n0021-9258 (Linking)",**

"ISSN" : "00219258", "PMID" : "7721867", "abstract" : "This study evaluated a rapid biomineralization phenomenon exhibited by an osteoblastic cell line, UMR 106-01 BSP, when treated with either organic phosphates [beta-glycerophosphate (beta-GP), Ser-P, or Thr-P], inorganic phosphate (P(i)), or calcium. In a dose-dependent manner, these agents (2-10 mM) stimulated confluent cultures to deposit mineral in the cell layer (ED50 of approximately 4.6 mM for beta-GP (30 +/- 2 nmol Ca2+/microgram DNA) and approximately 3.8 mM (29 +/- 2 nmol Ca2+/microgram DNA) for P(i)) with a plateau in mineral formation by 20 h (ET50 approximately 12-15 h). beta-GP or P(i) treatment yielded mineral crystals having an x-ray diffraction pattern similar to normal human bone. Alizarin red-S histology demonstrated calcium mineral deposition in the extracellular matrix and what appeared to be intracellular paranuclear staining. Electron microscopy revealed small, needle-like crystals associated with fibrillar, extracellular matrix deposits and intracellular spherical structures. Mineral formation was inhibited by levamisole (ED50 approximately 250 microM), pyrophosphate (ED50 approximately 1-10 microM), actinomycin C1 (500 ng/ml), cycloheximide (50 micrograms/ml), or brefeldin A (1 microgram/ml). These results indicate that UMR 106-01 BSP cells form a bio-apatitic mineralized matrix upon addition of supplemental phosphate. This process involves alkaline phosphatase activity, ongoing RNA and protein synthesis, as well as Golgi-mediated processing and secretion.", "author" : [{ "dropping-particle" : "", "family" : "Stanford", "given" : "C. M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jacobson", "given" : "P. a.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Eanes", "given" : "E. D.", "non-dropping-particle" : "", "parse-names"

: false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lembke", "given" : "L. a.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Midura", "given" : "R. J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "16", "issued" : { "date-parts" : [["1995"]] }, "page" : "9420-9428", "title" : "Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 106-01 BSP)", "type" : "article", "volume" : "270" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e9bae821-b265-418b-a13e-c47b53cf06c4"] }], "mendeley" : { "formattedCitation" : "[41]", "plainTextFormattedCitation" : "[41]", "previouslyFormattedCitation" : "[41]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }}. Fixed cells were incubated with 40 mM ARS (Sigma Aldrich) in 0.1 M Na2HPO4, pH 4.3 (10 minutes, room temperature) protected from light, with gentle shaking. After five washes with distilled water, cells were incubated in PBS for 15 minutes and ARS-calcium complex deposits were imaged in a CKX415F microscope (Olympus).

Oil red staining

For lipid droplet staining, WJ-MSC were fixed in 4% PFA and stained with a 0.2% Oil red O solution in isopropanol (Sigma Aldrich) for 30 min at RT with gentle shaking. Cells were then washed with distilled water prior to imaging.

Data analysis

In all experiments, data correspond to mean ±SEM of at least 3 biological replicates. Statistical analyses and plots were performed using GraphPad Prism 6 software (GraphPad

software, Inc). One way ANOVA followed by Tukey post hoc test or Student's T test were performed to determine statistical significance. Images were processed and figures constructed using Photoshop CS2 software (Adobe).

RESULTS

RUNX2/p57 overexpression results in enhanced osteoblast differentiation of WJ-MSC.

To assess the effect of RUNX2/p57 in WJ-MSC OD, we first inquired whether RUNX2/p57 overexpression enhances their differentiation potential as described for other MSC {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : {

"DOI" : "10.1016/j.biomaterials.2010.09.042", "ISBN" : "1878-5905 (Electronic)\\r0142-9612 (Linking)", "ISSN" : "01429612", "PMID" : "20947160", "abstract" : "In the present study, we tested the hypothesis that electroporation-mediated transfer of Runx2, Osterix, or both genes enhances the in vitro and in vivo osteogenesis from adipose stem cells (ASCs). ASCs were transfected with Runx2, Osterix, or both genes using electroporation, and further cultured in monolayer or in PLGA scaffold under osteogenic medium for 14 days, then analyzed for in vitro osteogenic differentiation. Transfected ASC-PLGA scaffold hybrids were also implanted on nude mice to test for in vivo ectopic bone formation. Runx2 and Osterix genes were strongly expressed in ASCs transfected with each gene on day 7, decreasing rapidly on day 14. Runx2 protein was strongly expressed in ASCs transfected with the Runx2 gene, while Osterix protein was strongly expressed in ASCs transfected with either or both Runx2 and Osterix genes. Overexpression of Runx2 and Osterix significantly increased the gene expression of osteogenic differentiation markers (alkaline phosphatase [ALP], osteocalcin [OCN], type I collagen [COL1A1], and bone sialoprotein [BSP]) in ASCs. Transfection of Runx2 and Osterix genes enhanced the protein expression of OCN, type I collagen, and BSP, as demonstrated by Western blot analysis, and ALP activity as well as enhancing mineralization in the monolayer culture and ASC-PLGA scaffold hybrids. Runx2- or Osterix-transfected ASC-PLGA scaffold hybrids promoted bone formation in nude mice after 6 weeks of in vivo implantation. ?? 2010 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Lee", "given" : "Jai Sun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "Jong Min", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "Il", "family" : "Im", "given" : "Gun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biomaterials", "id" : "ITEM-

1", "issue" : "3", "issued" : { "date-parts" : [["2011"]] }, "page" : "760-768", "publisher" : "Elsevier Ltd", "title" : "Electroporation-mediated transfer of Runx2 and Osterix genes to enhance osteogenesis of adipose stem cells", "type" : "article-journal", "volume" : "32" }, "uris" : ["http://www.mendeley.com/documents/?uuid=87d20448-41e3-4b1a-af25-e93dc2453dda"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.biomaterials.2010.03.019", "ISBN" : "1878-5905 (Electronic)\\r0142-9612 (Linking)", "ISSN" : "01429612", "PMID" : "20413153", "abstract" : "Adipose tissue contains multipotent mesenchymal stem cells (MSCs) that are able to differentiate into various tissues. Bone morphogenetic protein 2 (BMP2) is known as one of the key osteogenesis induction factors in MSCs. Recently, several new transcription factors that contribute to osteogenic differentiation have been reported, among them Runx2, Osterix, and Dlx5. We hypothesized that adipose-derived stromal cells (ASCs) could be induced to efficiently differentiate into osteocytes by the co-expression of the BMP2 and Runx2 genes. To prove this hypothesis, we constructed a bicistronic vector encoding the BMP2 and Runx2 genes linked to the 'self-cleaving' 2A peptide sequence. BMP2/Runx2-ASCs showed a gradual increase in alkaline phosphatase activity for two weeks. RT-PCR analysis and alizarin red staining revealed a high expression of osteogenesis-related markers (osteopontin, osteocalcin and collagen type I) and increased mineralization in BMP2/Runx2-ASCs compared to BMP2-ASCs. Six weeks after in vivo transplantation, BMP2/Runx2-ASCs also showed a significant increase in bone formation compared to ASCs and BMP2-ASCs. These findings demonstrate that the co-transfection of two osteogenic lineage-determining genes can enhance osteogenic differentiation of ASCs. ?? 2010 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Lee", "given" : "Suk Jun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-

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"type" : "article-journal", "volume" : "12" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6606259d-6e0e-45d1-a866-cca50206c80a"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1007/s12015-011-9337-4", "ISBN" : "1550-8943", "ISSN" : "15508943", "PMID" : "22139789", "abstract" : "Cbfa1/Runx2 is a bone transcription factor homologous to the Drosophila protein, Runt. Runx2 is a master gene that encodes for a protein involved in the osteogenic differentiation process from mesenchymal precursors. It is known that in Cbfa1 deficient mice (Cbfa1(-/-)) the lack of mature osteoblasts is associated to incomplete bone mineralization. An important aim of modern biology is the development of new molecular tools for identification of therapeutic approaches. Recent discoveries in cell and molecular biology enabled researchers in the bone tissue-engineering field to develop new strategies for gene and cell-based therapies. This review summarizes the process of osteogenic differentiation from mesenchymal stem cells and the importance of bone regeneration is discussed. In particular, given the increasing interest in the study of the transcription factor Runx2, this review highlights the role of this target gene and addresses recent strategies using Runx2 for bone regeneration.", "author" : [{ "dropping-particle" : "", "family" : "Carbonare", "given" : "Luca Dalle", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Innamorati", "given" : "Giulio", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Valenti", "given" : "Maria Teresa", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem Cell Reviews and Reports", "id" : "ITEM-4", "issued" : { "date-parts" : [["2012"]] }, "page" : "891-897", "title" : "Transcription Factor Runx2 and its Application to Bone Tissue Engineering", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=48dae6bf-e2e4-4dab-be82-9cf0b147c475"]

}], "mendeley" : { "formattedCitation" : "[27\u201330]", "plainTextFormattedCitation" : "[27\u201330]", "previouslyFormattedCitation" : "[27\u201330]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. We transduced WJ-MSC with lentiviral particles carrying a bicistronic vector encoding both RUNX2/p57 and GFP proteins (LV-RUNX2). A scheme of the experimental strategy is presented in Fig 1A. WJ-MSC transduction efficiency was confirmed for empty (LV-Ctrl) or LV-RUNX2 lentiviral particles (Fig S2) by determining RUNX2/p57 protein levels and its intracellular distribution after four days of infection through western blot (Fig 1B) and immunofluorescence (Fig 1C) analyses, respectively. WJ-MSC transduction results in high RUNX2 protein expression in WJ-MSCs (Fig 1B) and as expected, this exogenous RUNX2/p57 protein was selectively located in the nucleus (Fig. 1C). We next determined the effect of RUNX2/p57 overexpression over downstream genes during OD of WJ-MSCs. For this, LV-Ctrl or LV-RUNX2 transduced WJ-MSCs were cultured for 15 days in control (*CT*) or osteogenic media (*OB*) and then RNA samples analyzed by RT-qPCR (Fig 1D-G). Although at this time the osteogenic media alone was not sufficient to induce the expression of several bone-related genes, RUNX2/p57 overexpression resulted in robust increases of its direct targets *SP7* (Fig 1D), *BSP* (Fig 1E) and *ALP* (Fig 1F), all genes associated with immature and early-mature OD. Nevertheless, the expression of *BGLAP* gene mRNA, a late bone phenotypic marker was not induced by sustained RUNX2/p57 overexpression in WJ-MSCs (Fig 1G). To control the overall effect on the osteoblast differentiation of these infected WJ-MSCs, we performed Alizarin red S (ARS) staining and extracellular matrix-associated calcium quantification ~~that~~ confirming that RUNX2/p57 overexpression increased ~~d~~ the WJ-MSCs osteoblast differentiation (Fig S3).

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In summary, these results indicate that RUNX2/p57 overexpression enhances the osteogenic potential of WJ-MSC.

***RUNX2/p57* is transiently expressed during osteoblast differentiation of WJ-MSC**

RUNX2/p57 expression has been extensively used as a marker of WJ-MSC OD **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1089/ten.TEA.2010.0224", "ISBN" : "1937-335X (Electronic)\\r1937-3341 (Linking)", "ISSN" : "1937-335X", "PMID" : "20673136", "abstract" : "Most tissue engineering studies use human bone marrow mesenchymal stem cells for differentiation into desirable lineages. We derived a novel stem cell from the human umbilical cord Wharton's jelly (hWJSC) that has numerous advantages over other stem cell types in that they can be harvested in abundance very efficiently and painlessly with no risk of patient morbidity, have prolonged stemness properties in vitro, are hypoimmunogenic, and can be differentiated into many tissue types in two-dimensional culture. We compared four different three-dimensional nanofibrous scaffolds (polycaprolactone [PCL], PCL/collagen [PCL/Coll], PCL/hydroxyapatite [PCL/HA], and PCL/Coll/HA) for the attachment, proliferation, differentiation, and mineralization of hWJSCs into an osteogenic lineage. The collagen-based scaffolds (PCL/Coll and PCL/Coll/HA) showed better cell attachment and proliferation than PCL and PCL/HA, with increases of 41.80% and 38.52%, respectively. hWJSCs cultured on PCL/Coll/HA in the osteogenic medium up to 21 days demonstrated increased alkaline phosphatase activity and greater expression of osteocalcin, mineralization, and osteogenic-related genes compared to controls. Given the advantages of hWJSCs over other stem cell types, we propose that hWJSCs may be efficiently differentiated into an osteogenic lineage on a three-dimensional PCL/Coll/HA nanofibrous

scaffold for the treatment of bone defects.", "author" : [{ "dropping-particle" : "", "family" : "Gauthaman", "given" : "Kalamegam", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Venugopal", "given" : "Jayarama Reddy", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yee", "given" : "Fong Chui", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Biswas", "given" : "Arijit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ramakrishna", "given" : "Seeram", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bongso", "given" : "Ariff", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Tissue engineering. Part A", "id" : "ITEM-1", "issue" : "1-2", "issued" : { "date-parts" : [["2011"]] }, "page" : "71-81", "title" : "Osteogenic differentiation of human Wharton's jelly stem cells on nanofibrous substrates in vitro.", "type" : "article-journal", "volume" : "17" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ea24f7c9-88f9-4f69-ba57-25b36c86ba66"] }, { "id" : "ITEM-2", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Wang", "given" : "Limin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dormer", "given" : "Nathan H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bonewald", "given" : "Lynda F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Detamore", "given" : "Michael S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Tissue engineering. Part A", "id" : "ITEM-2", "issue" : "6", "issued" : { "date-parts" : [["2010"]] }, "page" : "1937-1948", "title" : "Osteogenic Differentiation of Human

Umbilical Cord Mesenchymal Stromal Cells in Polyglycolic Acid Scaffolds Limin", "type" : "article-journal", "volume" : "16" }, "uris" : ["http://www.mendeley.com/documents/?uuid=3ef17976-b50e-4506-89a8-793c0086cc84"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.7150/ijbs.6.499", "ISSN" : "1449-2288", "abstract" : "Multipotent mesenchymal stromal cells (MSCs) from Wharton's jelly (WJ) of umbilical cord bear higher proliferation rate and self-renewal capacity than adult tissue-derived MSCs and are a primitive stromal cell population. Stem cell niche or physiological microenvironment plays a crucial role in maintenance of stem cell properties and oxygen concentration is an important component of the stem cell niche. Low oxygen tension or hypoxia is prevalent in the microenvironment of embryonic stem cells and many adult stem cells at early stages of development. Again, in vivo, MSCs are known to home specifically to hypoxic events following tissue injuries. Here we examined the effect of hypoxia on proliferation and in vitro differentiation potential of WJ-MSCs. Under hypoxia, WJ-MSCs exhibited improved proliferative potential while maintaining multi-lineage differentiation potential and surface marker expression. Hypoxic WJ-MSCs expressed higher mRNA levels of hypoxia inducible factors, notch receptors and notch downstream gene HES1. Gene expression profile of WJ-MSCs exposed to hypoxia and normoxia was compared and we identified a differential gene expression pattern where several stem cells markers and early mesodermal/endothelial genes such as DESMIN, CD34, ACTC were upregulated under hypoxia, suggesting that in vitro culturing of WJ-MSCs under hypoxic conditions leads to adoption of a mesodermal/endothelial fate. Thus, we demonstrate for the first time the effect of hypoxia on gene expression and growth kinetics of WJ-MSCs. Finally, although WJ-MSCs do not induce teratomas, under stressful and long-term culture conditions, MSCs can occasionally undergo transformation. Though there were no

chromosomal abnormalities, certain transformation markers were upregulated in a few of the samples of WJ-MSCs under hypoxia.", "author" : [{ "dropping-particle" : "", "family" : "Nekanti", "given" : "Usha", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dastidar", "given" : "Sumitava", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Venugopal", "given" : "Parvathy", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Totey", "given" : "Satish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ta", "given" : "Malancha", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "International Journal of Biological Sciences", "id" : "ITEM-3", "issue" : "5", "issued" : { "date-parts" : [["2010"]] }, "page" : "499-512", "title" : "Increased Proliferation and Analysis of Differential Gene Expression in Human Wharton \u2019 s Jelly-derived Mesenchymal Stromal Cells under Hypoxia", "type" : "article-journal", "volume" : "6" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6373ccf8-300c-4509-b337-7093a3adf39c"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.2147/SCCAA.S17548", "ISSN" : "1178-6957", "PMID" : "24198529", "abstract" : "Mesenchymal stem cells (MSCs) have become an attractive tool for tissue engineering and targets in clinical transplantation due to their regeneration potential and immuno-suppressive capacity. Although MSCs derived from bone marrow are the most widely used, their harvest requires an invasive procedure. The umbilical cord, which is discarded at birth, can provide an inexhaustible source of stem cells for therapy. The Wharton's jelly-derived MSCs (WJ-MSCs), from the umbilical cord, have been shown to have faster proliferation rates and greater expansion capability compared with adult MSCs. The standard isolation and in vitro culture protocol for WJ-

MSCs utilizes fetal bovine serum (FBS) or calf serum as a nutrient supplement. However, FBS raises potential safety concerns such as transmission of viral/prion disease and may initiate xenogeneic immune reactions against bovine antigens. Therefore, for therapeutic applications, there is an urgent requirement to establish an alternative nutrient supplement which would favor cell proliferation, retain MSC characteristics, and prove safe in human subjects. In the present study, we isolated and expanded WJ-MSCs in 5% pooled, allogeneic human serum (HS) supplemented with 2 ng/mL of basic fibroblast growth factor. For cell dissociation, porcine trypsin was replaced with TrypLE, a recombinant enzyme, and a protease-free protocol was adapted for isolation of MSCs from WJ. We determined their growth kinetics, in vitro differentiation potential, surface marker expression, and colony-forming unit potential and compared them against standard WJ-MSC cultures expanded in 10% FBS. All these parameters matched quite well between the two MSC populations. To test whether there is any alteration in gene expression on switching from FBS to HS, we analyzed a panel of stem cell and early lineage markers using Taqman\u00ae low density array. No significant deviation in gene expression was observed between the two populations. Thus we established an efficient, complete xeno-free protocol for propagation of human WJ-MSCs.", "author" : [{ "dropping-particle" : "", "family" : "Venugopal", "given" : "Parvathy", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Balasubramanian", "given" : "Sudha", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "Sen", "family" : "Majumdar", "given" : "Anish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ta", "given" : "Malancha", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cells and cloning : advances and applications", "id" : "ITEM-4",

"issued" : { "date-parts" : [["2011"]] }, "page" : "39-50", "title" : "Isolation, characterization, and gene expression analysis of Wharton's jelly-derived mesenchymal stem cells under xeno-free culture conditions.", "type" : "article-journal", "volume" : "4" }, "uris" : ["http://www.mendeley.com/documents/?uuid=7589a71a-a2f2-429d-b6b0-235e05cd6833"] }, { "id" : "ITEM-5", "itemData" : { "DOI" : "10.1186/s13287-015-0263-2", "ISSN" : "1757-6512", "PMID" : "26718750", "abstract" : "BACKGROUND: Due to their intrinsic properties, stem cells are promising tools for new developments in tissue engineering and particularly for cartilage tissue regeneration. Although mesenchymal stromal/stem cells from bone marrow (BM-MSC) have long been the most used stem cell source in cartilage tissue engineering, they have certain limits. Thanks to their properties such as low immunogenicity and particularly chondrogenic differentiation potential, mesenchymal stromal/stem cells from Wharton's jelly (WJ-MSC) promise to be an interesting source of MSC for cartilage tissue engineering. METHODS: In this study, we propose to evaluate chondrogenic potential of WJ-MSC embedded in alginate/hyaluronic acid hydrogel over 28 days. Hydrogels were constructed by the original spraying method. Our main objective was to evaluate chondrogenic differentiation of WJ-MSC on three-dimensional scaffolds, without adding growth factors, at transcript and protein levels. We compared the results to those obtained from standard BM-MSC. RESULTS: After 3 days of culture, WJ-MSC seemed to be adapted to their new three-dimensional environment without any detectable damage. From day 14 and up to 28 days, the proportion of WJ-MSC CD73(+), CD90(+), CD105(+) and CD166(+) decreased significantly compared to monolayer marker expression. Moreover, WJ-MSC and BM-MSC showed different phenotype profiles. After 28 days of scaffold culture, our results showed strong upregulation of cartilage-specific transcript expression. WJ-MSC exhibited greater type II

collagen synthesis than BM-MSC at both transcript and protein levels. Furthermore, our work highlighted a relevant result showing that WJ-MSC expressed Runx2 and type X collagen at lower levels than BM-MSC. CONCLUSIONS: Once seeded in the hydrogel scaffold, WJ-MSC and BM-MSC have different profiles of chondrogenic differentiation at both the phenotypic level and matrix synthesis. After 4 weeks, WJ-MSC, embedded in a three-dimensional environment, were able to adapt to their environment and express specific cartilage-related genes and matrix proteins. Today, WJ-MSC represent a real alternative source of stem cells for cartilage tissue engineering.", "author" : [{ "dropping-particle" : "", "family" : "Reppel", "given" : "Lo\u00efc", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schiavi", "given" : "Jessica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Charif", "given" : "Naceur", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Leger", "given" : "L\u00e9onore", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yu", "given" : "Hao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pinzano", "given" : "Astrid", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henrionnet", "given" : "Christel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stoltz", "given" : "Jean-Fran\u00e7ois", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bensoussan", "given" : "Dani\u00e8le", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huselstein", "given" : "C\u00e9line", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research

& therapy", "id" : "ITEM-5", "issue" : "1", "issued" : { "date-parts" : [["2015", "1"]] }, "page" : "260", "title" : "Chondrogenic induction of mesenchymal stromal/stem cells from Wharton's jelly embedded in alginate hydrogel and without added growth factor: an alternative stem cell source for cartilage tissue engineering.", "type" : "article-journal", "volume" : "6" }, "uris" : ["http://www.mendeley.com/documents/?uuid=54e9b116-3297-4422-be32-80177d52d80e"] }], "mendeley" : { "formattedCitation" : "[42\u201346]", "plainTextFormattedCitation" : "[42\u201346]", "previouslyFormattedCitation" : "[42\u201346]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . However, *RUNX2/p57* expression dynamics during WJ-MSC OD progress is largely unexplored. To determine endogenous *RUNX2/p57* expression during osteoblastic differentiation at mRNA (Fig 2A) and protein (Fig 2B) levels, WJ-MSCs were cultured for up to 21 days in CT or OB media and samples analyzed by RT-qPCR and western blot, respectively. OB media treatment resulted in a maximal *RUNX2/p57* induction after 4 days of differentiation, declining afterwards (Fig 2A and 2B). This limited expression of *RUNX2/p57* in WJ-MSCs was paralleled by only minor changes in expression of its downstream osteogenic and chondrogenic target genes (Fig S4). These results showed that *RUNX2/p57* is induced only transiently during early stages of WJ-MSC osteogenic commitment without a significant activation of downstream gene targets. This raises the possibility that repressive mechanisms may be restricting *RUNX2/p57* gene activation and therefore diminishing a full engagement with the osteoblastic program. We have previously shown that Runx2/p57 gene expression during osteogenic lineage commitment is tightly controlled by epigenetic mechanisms **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258",

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therapies. However, the molecular mechanisms involved in their osteogenic conversion are poorly understood. Particularly, epigenetic control operating at the promoter regions of the two master regulators of the osteogenic program, RUNX2/P57 and SP7 has not yet been described in WJ-MSCs. Via quantitative PCR profiling and chromatin immunoprecipitation (ChIP) studies here we analyze the ability of WJ-MSCs to engage osteoblast lineage. In undifferentiated WJ-MSCs, RUNX2/P57 P1 and SP7 promoters are found deprived of significant levels of the histone post-translational marks that are normally associated with transcriptionally active genes (H3ac, H3K27ac and H3K4me3). Moreover, the RUNX2 P1 promoter lacks two relevant histone repressive marks (H3K9me3 and H3K27me3). Importantly, RUNX2 P1 promoter is found highly enriched in the H3K4me1 mark, which has been shown recently to mediate gene repression of key regulatory genes. Upon induction of WJ-MSCs osteogenic differentiation, we found that RUNX2/P57, but not SP7 gene expression is strongly activated, in a process that is accompanied by enrichment of activating histone marks (H3K4me3, H3ac, and H3K27ac) at the P1 promoter region. Histone mark analysis showed that SP7 gene promoter is robustly enriched in epigenetic repressive marks that may explain its poor transcriptional response to osteoblast differentiating media. Together these results point to critical regulatory steps during epigenetic control of WJ-MSCs osteogenic lineage commitment that are relevant for future applications in regenerative medicine. ", "author" : [{ "dropping-particle" : "", "family" : "Sepulveda", "given" : "Hugo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" :

"", "family" : "Bustos", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aedo", "given" : "S\u00f3crates", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Veronica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-2", "issued" : { "date-parts" : [["2016", "9", "30"]] }, "title" : "Epigenetic Signatures at the RUNX2-P1 and Sp7 Gene Promoters Control Osteogenic Lineage Commitment of Umbilical Cord-Derived Mesenchymal Stem Cells.", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4a544d50-d112-39c5-892a-27c2d48f44cd"] }], "mendeley" : { "formattedCitation" : "[20, 47]", "plainTextFormattedCitation" : "[20, 47]", "previouslyFormattedCitation" : "[20, 47]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. To address this possibility in WJ-MSCs differentiation, we analyzed the enrichment of the activating histone marks H3K4me3 and H3K27Ac at the *RUNX2* P1 promoter by chromatin immunoprecipitation (ChIP) (Fig 2C-D). As a control, we also analyzed the enrichment of these marks at the active *GAPDH* promoter (Fig 2C-D, right). Significant enrichment of both H3K4me3 and H3K27Ac marks were detected at the *RUNX2* P1 promoter in WJ-MSCs following incubation with OB media for 2 days (Fig 2C-D, left). However, an important reduction of H3K4me3 at this promoter was detected from 4 days

onwards, consistent with the *RUNX2/p57* reduction observed at mRNA and protein levels (Fig 2A-B). As previous reports had demonstrated that deposition of H3K4me3 at *RUNX2* P1 promoter is a key regulatory step during *RUNX2/p57* gene activation { ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Rojas", "given" : "Adriana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez", "given" : "Berta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Gary S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J.", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Allende", "given" : "Miguel L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["2015"]] }, "page" : "jbc.M115.657825", "title" : "Epigenetic Control of the Bone-master Runx2 Gene During Osteoblast-lineage Commitment by the Histone Demethylase JARID1B/KDM5B", "type" : "article-journal" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=062295d0-a558-4179-ada0-1334ee8ab402"] }], "mendeley" : { "formattedCitation" : "[47]", "plainTextFormattedCitation" : "[47]", "previouslyFormattedCitation" : "[47]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** , the results in WJ-MSCs suggest that a repressive mechanism involving the removal of this activating epigenetic mark may be operating at the RUNX2 P1 promoter.

The epigenetic repressor JARID1B is enriched at the RUNX2 promoter in WJ-MSC

We previously reported that JARID1B functions as a brake for *Runx2/p57* expression in murine MSC cell lines, reducing the H3K4me3 levels at the *Runx2* P1 promoter **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Rojas", "given" : "Adriana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez", "given" : "Berta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Gary S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J.", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle"

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Although there is an intrinsic WJ-MSCs heterogeneity that is inherent to these human primary cultures, we neither detect important differences in *JARID1B* mRNA (Fig 3F) nor in JARID1B protein (Fig 3G) expression among several WJ-MSCs biological replicates. Together, this evidence suggests that a JARID1B-mediated epigenetic downregulation of *RUNX2/p57* expression is conserved in human WJ-MSCs.

JARID1B loss of function results in enhanced expression of the bone-master gene *RUNX2/p57* in WJ-MSCs.

As we found that RUNX2 is a critical target to enhance the commitment of WJ-MSC to the osteogenic lineage, it became relevant to manipulate JARID1B function, by either inhibiting its catalytic activity or by reducing its expression levels. We first inhibited the activity of the JARID1B enzyme in WJ-MSCs using the recently described epi-drug PBIT which selectively inhibits this histone demethylase **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M112.419861", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Sayegh", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cao", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zou", "given" : "M. R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morales", "given" : "A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Blair", "given" : "L. P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Norcia", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hoyer", "given" : "D.", "non-

dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tackett", "given" : "a. J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Merkel", "given" : "J. S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yan", "given" : "Q.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "13", "issued" : { "date-parts" : [["2013"]] }, "page" : "9408-9417", "title" : "Identification of Small Molecule Inhibitors of Jumonji AT-rich Interactive Domain 1B (JARID1B) Histone Demethylase by a Sensitive High Throughput Screen", "type" : "article-journal", "volume" : "288" }, "uris" : ["http://www.mendeley.com/documents/?uuid=1114722f-bf90-481b-bdb5-095d7ccbf4fb"] }], "mendeley" : { "formattedCitation" : "[37]", "plainTextFormattedCitation" : "[37]", "previouslyFormattedCitation" : "[37]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}**. Two days of culturing WJ-MSCs in the presence of PBIT, resulted in globally increased levels of the H3K4me3 histone mark (Fig 4A), indicating a broad effect in JARID1B dependent histone modification. Next, we analyzed the effect of PBIT on *RUNX2/p57* mRNA expression in cells grown in the presence of control or OB media. We found that PBIT treatment resulted in a significant increase in *RUNX2/p57* mRNA (Fig 4B). Moreover, this enhanced *RUNX2/p57* expression was paralleled by significant enrichments of H3K4me3 at the RUNX2 P1 promoter (Fig 4C). Interestingly, these changes were not associated with significant variations of H3K27Ac enrichment at this P1 promoter (Fig 4D), another key epigenetic mark associated with activation of *RUNX2/p57* gene expression **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" :

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results further demonstrate that H3K4me3 is directly associated with *RUNX2/p57* gene expression and that JARID1B-activity contributes to control the levels of this histone mark at the RUNX2 P1 promoter in WJ-MSCs.

We next analyzed the effect of *JARID1B* silencing using lentiviral particles carrying shRNAs against this gene (shJARID1B). Transduction of shJARID1B in WJ-MSCs resulted in reduced JARID1B mRNA and protein expression (Fig 5A left and right, respectively) relative to cells infected with control shRNAs (shCtrl). After 48 hours of incubation in the presence of control or OB media, infected cells retained the reduced levels of JARID1B (Fig 5B). Importantly, JARID1B-knockdown resulted in a significant increase in *RUNX2/p57* mRNA expression only in WJ-MSCs grown in OB media (Fig 5C). ChIP analysis revealed that this RUNX2/p57 gene activation was accompanied with increased H3K4me3 and H3K27Ac at RUNX2 P1 promoter (Fig 5D and 5E, respectively). In agreement with previous reports **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Rojas", "given" : "Adriana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez", "given" : "Berta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Gary S.", "non-dropping-particle" : "", "parse-names" :

false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J.", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Allende", "given" : "Miguel L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["2015"]] }, "page" : "jbc.M115.657825", "title" : "Epigenetic Control of the Bone-master Runx2 Gene During Osteoblast-lineage Commitment by the Histone Demethylase JARID1B/KDM5B", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=062295d0-a558-4179-ada0-1334ee8ab402"] }], "mendeley" : { "formattedCitation" : "[47]", "plainTextFormattedCitation" : "[47]", "previouslyFormattedCitation" : "[47]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** these results further demonstrate that the release of JARID1B from the P1 promoter results in enhanced activity/accessibility of the histone acetylation machinery that deposits H3K27Ac at RUNX2 P1 promoter and enhances RUNX2 transcription.

Taken together, our pharmacological and gene-silencing strategies consistently showed that JARID1B loss of function results in increased *RUNX2/p57* transcription in WJ-MSC. This epigenetics-mediated enhancement of endogenous *RUNX2/p57* expression may lay the foundations for increasing the osteoblastic potential of WJ-MSC for bone tissue engineering.

DISCUSSION

Bone tissue engineering has emerged as a valuable approach for tissue replacement upon massive bone injury. Bone tissue engineering requires a cell source and also a precise knowledge of molecular mechanisms governing OD. We and others propose WJ-MSC as convenient cell sources for these purposes **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.4252/wjsc.v7.i1.149", "ISSN" : "1948-0210", "PMID" : "25621114", "abstract" : "In facing the mounting clinical challenge and suboptimal techniques of craniofacial bone defects resulting from various conditions, such as congenital malformations, osteomyelitis, trauma and tumor resection, the ongoing research of regenerative medicine using stem cells and concurrent advancement in biotechnology have shifted the focus from surgical reconstruction to a novel stem cell-based tissue engineering strategy for customized and functional craniofacial bone regeneration. Given the unique ontogenetical and cell biological properties of perinatal stem cells, emerging evidence has suggested these extraembryonic tissue-derived stem cells to be a promising cell source for extensive use in regenerative medicine and tissue engineering. In this review, we summarize the current achievements and obstacles in stem cell-based craniofacial bone regeneration and subsequently we address the characteristics of various types of perinatal stem cells and their novel application in tissue engineering of craniofacial bone. We propose the promising feasibility and scope of perinatal stem cell-based craniofacial bone tissue engineering for future clinical application.", "author" : [{ "dropping-particle" : "", "family" : "Si", "given" : "Jia-Wen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wang", "given" : "Xu-Dong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

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in EU and 131 million worldwide give a unique opportunity to collect lifesaving Wharton’s jelly derived mesenchymal stem cells (WJ-MSC). Evidences that these cells possess therapeutic properties are constantly accumulating. Collection of WJ-MSC is done at the time of delivery and it is easy and devoid of side effects associated with collection of adult stem cells from bone marrow or adipose tissue. Likewise, their rate of proliferation, immune privileged status, lack of ethical concerns, nontumorigenic properties make them ideal for both autologous and allogeneic use in regenerative medicine applications. This review provides an outline of the recent findings related to WJ-MSC therapeutic effects and possible advantage they possess over MSC from other sources. Results of first clinical trials conducted to treat immune disorders are highlighted.", "author" : [{ "dropping-particle" : "", "family" : "Kalaszczynska", "given" : "Ilona", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ferdyn", "given" : "Katarzyna", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "BioMed Research International", "id" : "ITEM-3", "issue" : "MARCH", "issued" : { "date-parts" : [["2015"]] }, "page" : "1-11", "title" : "Wharton\u2019s Jelly Derived Mesenchymal Stem Cells: Future of Regenerative Medicine? Recent Findings and Clinical Significance", "type" : "article-journal", "volume" : "2015" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b05cc19b-11a7-41d8-88c3-b2d808863eb0"] }], "mendeley" : { "formattedCitation" : "[11, 17, 48]", "plainTextFormattedCitation" : "[11, 17, 48]", "previouslyFormattedCitation" : "[11, 17, 48]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Because WJ-MSCs have a restricted bone potential, we demonstrate a regulatory pathway that may set the basis to overcome the intrinsic limitations of these cells to engage osteoblast differentiation.

RUNX2 transcription factor can direct WJ-MSCs towards osteoblast lineage commitment.

Our experiments demonstrate that *RUNX2/p57* overexpression is capable to induce the WJ-MSCs into osteoblast committed cells. RUNX2 overexpression in WJ-MSCs confirms the key role of this gene in promoting the OD program. Engagement of these cells is reflected by the expression of early, but not late, osteoblastic markers. These results are in line with previous data showing that long-lasting overexpression of RUNX2 inhibits osteoblast maturation **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1083/jcb.200105052", "ISSN" : "0021-9525", "PMID" : "11581292", "abstract" : "Targeted disruption of core binding factor alpha1 (Cbfa1) showed that Cbfa1 is an essential transcription factor in osteoblast differentiation and bone formation. Furthermore, both in vitro and in vivo studies showed that Cbfa1 plays important roles in matrix production and mineralization. However, it remains to be clarified how Cbfa1 controls osteoblast differentiation, bone formation, and bone remodelling. To understand fully the physiological functions of Cbfa1, we generated transgenic mice that overexpressed Cbfa1 in osteoblasts using type I collagen promoter. Unexpectedly, Cbfa1 transgenic mice showed osteopenia with multiple fractures. Cortical bone, which was thin, porous, and enriched with osteopontin, was invaded by osteoclasts, despite the absence of acceleration of osteoclastogenesis. Although the number of neonatal osteoblasts was increased, their function was impaired in matrix production and mineralization. Furthermore, terminally differentiated osteoblasts, which strongly express osteocalcin, and osteocytes were diminished greatly, whereas less mature osteoblasts expressing osteopontin accumulated in adult bone. These data indicate that immature organization of cortical bone, which was

caused by the maturational blockage of osteoblasts, led to osteopenia and fragility in transgenic mice, demonstrating that Cbfa1 inhibits osteoblast differentiation at a late stage.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Toyosawa", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Furuichi", "given" : "T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kanatani", "given" : "N", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yoshida", "given" : "C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Liu", "given" : "Y", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Himeno", "given" : "M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Narai", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yamaguchi", "given" : "A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Komori", "given" : "T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of cell biology", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2001", "10", "1"]] }, "page" : "157-66", "title" : "Overexpression of Cbfa1 in osteoblasts inhibits osteoblast maturation and causes osteopenia with multiple fractures.", "type" : "article-journal", "volume" : "155" }, "uris" : ["http://www.mendeley.com/documents/?uuid=328b30da-cdf2-3c17-b59b-659cfbe8135e"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1002/dvdy.21187", "ISSN" : "1058-8388", "PMID" : "17497678", "abstract" : "Runx2 is an essential transcription factor for osteoblast

differentiation. However, the functions of Runx2 in postnatal bone development remain to be clarified. Introduction of dominant-negative (dn)-Runx2 did not inhibit Col1a1 and osteocalcin expression in mature osteoblastic cells. In transgenic mice that expressed dn-Runx2 in osteoblasts, the trabecular bone had increased mineralization, increased volume, and features of compact bone, and the expression of major bone matrix protein genes was relatively maintained. After ovariectomy, neither osteolysis nor bone formation was enhanced and bone was relatively conserved. In wild-type mice, Runx2 was strongly expressed in immature osteoblasts but downregulated during osteoblast maturation. These findings indicate that the maturity and turnover rate of bone are determined by the level of functional Runx2 and Runx2 is responsible for bone loss in estrogen deficiency, but that Runx2 is not essential for maintenance of the expression of major bone matrix protein genes in postnatal bone development and maintenance.", "author" : [{ "dropping-particle" : "", "family" : "Maruyama", "given" : "Zenjiro", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yoshida", "given" : "Carolina A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Furuichi", "given" : "Tatsuya", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Amizuka", "given" : "Norio", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ito", "given" : "Masako", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fukuyama", "given" : "Ryo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Miyazaki", "given" : "Toshihiro", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kitaura", "given" : "Hideki", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" :

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differentiation program {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1128/MCB.24.20.9248-9261.2004", "ISSN" : "0270-7306", "PMID" : "15456894", "abstract" : "Genetic studies show that Msx2 and Dlx5 homeodomain (HD) proteins support skeletal development, but null mutation of the closely related Dlx3 gene results in early embryonic lethality. Here we find that expression of Dlx3 in the mouse embryo is associated with new bone formation and regulation of osteoblast differentiation. Dlx3 is expressed in osteoblasts, and overexpression of Dlx3 in osteoprogenitor cells promotes, while specific knock-down of Dlx3 by RNA interference inhibits, induction of osteogenic markers. We characterized gene regulation by Dlx3 in relation to that of Msx2 and Dlx5 during osteoblast differentiation. Chromatin immunoprecipitation assays revealed a molecular switch in HD protein association with the bone-specific osteocalcin (OC) gene. The transcriptionally repressed OC gene was occupied by Msx2 in proliferating osteoblasts, while Dlx3, Dlx5, and Runx2 were recruited postproliferatively to initiate transcription. Dlx5 occupancy increased over Dlx3 in mature osteoblasts at the mineralization stage of differentiation, coincident with increased RNA polymerase II occupancy. Dlx3 protein-DNA interactions stimulated OC promoter activity, while Dlx3-Runx2 protein-protein interaction reduced Runx2-mediated transcription. Deletion analysis showed that the Dlx3 interacting domain of Runx2 is from amino acids 376 to 432, which also include the transcriptionally active subnuclear targeting sequence (376 to 432). Thus, we provide cellular and molecular evidence for Dlx3 in regulating osteoprogenitor cell differentiation and for both positive and negative regulation of gene transcription. We propose that multiple HD proteins in osteoblasts constitute a regulatory network that mediates development of the bone phenotype through the sequential association of distinct HD proteins with promoter regulatory elements.", "author" : [{

"dropping-particle" : "", "family" : "Hassan", "given" : "Mohammad Q", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Javed", "given" : "Amjad", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morasso", "given" : "Maria I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Karlin", "given" : "Jeremy", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Gary S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular and cellular biology", "id" : "ITEM-1", "issue" : "20", "issued" : { "date-parts" : [["2004", "10"]] }, "page" : "9248-61", "publisher" : "American Society for Microbiology (ASM)", "title" : "Dlx3 transcriptional regulation of osteoblast differentiation: temporal recruitment of Msx2, Dlx3, and Dlx5 homeodomain proteins to chromatin of the osteocalcin gene.", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9ab886f9-8af3-3309-bb88-440f8699f819"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1210/mend.11.11.0011", "ISSN" : "0888-8809", "PMID" : "9328350", "abstract" : "Two homeotic genes, Dlx and Msx, appear to regulate development of mineralized tissues, including bone, cartilage, and tooth. Expression of Msx-1 and Msx-2 has been studied during development of the osteoblast

phenotype, but the role of Dlx in this context and in the regulation of bone-expressed genes is unknown. We used targeted differential display to isolate homeotic genes of the Dlx family that are expressed at defined stages of osteoblast differentiation. These studies were carried out with fetal rat calvarial cells that produce bone-like tissue in vitro. We observed a mineralization stage-specific mRNA and cloned the corresponding cDNA, which represents the rat homolog of Dlx-5. Northern blot analysis and competitive RT-PCR demonstrated that Dlx-5 and the bone-specific osteocalcin genes exhibit similar up-regulated expression during the mineralization period of osteoblast differentiation. This expression pattern differs from that of Msx-2, which is found predominantly in proliferating osteoblasts. Several approaches were pursued to determine functional consequences of Dlx-5 expression on osteocalcin transcription. Constitutive expression of Dlx-5 in ROS 17/2.8 cells decreased osteocalcin promoter activity in transient assays, and conditional expression of Dlx-5 in stable cell lines reduced endogenous mRNA levels. Consistent with this finding, antisense inhibition of Dlx-5 increased osteocalcin gene transcription. Osteocalcin promoter deletion analysis and binding of the in vitro translation product of Dlx-5 demonstrated that repressor activity was targeted to a single homeodomain-binding site, located in OC-Box I (-99 to -76). These findings demonstrate that Dlx-5 represses osteocalcin gene transcription. However, the coupling of increased Dlx-5 expression with progression of osteoblast differentiation suggests an important role in promoting expression of the mature bone cell phenotype.", "author" : [{ "dropping-particle" : "", "family" : "Ryoo", "given" : "H. M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hoffmann", "given" : "H. M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Beumer", "given" : "T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : ""

}, { "dropping-particle" : "", "family" : "Frenkel", "given" : "B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Towler", "given" : "D. A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "G. S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "J. L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "A. J.", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "J. B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular Endocrinology", "id" : "ITEM-2", "issue" : "11", "issued" : { "date-parts" : [["1997", "10"]] }, "page" : "1681-1694", "title" : "Stage-Specific Expression of Dlx-5 during Osteoblast Differentiation: Involvement in Regulation of Osteocalcin Gene Expression", "type" : "article-journal", "volume" : "11" }, "uris" : ["http://www.mendeley.com/documents/?uuid=03eba2e7-1dba-310d-85d7-2e356dee4d41"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1074/jbc.M502267200", "ISSN" : "0021-9258", "PMID" : "16115867", "abstract" : "Two major isoforms of the Runx2 gene are expressed by alternative promoter usage: Runx2 type I (Runx2-I) is derived from the proximal promoter (P2), and Runx2 type II (Runx2-II) is produced by the distal promoter (P1). Our previous results indicate that Dlx5 mediates BMP-2-induced Runx2 expression and osteoblast differentiation (Lee, M.-H., Kim, Y-J., Kim, H-J., Park, H-D., Kang, A-R., Kyung, H.-M., Sung, J-H., Wozney, J. M., Kim, H-J., and Ryoo, H-M. (2003) J. Biol. Chem. 278, 34387-34394). However, little is known of the molecular mechanisms by which Dlx5 up-regulates Runx2 expression in BMP-2 signaling. Here, Runx2-II expression was found to be specifically stimulated by BMP-2 treatment or by Dlx5 overexpression. In

addition, BMP-2, Dlx5, and Runx2-II were found to be expressed in osteogenic fronts and parietal bones of the developing cranial vault and Runx2-I and Msx2 in the sutural mesenchyme. Furthermore, Runx2 P1 promoter activity was strongly stimulated by Dlx5 overexpression, whereas Runx2 P2 promoter activity was not. Runx2 P1 promoter deletion analysis indicated that the Dlx5-specific response is due to sequences between -756 and -342 bp of the P1 promoter, where three Dlx5-response elements are located. Dlx5 responsiveness to these elements was confirmed by gel mobility shift assay and site-directed mutagenesis. Moreover, Msx2 specifically suppressed the Runx2 P1 promoter, and the responsible region overlaps with that recognized by Dlx5. In summary, Dlx5 specifically transactivates the Runx2 P1 promoter, and its action on the P1 promoter is antagonized by Msx2.", "author" : [{ "dropping-particle" : "", "family" : "Lee", "given" : "Mi-Hye", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kim", "given" : "Youn-Jeong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yoon", "given" : "Won-Joon", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kim", "given" : "Jee-In", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kim", "given" : "Byung-Gyu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hwang", "given" : "Yoo-Seok", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wozney", "given" : "John M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chi", "given" : "Xin-Zi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bae", "given" : "Suk-Chul", "non-dropping-particle" : "", "parse-names" : false, "suffix" :

"" }, { "dropping-particle" : "", "family" : "Choi", "given" : "Kang-Young", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cho", "given" : "Je-Yoel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Choi", "given" : "Je-Yong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ryoo", "given" : "Hyun-Mo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of biological chemistry", "id" : "ITEM-3", "issue" : "42", "issued" : { "date-parts" : [["2005", "10", "21"]] }, "page" : "35579-87", "title" : "Dlx5 specifically regulates Runx2 type II expression by binding to homeodomain-response elements in the Runx2 distal promoter.", "type" : "article-journal", "volume" : "280" }, "uris" : ["http://www.mendeley.com/documents/?uuid=2ed20a97-5724-4736-9715-fbeeadc1c309"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1089/ten.TEB.2012.0527", "ISBN" : "1937-3376 (Electronic)\\r1937-3368 (Linking)", "ISSN" : "1937-3376", "PMID" : "23150948", "abstract" : "The development of the musculoskeletal system is a complex process that involves very precise control of bone formation and growth as well as remodeling during postnatal life. Although the understanding of the transcriptional mechanisms of osteogenesis has increased considerably, the molecular regulatory basis, especially the gene regulatory network of osteogenic differentiation, is still poorly understood. This review provides the reader with an overview of the key transcription factors that govern bone formation, highlighting their function and regulation linked to Runt-related transcription factor 2 (Runx2). Runx2 as the master transcription factor of osteoblast differentiation, Twist, Msh homeobox 2 (Msx2), and promyelocytic leukemia zinc-finger protein (PLZF) acting upstream of Runx2, Osterix (Osx) acting downstream of Runx2, and activating transcription factor 4 (ATF4) and zinc-

finger protein 521 (ZFP521) acting as cofactors of Runx2 are discussed, and their relevance for tissue engineering is presented. References are provided for more in-depth personal study.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Tong Ming", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "Eng Hin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Tissue engineering. Part B, Reviews", "id" : "ITEM-4", "issue" : "3", "issued" : { "date-parts" : [["2013"]] }, "page" : "254-63", "title" : "Transcriptional regulatory cascades in Runx2-dependent bone development.", "type" : "article-journal", "volume" : "19" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ff0b15ee-bdc3-441a-9776-71f48b854f3e"] }], "mendeley" : { "formattedCitation" : "[51\u201354]", "plainTextFormattedCitation" : "[51\u201354]", "previouslyFormattedCitation" : "[51\u201354]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

RUNX2 and JARID1B during WJ-MSC osteoblast differentiation

Due to the pivotal role played by RUNX2 in OD we aimed to increase the endogenous RUNX2 expression in WJ-MSC. Nevertheless, we determined that specific restrictions in WJ-MSC will still need to be overcome to further enhance their terminal osteoblast differentiation potential. Despite to this limited acquisition of the osteoblastic gene expression program in WJ-MSC, late OD hallmarks have been detected in a small percentage of the cell populations by staining methods **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1007/s10456-014-9432-7", "ISSN" : "1573-7209", "PMID" : "24728929", "abstract" : "Disorders in skin wound

healing are a major health problem that requires the development of innovative treatments. The use of biomaterials as an alternative of skin replacement has become relevant, but its use is still limited due to poor vascularization inside the scaffolds, resulting in insufficient oxygen and growth factors at the wound site. In this study, we have developed a cell-based wound therapy consisting of the application of collagen-based dermal scaffolds containing mesenchymal stem cells from Wharton's jelly (WJ-MSC) in an immunocompetent mouse model of angiogenesis. From our comparative study on the secretion profile between WJ-MSC and adipose tissue-derived MSC, we found a stronger expression of several well-characterized growth factors, such as VEGF-A, angiopoietin-1 and aFGF, which are directly linked to angiogenesis, in the culture supernatant of WJ-MSC, both on monolayer and 3D culture conditions. WJ-MSC proved to be angiogenic both in vitro and in vivo, through tubule formation and CAM assays, respectively. Moreover, WJ-MSC consistently improved the healing response in vivo in a mouse model of human-like dermal repair, by triggering angiogenesis and further providing a suitable matrix for wound repair, without altering the inflammatory response in the animals. Since these cells can be easily isolated, cultured with high expansion rates and cryopreserved, they represent an attractive stem cell source for their use in allogeneic cell transplant and tissue engineering.", "author" : [{ "dropping-particle" : "", "family" : "Edwards", "given" : "Sandra S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zavala", "given" : "Gabriela", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Elliott", "given" : "Mat\u00edas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mart\u00ednez", "given" : "Samuel",

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osteoblast lineage. In undifferentiated WJ-MSCs, RUNX2/P57 P1 and SP7 promoters are found deprived of significant levels of the histone post-translational marks that are normally associated with transcriptionally active genes (H3ac, H3K27ac and H3K4me3). Moreover, the RUNX2 P1 promoter lacks two relevant histone repressive marks (H3K9me3 and H3K27me3). Importantly, RUNX2 P1 promoter is found highly enriched in the H3K4me1 mark, which has been shown recently to mediate gene repression of key regulatory genes. Upon induction of WJ-MSCs osteogenic differentiation, we found that RUNX2/P57, but not SP7 gene expression is strongly activated, in a process that is accompanied by enrichment of activating histone marks (H3K4me3, H3ac, and H3K27ac) at the P1 promoter region. Histone mark analysis showed that SP7 gene promoter is robustly enriched in epigenetic repressive marks that may explain its poor transcriptional response to osteoblast differentiating media. Together these results point to critical regulatory steps during epigenetic control of WJ-MSCs osteogenic lineage commitment that are relevant for future applications in regenerative medicine. This article is protected by copyright. All rights reserved.", "author" : [{ "dropping-particle" : "", "family" : "Sepulveda", "given" : "Hugo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bustos", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aedo", "given" : "S\u00f3crates", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "",

"family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Veronica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "9", "30"]] }, "title" : "Epigenetic Signatures at the RUNX2-P1 and Sp7 Gene Promoters Control Osteogenic Lineage Commitment of Umbilical Cord-Derived Mesenchymal Stem Cells.", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4a544d50-d112-39c5-892a-27c2d48f44cd"] }], "mendeley" : { "formattedCitation" : "[20]", "plainTextFormattedCitation" : "[20]", "previouslyFormattedCitation" : "[20]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Alternatively, these results may be indicating that there is a small subpopulation of cells within our WJ-MSC primary cultures that are less refractory to upregulate the expression of these osteogenic master regulators in OB growing conditions. Future studies will be required to address these possibilities.

Previously, we demonstrated that the *RUNX2* P1 promoter does not contain repressive histone marks (H3K9me3 and H3K27me3) in WJ-MSCs **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcp.25627", "ISSN" : "1097-4652", "PMID" : "27689934", "abstract" : "Wharton's Jelly mesenchymal stem cells (WJ-MSCs) are an attractive potential source of multipotent stem cells for bone tissue replacement therapies. However, the molecular mechanisms involved in their osteogenic conversion are poorly understood. Particularly, epigenetic control operating at the promoter

regions of the two master regulators of the osteogenic program, RUNX2/P57 and SP7 has not yet been described in WJ-MSCs. Via quantitative PCR profiling and chromatin immunoprecipitation (ChIP) studies here we analyze the ability of WJ-MSCs to engage osteoblast lineage. In undifferentiated WJ-MSCs, RUNX2/P57 P1 and SP7 promoters are found deprived of significant levels of the histone post-translational marks that are normally associated with transcriptionally active genes (H3ac, H3K27ac and H3K4me3). Moreover, the RUNX2 P1 promoter lacks two relevant histone repressive marks (H3K9me3 and H3K27me3). Importantly, RUNX2 P1 promoter is found highly enriched in the H3K4me1 mark, which has been shown recently to mediate gene repression of key regulatory genes. Upon induction of WJ-MSCs osteogenic differentiation, we found that RUNX2/P57, but not SP7 gene expression is strongly activated, in a process that is accompanied by enrichment of activating histone marks (H3K4me3, H3ac, and H3K27ac) at the P1 promoter region. Histone mark analysis showed that SP7 gene promoter is robustly enriched in epigenetic repressive marks that may explain its poor transcriptional response to osteoblast differentiating media. Together these results point to critical regulatory steps during epigenetic control of WJ-MSCs osteogenic lineage commitment that are relevant for future applications in regenerative medicine. ", "author" : [{ "dropping-particle" : "", "family" : "Sepulveda", "given" : "Hugo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Prieto", "given" : "Catalina P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bustos", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" :

"Aedo", "given" : "S\u00f3crates", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Veronica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "9", "30"]] }, "title" : "Epigenetic Signatures at the RUNX2-P1 and Sp7 Gene Promoters Control Osteogenic Lineage Commitment of Umbilical Cord-Derived Mesenchymal Stem Cells.", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4a544d50-d112-39c5-892a-27c2d48f44cd"] }], "mendeley" : { "formattedCitation" : "[20]", "plainTextFormattedCitation" : "[20]", "previouslyFormattedCitation" : "[20]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Moreover, following 48 h of OD stimulation, significant *RUNX2/p57* mRNA can be detected and associated with enrichment of activating histone modifications (H3K4me3 and H3K27Ac) at RUNX2 P1 promoter **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcp.25627", "ISSN" : "1097-4652", "PMID" : "27689934", "abstract" : "Wharton's Jelly mesenchymal stem cells (WJ-MSCs) are an attractive potential source of multipotent stem cells for bone tissue replacement therapies. However, the molecular mechanisms involved in their osteogenic conversion are poorly understood. Particularly, epigenetic control operating at the promoter regions of the two master regulators of the osteogenic program, RUNX2/P57 and SP7 has not yet been

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"dropping-particle" : "", "family" : "Lattus", "given" : "Jos\u00e9", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palma", "given" : "Veronica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of cellular physiology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "9", "30"]] }, "title" : "Epigenetic Signatures at the RUNX2-P1 and Sp7 Gene Promoters Control Osteogenic Lineage Commitment of Umbilical Cord-Derived Mesenchymal Stem Cells.", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4a544d50-d112-39c5-892a-27c2d48f44cd"] }], "mendeley" : { "formattedCitation" : "[20]", "plainTextFormattedCitation" : "[20]", "previouslyFormattedCitation" : "[20]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Here we show that this *RUNX2/p57* gene activation is only transient and correlated with transitory H3K4me3 enrichment. This impairment of WJ-MSCs to acquire a stable active configuration of RUNX2 P1 promoter and the absence of classical repressive histone marks on this promoter is correlated with repression of RUNX2 expression in these cells. As described previously in murine mesenchymal cells **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Rojas", "given" : "Adriana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez",

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(Electronic)\\r0261-4189 (Linking)", "ISSN" : "1460-2075", "PMID" : "22020125", "abstract" : "H3K4 methylation is associated with active transcription and in combination with H3K27me3 thought to keep genes regulating development in a poised state. The contribution of enzymes regulating trimethylation of lysine 4 at histone 3 (H3K4me3) levels to embryonic stem cell (ESC) self-renewal and differentiation is just starting to emerge. Here, we show that the H3K4me2/3 histone demethylase Jarid1b (Kdm5b/Plu1) is dispensable for ESC self-renewal, but essential for ESC differentiation along the neural lineage. By genome-wide location analysis, we demonstrate that Jarid1b localizes predominantly to transcription start sites of genes encoding developmental regulators, of which more than half are also bound by Polycomb group proteins. Virtually all Jarid1b target genes are associated with H3K4me3 and depletion of Jarid1b in ESCs leads to a global increase of H3K4me3 levels. During neural differentiation, Jarid1b-depleted ESCs fail to efficiently silence lineage-inappropriate genes, specifically stem and germ cell genes. Our results delineate an essential role for Jarid1b-mediated transcriptional control during ESC differentiation.", "author" : [{ "dropping-particle" : "", "family" : "Schmitz", "given" : "Sandra U", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Albert", "given" : "Mareike", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Malatesta", "given" : "Martina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morey", "given" : "Lluis", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "V", "family" : "Johansen", "given" : "Jens", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bak", "given" : "Mads", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tommerup", "given" :

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(EB) formation, showed that KDM5b reduced the terminally differentiated cells and increased proliferating progenitors. These were achieved by two mechanisms, blocking of the upregulation of cell lineage markers and maintenance of cyclins, that allowed cells to escape differentiation and remain uncommitted. Additionally, EBs maintain high levels of Oct4 and Nanog and can be dissociated to reestablish highly proliferative cultures. The persistence of uncommitted progenitors may be due to the direct regulation of the Tcf/Lef family member mTcf3/hTcf7L1, an upstream regulator of Nanog expression. These findings demonstrate a role for KDM5b in the choice between proliferation and differentiation during development.", "author" : [{ "dropping-particle" : "", "family" : "Dey", "given" : "Bijan K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stalker", "given" : "Leanne", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schnerch", "given" : "Angelique", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bhatia", "given" : "Mickie", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Taylor-Papidimitriou", "given" : "Joyce", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wynder", "given" : "Christopher", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular and cellular biology", "id" : "ITEM-3", "issue" : "17", "issued" : { "date-parts" : [["2008", "9", "1"]] }, "page" : "5312-27", "title" : "The histone demethylase KDM5b/JARID1b plays a role in cell fate decisions by blocking terminal differentiation.", "type" : "article-journal", "volume" : "28" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e602ff29-3b77-3b9f-96be-75d4025efd78"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1128/MCB.00692-13", "ISSN" : "1098-

5549", "PMID" : "24100015", "abstract" : "Embryonic stem (ES) cell pluripotency is thought to be regulated in part by H3K4 methylation. However, it is unclear how H3K4 demethylation contributes to ES cell function and participates in induced pluripotent stem (iPS) cell reprogramming. Here, we show that KDM5B, which demethylates H3K4, is important for ES cell differentiation and presents a barrier to the reprogramming process. Depletion of Kdm5b leads to an extension in the self-renewal of ES cells in the absence of LIF. Transcriptome analysis revealed the persistent expression of pluripotency genes and underexpression of developmental genes during differentiation in the absence of Kdm5b, suggesting that KDM5B plays a key role in cellular fate changes. We also observed accelerated reprogramming of differentiated cells in the absence of Kdm5b, demonstrating that KDM5B is a barrier to the reprogramming process. Expression analysis revealed that mesenchymal master regulators associated with the epithelial-to-mesenchymal transition (EMT) are downregulated during reprogramming in the absence of Kdm5b. Moreover, global analysis of H3K4me3/2 revealed that enhancers of fibroblast genes are rapidly deactivated in the absence of Kdm5b, and genes associated with EMT lose H3K4me3/2 during the early reprogramming process. These findings provide functional insight into the role for KDM5B in regulating ES cell differentiation and as a barrier to the reprogramming process.", "author" : [{ "dropping-particle" : "", "family" : "Kidder", "given" : "Benjamin L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Gangqing", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yu", "given" : "Zu-Xi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Liu", "given" : "Chengyu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhao", "given" : "Keji", "non-

dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular and cellular biology", "id" : "ITEM-4", "issue" : "24", "issued" : { "date-parts" : [["2013", "12", "15"]] }, "page" : "4793-810", "title" : "Extended self-renewal and accelerated reprogramming in the absence of Kdm5b.", "type" : "article-journal", "volume" : "33" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ca525fc2-b921-37c3-af19-3b54dee2c2cb"] }, { "id" : "ITEM-5", "itemData" : { "DOI" : "10.1182/blood-2013-04-496281", "ISSN" : "0006-4971", "PMID" : "23777767", "abstract" : "Histone methylation is a dynamic and reversible process proposed to directly impact on stem cell fate. The Jumonji (JmjC) domain-containing family of demethylases comprises 27 members that target mono-, di-, and trimethylated lysine residues of histone (or nonhistone) proteins. To evaluate their role in regulation of hematopoietic stem cell (HSC) behavior, we performed an in vivo RNAi-based functional screen and demonstrated that Jarid1b and Jhdm1f play opposing roles in regulation of HSC activity. Decrease in Jarid1b levels correlated with an in vitro expansion of HSCs with preserved long-term in vivo lymphomyeloid differentiation potential. Through RNA sequencing analysis, Jarid1b knockdown was associated with increased expression levels of several HSC regulators (Hoxa7, Hoxa9, Hoxa10, Hes1, Gata2) and reduced levels of differentiation-associated genes. shRNA against Jhdmlf, in contrast, impaired hematopoietic reconstitution of bone marrow cells. Together, our studies identified Jarid1b as a negative regulator of HSC activity and Jhdmlf as a positive regulator of HSC activity.", "author" : [{ "dropping-particle" : "", "family" : "Cellot", "given" : "S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hope", "given" : "K. J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chagraoui", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

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language/schema/raw/master/csl-citation.json" }**}**. Moreover, the constitutive JARID1B expression observed in presence of OB medium evidences an elevated concentration of this RUNX2 gene-repressor during WJ-MSCs osteoblast lineage commitment. The correlation found between JARID1B-enrichment at RUNX2 P1 promoter and reduced RUNX2 gene expression supports the existence of a repressive mechanism mediated by JARID1B controlling the RUNX2 P1 promoter in WJ-MSCs.

The use of the epi-drug PBIT to inhibit JARID1B activity further confirmed the functional role of JARID1B over *RUNX2/p57* gene expression. This JARID1B-inhibition also demonstrates that abrogation of JARID1B activity does not affect the H3K27Ac levels suggesting that enrichment of H3K4me3 is not sufficient to upregulate the p300-mediated increase in H3K27Ac at Runx2 P1 promoter that normally accompanies transcriptional activation of this gene **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1074/jbc.M115.657825", "ISSN" : "0021-9258", "author" : [{ "dropping-particle" : "", "family" : "Rojas", "given" : "Adriana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aguilar", "given" : "Rodrigo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Henriquez", "given" : "Berta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lian", "given" : "Jane B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Janet L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stein", "given" : "Gary S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wijnen", "given" : "Andre J.", "non-dropping-particle"

: "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zundert", "given" : "Brigitte", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Allende", "given" : "Miguel L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Montecino", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Biological Chemistry", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["2015"]] }, "page" : "jbc.M115.657825", "title" : "Epigenetic Control of the Bone-master Runx2 Gene During Osteoblast-lineage Commitment by the Histone Demethylase JARID1B/KDM5B", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=062295d0-a558-4179-ada0-1334ee8ab402"] }], "mendeley" : { "formattedCitation" : "[47]", "plainTextFormattedCitation" : "[47]", "previouslyFormattedCitation" : "[47]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Thus, our data suggests that it is JARID1B binding to the P1 promoter and not just inhibition of JARID1B activity that mediates the blockage for p300 recruitment. Thus, it is tempting to speculate that a required condition for a significant *RUNX2/p57* gene activation is the proper release of JARID1B from the P1 promoter. Additionally, because other JARID1 sub-family members have been found regulating MSC lineage commitment **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1186/s12885-015-1798-4", "ISSN" : "1471-2407", "author" : [{ "dropping-particle" : "", "family" : "Ji", "given" : "Xuening", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jin", "given" : "Shi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Qu", "given" : "Xiaotong", "non-

dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Li", "given" : "Kejun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wang", "given" : "Hongjiang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "He", "given" : "Hui", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Guo", "given" : "Fuchao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dong", "given" : "Lei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "BMC Cancer", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2015", "12", "26"]] }, "page" : "801", "title" : "Lysine-specific demethylase 5C promotes hepatocellular carcinoma cell invasion through inhibition BMP7 expression", "type" : "article-journal", "volume" : "15" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a27ace4d-53ce-30db-8e8e-5a1bf55ef088"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/cddis.2016.238", "ISSN" : "2041-4889", "author" : [{ "dropping-particle" : "", "family" : "Wang", "given" : "Chuandong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wang", "given" : "Jing", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Li", "given" : "Jiao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Guoli", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shan", "given" : "Shengzhou", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Li", "given" : "Qingfeng", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhang", "given" : "Xiaoling", "non-dropping-

particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Cell Death and Disease", "id" : "ITEM-2", "issue" : "8", "issued" : { "date-parts" : [["2016", "8", "11"]] }, "page" : "e2335", "publisher" : "Nature Publishing Group", "title" : "KDM5A controls bone morphogenic protein 2-induced osteogenic differentiation of bone mesenchymal stem cells during osteoporosis", "type" : "article-journal", "volume" : "7" }, "uris" : ["http://www.mendeley.com/documents/?uuid=166ada46-8cd5-3975-a3ad-43b1882e595a"] }], "mendeley" : { "formattedCitation" : "[60, 61]", "plainTextFormattedCitation" : "[60, 61]", "previouslyFormattedCitation" : "[60, 61]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, it is possible that RUNX2-repression in WJ-MSCs could also involve the activity of the JARID1A/C proteins. However, it appears that these other two H3K4me3 demethylases are unable to fully compensate lack of JARID1B function in JARID1B null mice **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.3892/ijo.2011.956", "ISSN" : "1019-6439", "PMID" : "21369698", "abstract" : "The four members of the JARID1/KDM5 family of proteins, a sub-group of the larger ARID (AT rich DNA binding domain) family, have been shown to demethylate trimethylated lysine 4 on histone 3 (H3K4me3), a chromatin mark associated with actively transcribed genes. In some lower organisms a single homologue of JARID1 is found, and functions of the four proteins found in mice and humans may be specific or overlapping. To investigate the function of the Jarid1B protein we examined the effects of deletion of the gene in mice. Systemic knock out of Jarid1b resulted in early embryonic lethality, whereas mice not expressing the related Jarid1A gene are viable and fertile. A second mouse strain expressing a Jarid1b gene with the ARID domain deleted was viable and fertile but displayed a mammary phenotype, where terminal end bud development and side branching

was delayed at puberty and in early pregnancy. Since development of terminal end buds are completely dependent on signalling from the estrogen receptor (ER\u03b1), we investigated the expression of a target gene (progesterone receptor) in the \u2206ARID mouse and found levels to be reduced as compared to wild-type. JARID1B is widely expressed in ER+ breast cancers and breast cancer cell lines, and interaction with ER\u03b1 was demonstrated by co-immunoprecipitations in cells transfected with tagged ER\u03b1 and JARID1B genes. Down-regulation of expression of JARID1B using shRNAi in MCF-7 cells resulted in a dramatic decrease in E2 stimulated tumour growth in nude mice. The data demonstrate a specific role for Jarid1B in early embryonic development, in the development and differentiation of the normal mammary gland, and in estrogen induced growth of ER+ breast cancer.", "author" : [{ "dropping-particle" : "", "family" : "Burchell", "given" : "Steven", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Spencer-Dene", "given" : "Bradley", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hall", "given" : "Debbie", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Santangelo", "given" : "Samantha", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosewell", "given" : "Ian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Guenatri", "given" : "Mounia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Beatson", "given" : "Richard", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Scibetta", "given" : "Angelo G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Burchell", "given" : "Joy M", "non-dropping-particle" : "", "parse-names" :

false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Taylor-Papadimitriou", "given" : "Joyce", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "International Journal of Oncology", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["2011", "5", "1"]] }, "page" : "1267-77", "title" : "PLU-1/JARID1B/KDM5B is required for embryonic survival and contributes to cell proliferation in the mammary gland and in ER+ breast cancer cells", "type" : "article-journal", "volume" : "38" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f195ccd3-4ab4-39c7-98e3-b8a1a0e4fca4"] }], "mendeley" : { "formattedCitation" : "[62]", "plainTextFormattedCitation" : "[62]", "previouslyFormattedCitation" : "[62]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Further research will be necessary to formally explore this possibility.

Biomedical significance of an epigenetic-mediated enhancement of RUNX2 expression in WJ-MSC

Epigenetic control of gene expression have emerged as a plausible target to modulate MSC fate for tissue engineering **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1186/s13287-015-0018-0", "ISSN" : "1757-6512", "PMID" : "25890062", "abstract" : "Mesenchymal stem cells (MSCs) hold great promise for therapeutic use in regenerative medicine and tissue engineering. A detailed understanding of the molecular processes governing MSC fate determination will be instrumental in the application of MSCs. Much progress has been made in recent years in defining the epigenetic events that control the differentiation of MSCs into different lineages. A complex network of transcription factors and histone modifiers, in concert with specific

transcriptional co-activators and co-repressors, activates or represses MSC differentiation. In this review, we summarize recent progress in determining the effects of histone-modifying enzymes on the multilineage differentiation of MSCs. In addition, we propose that the manipulation of histone signatures associated with lineage-specific differentiation by small molecules has immense potential for the advancement of MSC-based regenerative medicine.", "author" : [{ "dropping-particle" : "", "family" : "Huang", "given" : "Biao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Li", "given" : "Gang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jiang", "given" : "Xiao Hua", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cell research & therapy", "id" : "ITEM-1", "issued" : { "date-parts" : [["2015", "1"]] }, "page" : "35", "title" : "Fate determination in mesenchymal stem cells: a perspective from histone-modifying enzymes.", "type" : "article-journal", "volume" : "6" }, "uris" : ["http://www.mendeley.com/documents/?uuid=eaf4fdd4-62b2-4cc5-9b81-a4ed12a7ba82"] }], "mendeley" : { "formattedCitation" : "[63]", "plainTextFormattedCitation" : "[63]", "previouslyFormattedCitation" : "[63]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Particularly, histone deacetylase (HDAC) modulation using HDAC-inhibitors has been shown to promote osteoblastic differentiation of AD-MSC **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcb.20544", "ISBN" : "0730-2312 (Print)\\r0730-2312 (Linking)", "ISSN" : "07302312", "PMID" : "16088945", "abstract" : "Valproic acid (VPA) has been used as an anticonvulsant agent for the treatment of epilepsy, as well as a mood stabilizer for the treatment of bipolar disorder, for several decades. The mechanism of action for these effects remains to be elucidated and is

most likely multifactorial. Recently, VPA has been reported to inhibit histone deacetylase (HDAC) and HDAC has been reported to play roles in differentiation of mammalian cells. In this study, the effects of HDAC inhibitors on differentiation and proliferation of human adipose tissue-derived stromal cells (hADSC) and bone marrow stromal cells (hBMSC) were determined. VPA increased osteogenic differentiation in a dose dependent manner. The pretreatment of VPA before induction of differentiation also showed stimulatory effects on osteogenic differentiation of hMSC. Trichostatin A (TSA), another HDAC inhibitor, also increased osteogenic differentiation, whereas valpromide (VPM), a structural analog of VPA which does not possess HDAC inhibitory effects, did not show any effect on osteogenic differentiation on hADSC. RT-PCR and Real-time PCR analysis revealed that VPA treatment increased osterix, osteopontin, BMP-2, and Runx2 expression. The addition of noggin inhibited VPA-induced potentiation of osteogenic differentiation. VPA inhibited proliferation of hADSC and hBMSC. Our results suggest that VPA enhance osteogenic differentiation, probably due to inhibition of HDAC, and could be useful for in vivo bone engineering using hMSC.", "author" : [{ "dropping-particle" : "", "family" : "Cho", "given" : "Hyun Hwa", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Park", "given" : "Hyung Taek", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kim", "given" : "Yeon Jeong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bae", "given" : "Yong Chan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Suh", "given" : "Kuen Taek", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jung", "given" : "Jin Sup", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Cellular

Biochemistry", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2005"]] }, "page" : "533-542", "title" : "Induction of osteogenic differentiation of human mesenchymal stem cells by histone deacetylase inhibitors", "type" : "article-journal", "volume" : "96" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9c32f966-7965-4d4d-b27e-7c0ed74c4ebb"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1089/scd.2012.0105", "ISBN" : "1557-8534 (Electronic)\\r1547-3287 (Linking)", "ISSN" : "1557-8534", "PMID" : "22873791", "abstract" : "Adult stem cells reside in many types of tissues and adult stem cell-based regenerative medicine holds great promise for repair of diseased tissues. Recently, adipose-derived stem cells (ADSCs) were found to be an appealing alternative to bone marrow stem cells (BMSCs) for tissue-engineered bone regeneration. Compared with BMSCs, ADSCs can be easily and abundantly available from adipose tissue. However, our previous study has discovered an important phenomenon that BMSCs have greater osteogenic potential than ADSCs in vitro. In this study, we aimed to explore its mechanism and improve the osteogenic potential of ADSCs for bone tissue regeneration. It has been reported that the epigenetic states could contribute to lineage-specific differentiation of adult stem cells. We observed that the epigenetic changes of BMSCs were much greater compared with ADSCs after a 3-day osteogenic induction. Runt-related transcription factor 2 (Runx2) is essential for osteoblast differentiation and bone formation. We found that BMSCs underwent more obvious epigenetic changes on the Runx2 promoter than ADSCs after osteogenic induction. These results suggest the epigenetic regulation involvement in Runx2 expression, and thus osteogenesis. We subsequently used a histone deacetylase inhibitor, trichostatin A (TSA), to promote the osteogenesis capacity of ADSCs. The results showed that TSA promoted rat ADSCs osteogenic differentiation by altering the epigenetic modifications on the Runx2 promoter

in a bone morphogenetic protein signaling-dependent manner.", "author" : [{ "dropping-particle" : "", "family" : "Hu", "given" : "Xiaoqing", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhang", "given" : "Xin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dai", "given" : "Linghui", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhu", "given" : "Jingxian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jia", "given" : "Zhuqing", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wang", "given" : "Weiping", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhou", "given" : "Chunyan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ao", "given" : "Yingfang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem cells and development", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2013"]] }, "page" : "248-55", "title" : "Histone deacetylase inhibitor trichostatin A promotes the osteogenic differentiation of rat adipose-derived stem cells by altering the epigenetic modifications on Runx2 promoter in a BMP signaling-dependent manner.", "type" : "article-journal", "volume" : "22" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b12769f3-d40e-4726-81c2-13993ba5e61a"] }], "mendeley" : { "formattedCitation" : "[64, 65]", "plainTextFormattedCitation" : "[64, 65]", "previouslyFormattedCitation" : "[64, 65]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Thus, JARID1B modulation can be now proposed as an additional and valuable tool to enhance the WJ-MSCs OD.

Considering the biomedical advantages of using WJ-MSCs for tissue engineering applications, an important benefit of our experiments utilizing PBIT is to set the basis for a future gene transfer-free strategy to produce JARID1B deficient cells. Use of epi-drugs can prevent risks associated with putative random insertion of DNA sequences at host genomes and can function as a homogeneous stimulus for whole stem cell populations. Importantly, other JARID1B inhibitors have shown evidence of specificity and efficacy to produce particular cellular effects **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.chembiol.2017.02.006", "ISSN" : "24519456", "PMID" : "28262558", "abstract" : "Methylation of lysine residues on histone tail is a dynamic epigenetic modification that plays a key role in chromatin structure and gene regulation. Members of the KDM5 (also known as JARID1) sub-family are 2-oxoglutarate (2-OG) and Fe(2+)-dependent oxygenases acting as histone 3 lysine 4 trimethyl (H3K4me3) demethylases, regulating proliferation, stem cell self-renewal, and differentiation. Here we present the characterization of KDOAM-25, an inhibitor of KDM5 enzymes. KDOAM-25 shows biochemical half maximal inhibitory concentration values of\u00a0<100\u00a0nM for KDM5A-D in\u00a0vitro, high selectivity toward other 2-OG oxygenases sub-families, and no off-target activity on a panel of 55 receptors and enzymes. In human cell assay systems, KDOAM-25 has a half maximal effective concentration of \u223c50\u00a0\u03bcM and good selectivity toward other demethylases. KDM5B is overexpressed in multiple myeloma and negatively correlated with the overall survival. Multiple myeloma MM1S cells treated with KDOAM-25 show increased global H3K4 methylation at transcriptional start sites and impaired proliferation.", "author" : [{ "dropping-particle" : "", "family" : "Tumber", "given" : "Anthony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nuzzi",

"given" : "Andrea", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hookway", "given" : "Edward S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hatch", "given" : "Stephanie B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Velupillai", "given" : "Srikannathasan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Johansson", "given" : "Catrine", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kawamura", "given" : "Akane", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Savitsky", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yapp", "given" : "Clarence", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Szykowska", "given" : "Aleksandra", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wu", "given" : "Na", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bountra", "given" : "Chas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Strain-Damerell", "given" : "Claire", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Burgess-Brown", "given" : "Nicola A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ruda", "given" : "Gian Filippo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fedorov", "given" : "Oleg", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Munro", "given" : "Shonagh", "non-dropping-

particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "England", "given" : "Katherine S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nowak", "given" : "Radoslaw P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schofield", "given" : "Christopher J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thangue", "given" : "Nicholas B.", "non-dropping-particle" : "La", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pawlyn", "given" : "Charlotte", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Davies", "given" : "Faith", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morgan", "given" : "Gareth", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Athanasou", "given" : "Nick", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "M\u00fcller", "given" : "Susanne", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Oppermann", "given" : "Udo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Brennan", "given" : "Paul E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Cell Chemical Biology", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2017", "3", "16"]] }, "page" : "371-380", "title" : "Potent and Selective KDM5 Inhibitor Stops Cellular Demethylation of H3K4me3 at Transcription Start Sites and Proliferation of MM1S Myeloma Cells", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f83d0b30-8807-3e93-b46f-158b8e97388e"] }], "mendeley" : { "formattedCitation" : "[66]", "plainTextFormattedCitation" : "[66]",

"previouslyFormattedCitation" : "[66]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }. Testing PBIT *in vivo* or the development of derivate JARID1B-inhibitors may be important for future clinical applications.

As bone tissue engineering comprehends the influence of cells, soluble factors and scaffolds, any future application of this knowledge would need to be tested in conjunction with suitable biomaterials. The efficacy of those biomaterials needs to consider the contribution of mechanical cues such as shear stress, substrate rigidity, and nanotopography {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1186/scrt158", "ISSN" : "1757-6512", "PMID" : "23369796", "abstract" : "Bone is a load-bearing tissue and physical forces play key roles in the development and maintenance of its structure. Mechanical cues can stimulate the expression of an osteogenic phenotype, enhance matrix and mineral deposition, and influence tissue organization to improve the functional outcome of engineered bone grafts. In recent years, a number of studies have investigated the effects of biophysical forces on the bone formation properties of osteoprogenitor cells. The application of physiologically relevant stimuli to tissue-engineered bone may be determined through observation and understanding of forces to which osteoblasts, osteoclasts, and osteocytes are exposed in native bone. Subsequently, these cues may be parameterized and their effects studied in well-defined in vitro systems. The osteo-inductive effects of three specific mechanical cues - shear stress, substrate rigidity, and nanotopography - on cells cultured in monolayer or in three-dimensional biomaterial scaffolds in vitro are reviewed. Additionally, we address the time-dependent effects of mechanical cues on vascular infiltration and de novo bone formation in acellular

scaffolds implanted into load-bearing sites in vivo. Recent studies employing cutting-edge advances in biomaterial fabrication and bioreactor design have provided key insights into the role of mechanical cues on cellular fate and tissue properties of engineered bone grafts. By providing mechanistic understanding, future studies may go beyond empirical approaches to rational design of engineering systems to control tissue development.", "author" : [{ "dropping-particle" : "", "family" : "Hung", "given" : "Ben P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hutton", "given" : "Daphne L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Grayson", "given" : "Warren L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Stem Cell Research & Therapy", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2013"]] }, "page" : "1-7", "title" : "Mechanical control of tissue-engineered bone", "type" : "article-journal", "volume" : "4" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6ac9742a-07d3-4c4b-9562-337c7f10a64d"] }], "mendeley" : { "formattedCitation" : "[67]", "plainTextFormattedCitation" : "[67]", "previouslyFormattedCitation" : "[67]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** in WJ-MSC bone differentiation. Specifically, recent evidence shows that hierarchical fibrous scaffolds favor osteogenic marker expression in WJ-MSC upon differentiation induction **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1088/1758-5090/7/3/035009", "ISSN" : "1758-5090", "PMID" : "26335618", "abstract" : "Hierarchical structures, constituted by polymeric nano and microfibers, have been considered promising scaffolds for tissue engineering strategies, mainly because they mimic, in some way, the complexity and nanoscale detail observed in real organs. The

chondrogenic potential of these scaffolds has been previously demonstrated, but their osteogenic potential is not yet corroborated. In order to assess if a hierarchical structure, with nanoscale details incorporated, is an improved scaffold for bone tissue regeneration, we evaluate cell adhesion, proliferation, and osteogenic differentiation of human Wharton's jelly derived stem cells (hWJSCs), seeded into hierarchical fibrous scaffolds. Biological data corroborates that hierarchical fibrous scaffolds show an enhanced cell entrapment when compared to rapid prototyped scaffolds without nanofibers. Furthermore, upregulation of bone specific genes and calcium phosphate deposition confirms the successful osteogenic differentiation of hWJSCs on these scaffolds. These results support our hypothesis that a scaffold with hierarchical structure, in conjugation with hWJSCs, represents a possible feasible strategy for bone tissue engineering applications.", "author" : [{ "dropping-particle" : "", "family" : "Canha-Gouveia", "given" : "Analuce", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rita Costa-Pinto", "given" : "Ana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Martins", "given" : "Albino M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Silva", "given" : "Nuno A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Faria", "given" : "Susana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sousa", "given" : "Rui A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Salgado", "given" : "Ant\u00f3nio J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sousa", "given" : "Nuno", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Reis", "given" :

"Rui L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Neves", "given" : "Nuno M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biofabrication", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2015"]] }, "page" : "035009", "publisher" : "IOP Publishing", "title" : "Hierarchical scaffolds enhance osteogenic differentiation of human Wharton's jelly derived stem cells.", "type" : "article-journal", "volume" : "7" }, "uris" : ["http://www.mendeley.com/documents/?uuid=2b22bd07-9130-41ff-922c-7727ff457d51"] }], "mendeley" : { "formattedCitation" : "[68]", "plainTextFormattedCitation" : "[68]", "previouslyFormattedCitation" : "[68]" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Thus, the *in vivo* functional result will depend on a combination of stem cell potential and mechanical stimuli.

In summary, here we demonstrate a new epigenetic route to overcome the intrinsic limitations of WJ-MSCs to be differentiated into osteoblasts that could increase the possibility of its successful application in bone therapies.

CONCLUSIONS

Our results collectively show for the first time that RUNX2/p57 overexpression increases WJ-MSC osteogenic differentiation. RUNX2/p57 is transiently up-regulated during early stages of WJ-MSC osteogenic differentiation. JARID1B is enriched at the RUNX2 P1 promoter and contributes to repress this promoter during WJ-MSC differentiation.

JARID1B loss of function via silencing or pharmacological inhibition results in an increased RUNX2/p57 expression.

ACKNOWLEDGEMENTS

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

REFERENCES

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FIGURE LEGENDS

Figure 1: Ectopic *RUNX2/p57* expression enhances osteoblast differentiation potential of WJ-MSCs.

(A) Scheme of the experiment. After 1 day of culture, 10^5 WJ-MSC were transduced for 4 days with the indicated lentiviral particles and then incubated for 15 days in control (*CT*) or osteoblastic media (*OB*). Samples were collected at indicated times for indicated analysis.

(B-C) RUNX2 overexpression via lentiviral transduction in WJ-MSCs. (B) WJ-MSCs transduced with lentiviral particles carrying a bicistronic vector encoding copGFP and RUNX2 (LV-RUNX2) or an empty sequence control (LV-Ctrl). Different WJ-MSC from umbilical cords randomly selected are indicated as #1-3. RUNX2 protein was detected by Western blot analysis exclusively in WJ-MSCs transduced with LV-RUNX2 vector. TFIIB was used as a loading control. (C) Fluorescence microscopy of transduced WJ-MSC reveals nuclear localization of ectopically expressed RUNX2/p57. Cop-GFP expression is shown as a transduction control. DAPI, nuclear marker.

(D-G) *RUNX2/p57* overexpression results in an increase in osteogenic markers in WJ-MSCs. SP7, BSP, ALP and BGLAP mRNA expression were analyzed by RT-qPCR. Results for different biological replicates are shown (#1-3). Normalized GAPDH gene expression is shown relative to LV-Ctrl infected cells cultured in CT.

Figure 2: RUNX2 expression is transiently induced in WJ-MSCs grown with osteoblastic media.

(A-B) *RUNX2/p57* expression is induced in early stages of WJ-MSC osteoblast differentiation. WJ-MSCs from 3 umbilical cords were induced to differentiate for the

indicated days *in vitro* (DIV) to determine (A) mRNA and (B) protein expression levels by RT-qPCR and western blot (WB), respectively. In (A), normalized GAPDH expression is shown relative to pretreated conditions (DIV 0). Statistical differences were determined relative to DIV 0. * $p \leq 0.05$; One way-ANOVA and Tukey post-hoc test. In WB analysis, LAMINB1 levels were determined as loading control.

(C-D) Enrichment of activating histone post-translational modifications (PTMs) at the RUNX2 P1 and GAPDH promoters during osteoblast differentiation of WJ-MSCs. Levels of tri-methylation of lysine 4 (H3Kme3) and acetylation of lysine 27 (H3K27Ac) of histone H3 were determined by chromatin immunoprecipitation assays (ChIP) using specific antibodies. Precipitated DNA was then analyzed by qPCR. Normal IgG was used as control. (C) H3K4me3 and (D) H3K27Ac enrichments at the RUNX2 P1 (left) and GAPDH (right) promoters. In all ChIP assays y axis represents percent of input DNA. Data represent the mean of independent biological replicates ±SEM. Statistical differences were determined relative to DIV 0. * $p \leq 0.05$; Student's t test.

Figure 3: JARID1B demethylase is expressed in WJ-MSCs and is enriched at the RUNX2 P1 promoter.

(A-B) JARID1B is constitutively expressed in WJ-MSC osteoblastic differentiation. (A) mRNA and (B) protein levels of JARID1B were determined in same conditions detailed in Fig 2A-B.

(C-D) JARID1B is located in WJ-MSC nucleus. (C) Confocal microscopy and (D) western blot analysis of subcellular fractions showing that JARID1B is located at the WJ-MSC nucleus. DAPI and LAMINB1, nuclear markers; tubulin, cytoplasmic marker.

(E) JARID1B binds to the RUNX2 P1 promoter in WJ-MSC. JARID1B enrichments at the RUNX2 P1 promoter were determined by ChIP in the conditions detailed in Fig 2C-D.

(F-G) JARID1B is expressed in WJ-MSC from different umbilical cords. (F) mRNA and (G) protein expression levels of JARID1B were analyzed in WJ-MSC from different biological replicates (symbolized as #1-4) by RT-qPCR and western blot. SAOS-2 and HUVEC cells were used as benchmarks for human JARID1B expression.

Figure 4: JARID1B inhibition results in enhanced *RUNX2/p57* expression in WJ-MSCs.

(A) Global H3K4me3 levels are increased in WJ-MSC upon treatment with the JARID1B-inhibitor PBIT. Purified histone samples obtained from WJ-MSCs cultured during 2 days in the presence of PBIT (1µM) or DMSO (vehicle) were analyzed by western blot using indicated antibodies. Total H3 histone levels are shown as a loading control.

(B) *RUNX2/p57* expression is increased in PBIT-treated WJ-MSCs. mRNAs extracted from WJ-MSCs cultured in the conditions described above (A) were analyzed by RT-qPCR. *RUNX2/p57* expression was normalized by *GAPDH* levels and shown as relative to undifferentiated (UD) pretreated WJ-MSCs. Data represents mean ±SEM; $n \geq 3$, *$p \leq 0.05$; Student's t test.

(C-D) Histone PTMs in PBIT-treated WJ-MSCs. Enrichment of histone PTMs at RUNX2 P1 promoter were determined by ChIP in samples of WJ-MSCs obtained in the conditions described above (A). Enrichment of (C) H3K4me3 and (D) H3K27Ac at the RUNX2 P1 promoter were determined as described in Fig 2C-D. Data represents mean ±SEM; $n \geq 3$, *$p \leq 0.05$; Student's t test.

Figure 5: *JARID1B* silencing results in an increase in *RUNX2/p57* expression and involves changes in the epigenetic state of the P1 promoter region.

(A) JARID1B-silencing in WJ-MSCs. WJ-MSCs were transduced with lentiviral particles carrying specific shRNAs against *JARID1B* (shJARID1B) or an unrelated sequence (shCtrl). Knockdown efficiency was determined at mRNA (left) and protein (right) levels by RT-qPCR and western blot, respectively.

(B-C) RUNX2/p57 expression is enhanced in JARID1B-silenced WJ-MSCs. WJ-MSCs transduced with sh*JARID1B* or shCtrl lentivirus were incubated in *CT* or *OB* media. (C) JARID1B and (D) RUNX2/p57 mRNA expression was determined by RT-qPCR and normalized against *GAPDH*. Relative mRNA expression was estimated relative to undifferentiated (UD) untreated WJ-MSCs. Data represents mean ±SEM; $n \geq 3$, ***≤0.001; Student's t test.

(D-E) JARID1B-silencing in WJ-MSCs induces significant changes in histone PTMs at the RUNX2 P1 promoter. Enrichments of (D) H3K4me3 and (E) H3K27Ac at the RUNX2 P1 promoter were determined by ChIP in samples from WJ-MSCs grown in the conditions indicated above (B-C). ChIP assays were performed as detailed in Fig 2C-D. Data represents mean ±SEM; $n \geq 3$, *p≤0.05; Student's t test.

FIGURE 1

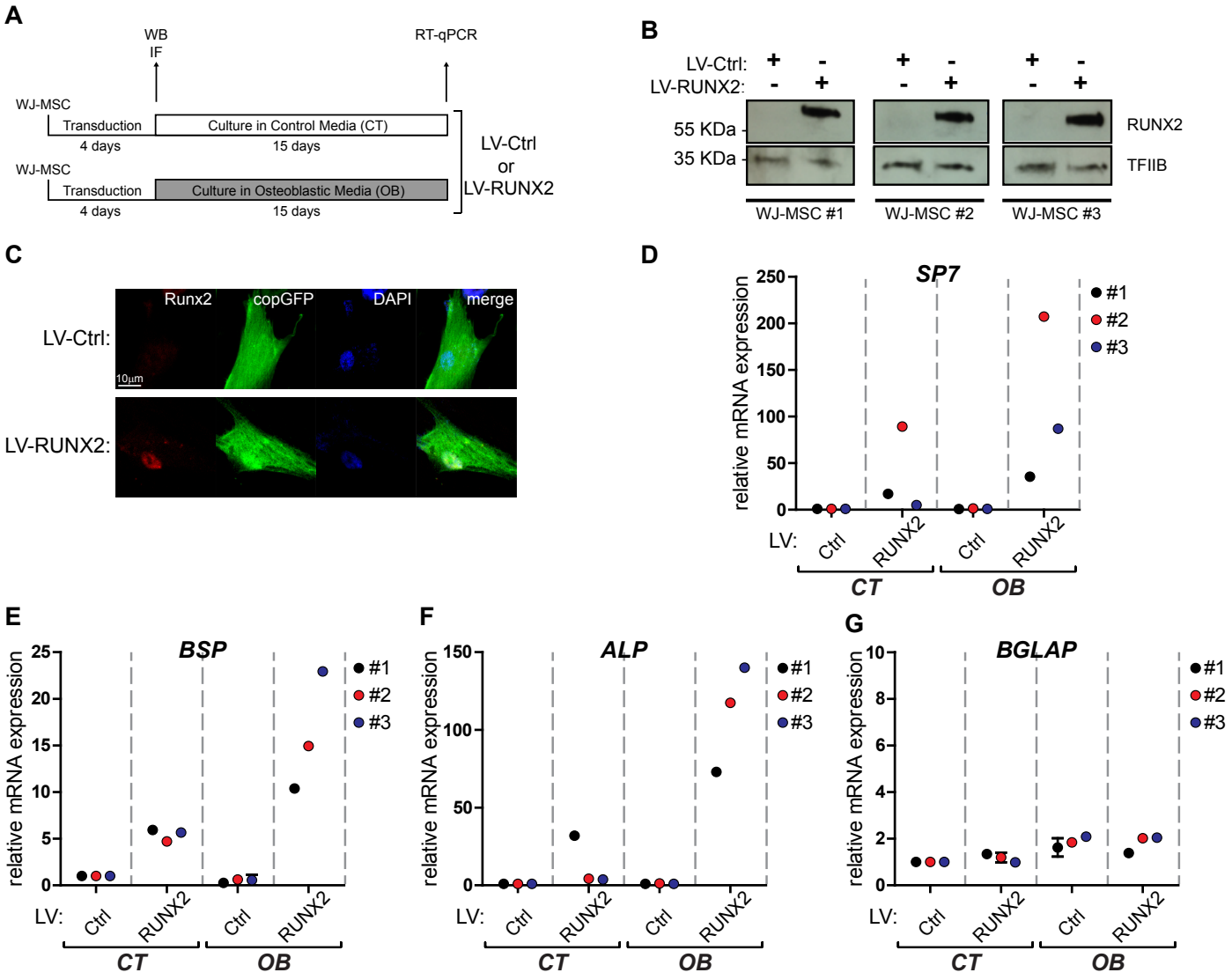

A
WB
IF
RT-qPCR
WJ-MSC
Transduction
4 days
Culture in Control Media (CT)
15 days
WJ-MSC
Transduction
4 days
Culture in Osteoblastic Media (OB)
15 days
LV-Ctrl
or
LV-RUNX2
B
LV-Ctrl:
LV-RUNX2:
55 KDa
35 KDa
RUNX2
TFIIB
WJ-MSC #1
WJ-MSC #2
WJ-MSC #3
C
LV-Ctrl:
LV-RUNX2:
Runx2
copGFP
DAPI
merge
10µm
D
SP7
relative mRNA expression
LV:
Ctrl
RUNX2
CT
OB
#1
#2
#3
E
BSP
F
ALP
G
BGLAP

FIGURE 2

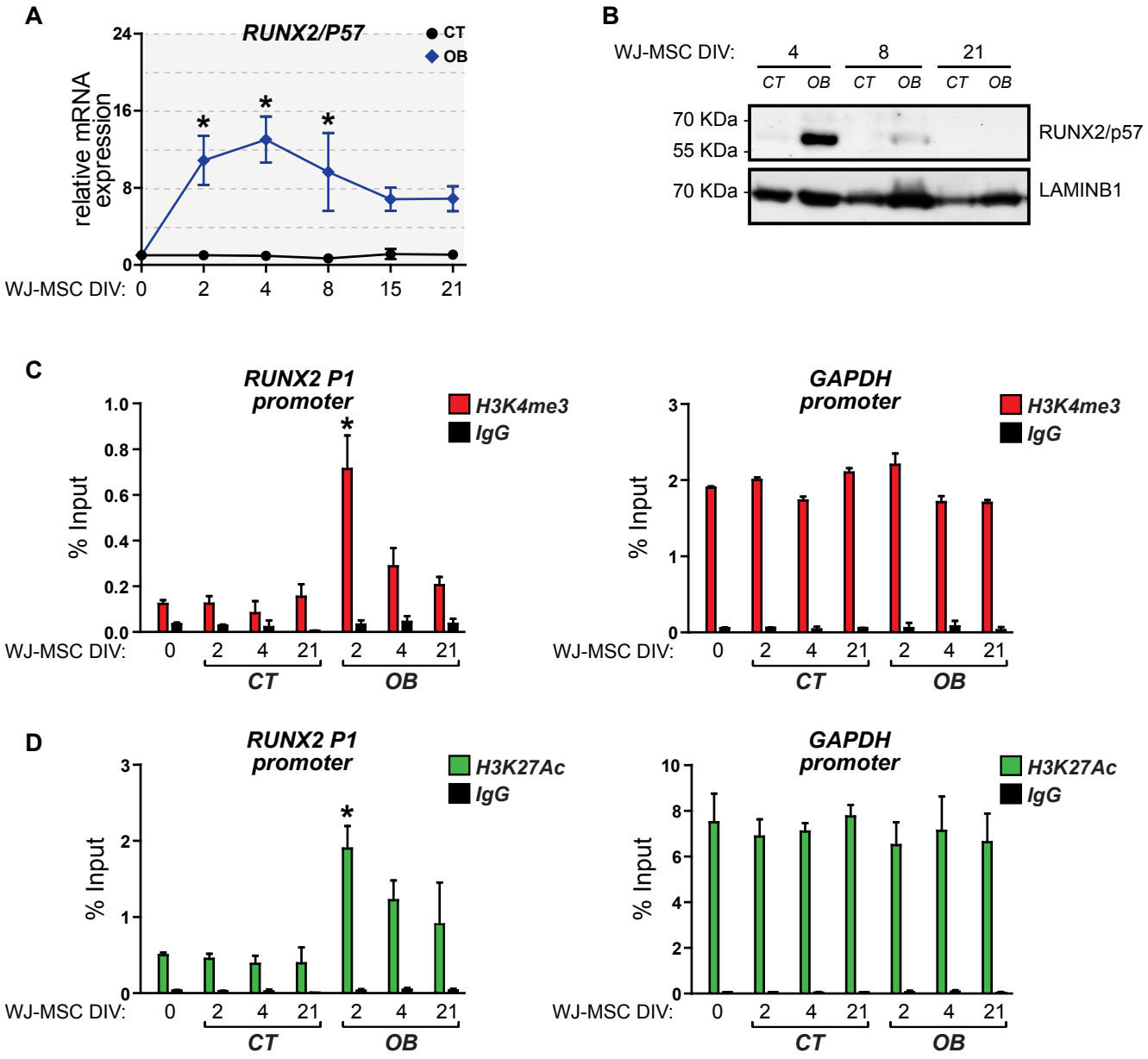

A
RUNX2/P57
CT
OB
relative mRNA expression
24
16
8
0
WJ-MSC DIV: 0 2 4 8 15 21
B
WJ-MSC DIV: 4 8 21
CT OB CT OB CT OB
70 KDa
55 KDa
RUNX2/p57
70 KDa
LAMINB1
C
RUNX2 P1 promoter
H3K4me3
IgG
% Input
1.0
0.8
0.6
0.4
0.2
0.0
WJ-MSC DIV: 0 2 4 21 2 4 21
CT
OB
GAPDH promoter
H3K4me3
IgG
% Input
3
2
1
0
WJ-MSC DIV: 0 2 4 21 2 4 21
CT
OB
D
RUNX2 P1 promoter
H3K27Ac
IgG
% Input
3
2
1
0
WJ-MSC DIV: 0 2 4 21 2 4 21
CT
OB
GAPDH promoter
H3K27Ac
IgG
% Input
10
8
6
4
2
0
WJ-MSC DIV: 0 2 4 21 2 4 21
CT
OB

FIGURE 3

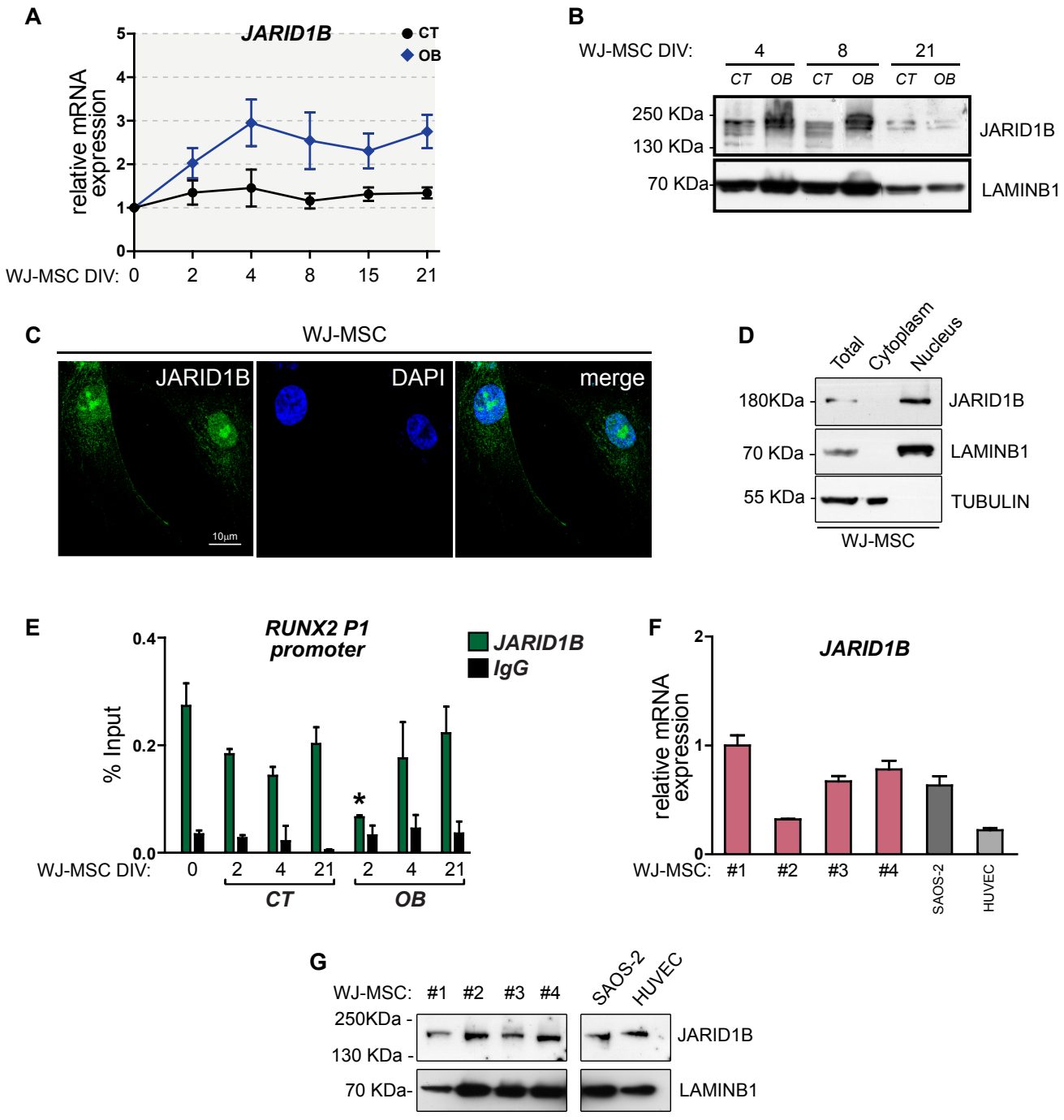

A
JARID1B
CT
OB
relative mRNA expression
WJ-MSC DIV: 0 2 4 8 15 21
B
WJ-MSC DIV: 4 8 21
CT OB CT OB CT OB
250 KDa
130 KDa
70 KDa
JARID1B
LAMINB1
C
WJ-MSC
JARID1B
DAPI
merge
10µm
D
Total
Cytoplasm
Nucleus
180KDa
70 KDa
55 KDa
JARID1B
LAMINB1
TUBULIN
WJ-MSC
E
RUNX2 P1 promoter
JARID1B
IgG
% Input
WJ-MSC DIV: 0 2 4 21 2 4 21
CT
OB
F
JARID1B
relative mRNA expression
WJ-MSC: #1 #2 #3 #4 SAOS-2 HUVEC
G
WJ-MSC: #1 #2 #3 #4 SAOS-2 HUVEC
250KDa
130 KDa
70 KDa
JARID1B
LAMINB1

FIGURE 4

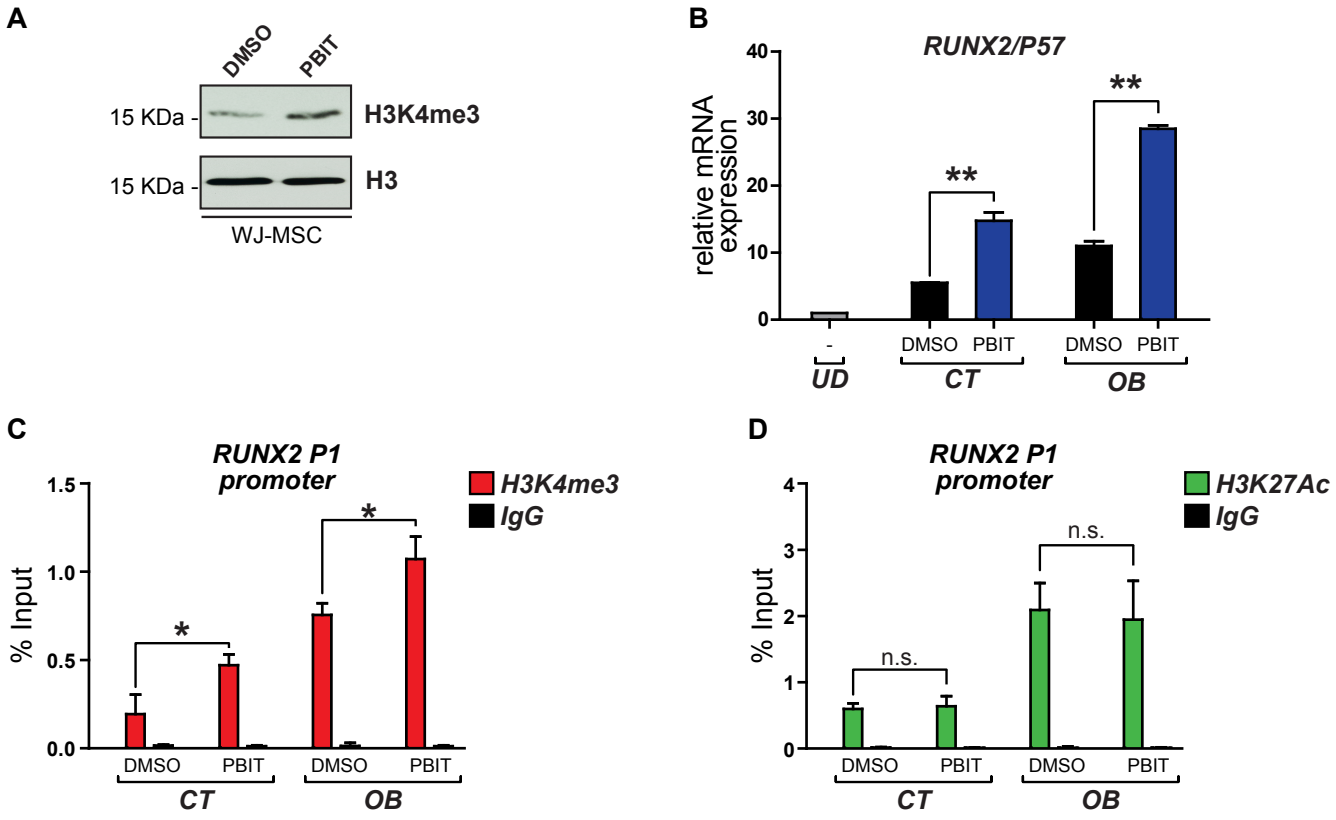

A
DMSO
PBIT
15 KDa -
H3K4me3
15 KDa -
H3
WJ-MSC
B
RUNX2/P57
relative mRNA expression
40
30
20
10
0
**
**
-
DMSO
PBIT
DMSO
PBIT
UD
CT
OB
C
RUNX2 P1 promoter
H3K4me3
IgG
% Input
1.5
1.0
0.5
0.0
*
*
DMSO
PBIT
DMSO
PBIT
CT
OB
D
RUNX2 P1 promoter
H3K27Ac
IgG
% Input
4
3
2
1
0
n.s.
n.s.
DMSO
PBIT
DMSO
PBIT
CT
OB

FIGURE 5

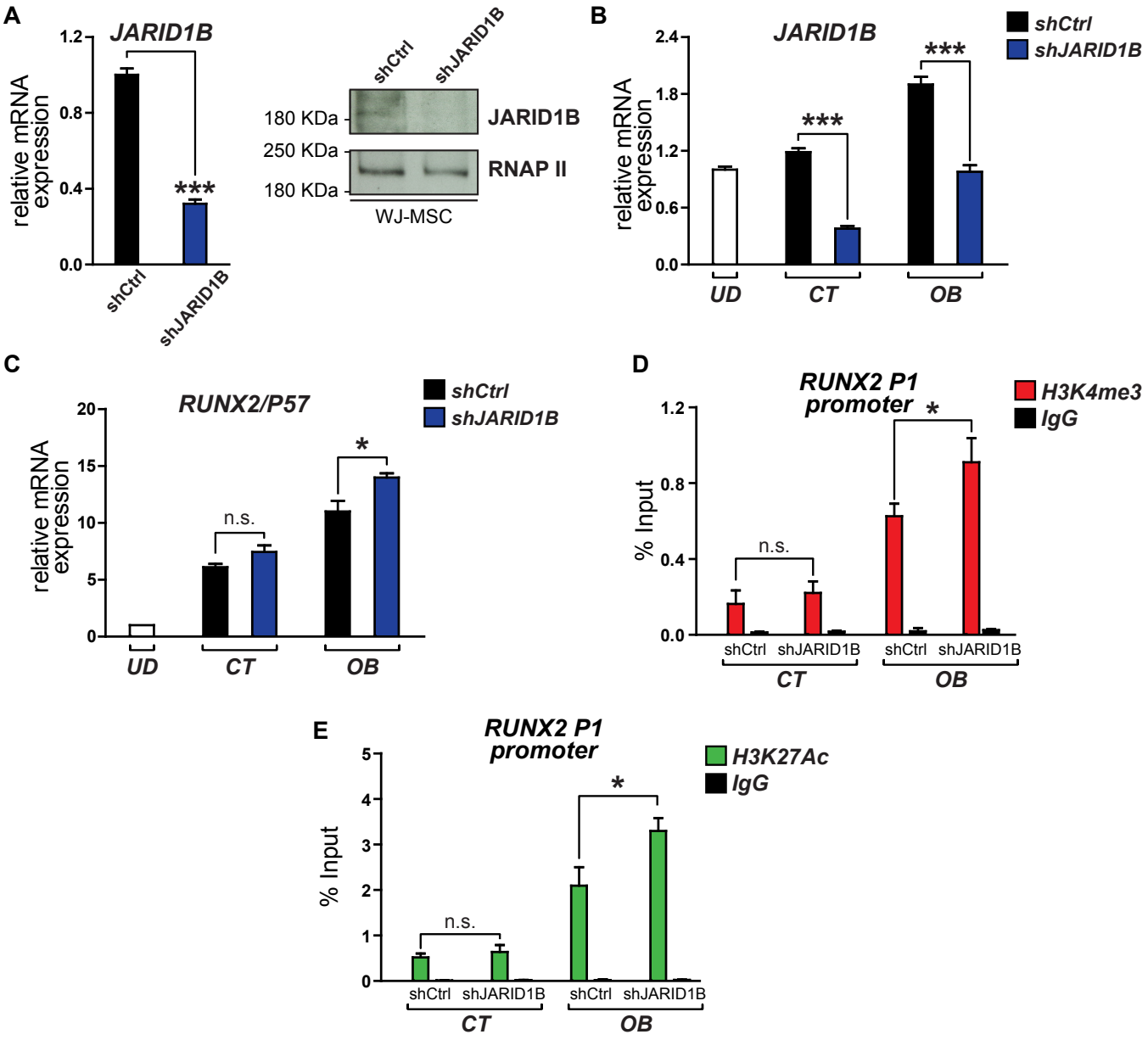

A
JARID1B
relative mRNA expression
1.2
0.8
0.4
0.0
***
shCtrl
shJARID1B
shCtrl
shJARID1B
180 KDa
250 KDa
180 KDa
JARID1B
RNAP II
WJ-MSC
B
JARID1B
shCtrl
shJARID1B
relative mRNA expression
2.4
1.8
1.2
0.6
0.0
***
***
UD
CT
OB
C
RUNX2/P57
shCtrl
shJARID1B
relative mRNA expression
20
15
10
5
0
n.s.
*
UD
CT
OB
D
RUNX2 P1 promoter
H3K4me3
IgG
% Input
1.2
0.8
0.4
0.0
n.s.
*
shCtrl
shJARID1B
shCtrl
shJARID1B
CT
OB
E
RUNX2 P1 promoter
H3K27Ac
IgG
% Input
5
4
3
2
1
0
n.s.
*
shCtrl
shJARID1B
shCtrl
shJARID1B
CT
OB

FIGURE S1

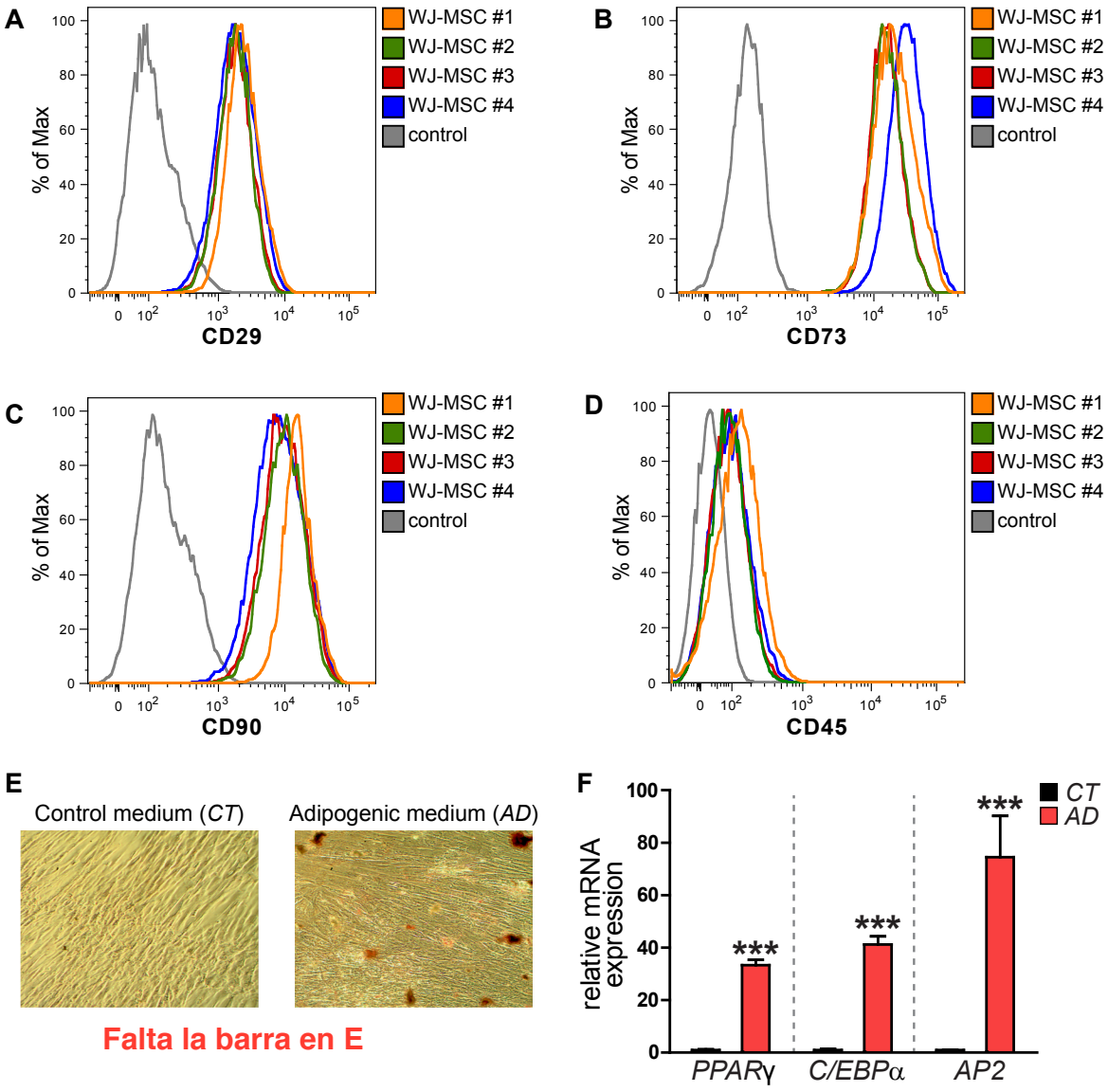


Figure S1: WJ-MSCs characterization. A-D. WJ-MSCs express phenotypic MSC surface markers. Flow cytometry histograms indicating enhanced expression of MSC markers CD29 (A), CD73 (B) and CD90 (C) and absence of the hematopoietic stem cell (HSC) marker CD45 (D). Four WJ-MSC biological replicates (WJ-MSC#1-4) were included in the analyses and PE-Cy5 was used as a negative control (control). E-F. Adipogenic differentiation of WJ-MSCs. Representative images of oil red stained WJ-MSCs grown in control (CT) or adipogenic medium (AD) (E). Increased expression of phenotypic markers in WJ-MSC induced to adipogenesis (F). RT-qPCR analysis showing the expression of early (PPARγ and C/EBPα) or late (AP2) adipogenic markers in WJ-MSC incubated in CT or AD media. Data represents mean ±SEM; n ≥ 3, ***p≤0.001; Student's t test.

Para E & F agregar 21 dias en leyenda , al menos para E
Confirmar para F tambien

FIGURE S2

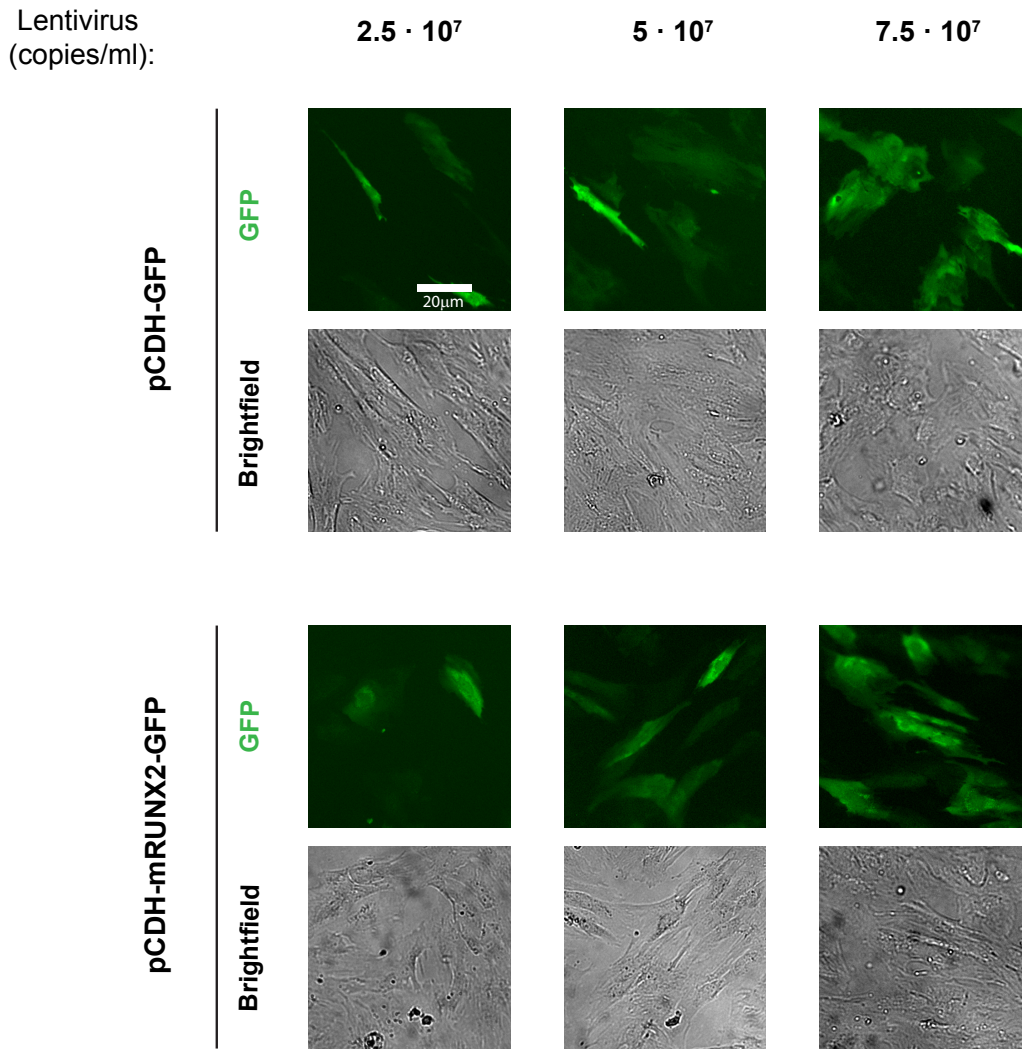
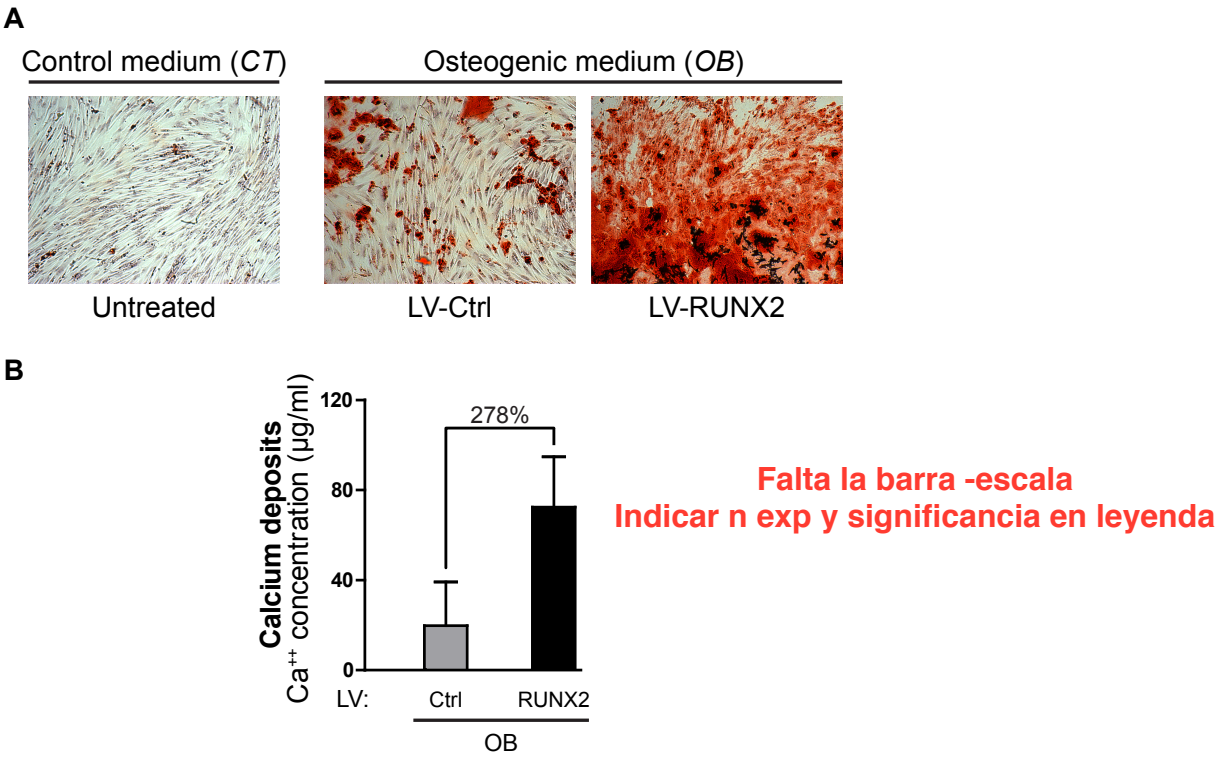


Figure S2: WJ-MSCs are efficiently transduced via lentiviral particles. Brightfield and fluorescence microscopy images showing infected WJ-MSCs with lentivirus CO (Upper panel) or RUNX2/p57 (Bottom panel) at the indicated lentiviral particle copies. Scale bar: 20 µm. Transduction efficiency was confirmed by GFP fluorescence observation in a CKX415F fluorescence microscope (Olympus).

FIGURE S3



Falta la barra -escala
Indicar n exp y significancia en leyenda

Figure S3: RUNX2/p57 overexpression leads to enhanced extracellular matrix mineralization in WJ-MSCs. A. Light microscopy images of Alizarin Red S (ARS) staining of calcium deposits in untreated or infected (LV-Ctrl or LV-RUNX2) WJ-MSCs cultured by 15 days at indicated conditions. B: Ca++ quantification of WJ-MSCs infected with LV-Ctrl or LV-RUNX2 lentivirus and cultured by 15 days in osteogenic medium.

FIGURE S4

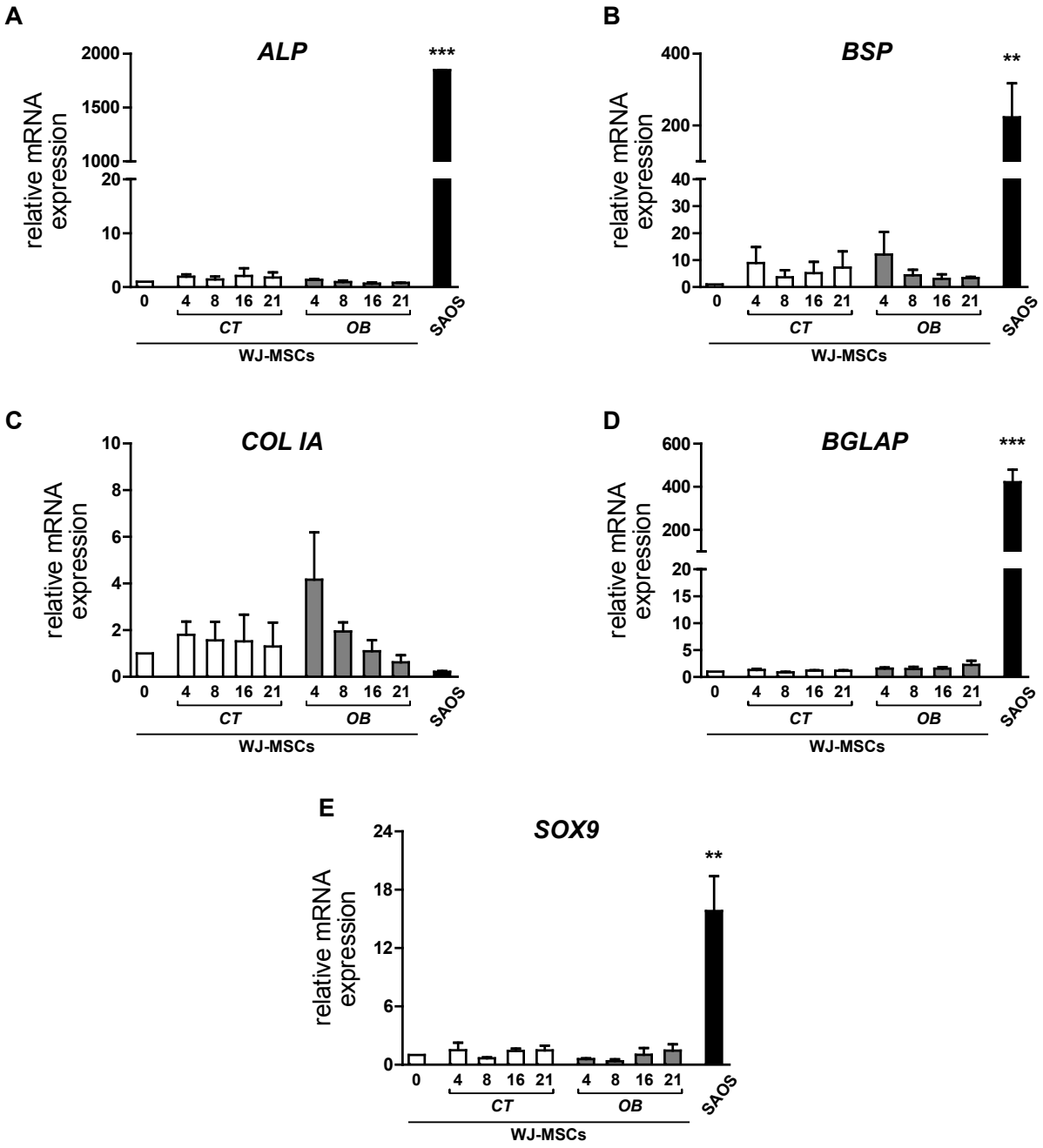


Figure S4: Late osteoblastic markers are not induced during WJ-MSC osteogenic differentiation. A-D: WJ-MSC from 3 umbilical cords randomly selected were incubated in the presence of control (CT) or osteoblastic (OB) media for the indicated times. mRNA expression of the osteoblastic markers ALP (A), BSP (B), COL1A (C) and BGLAP (D) as well as the chondrogenic marker SOX9 (E) were analyzed by RT-qPCR. SAOS-2 cells were used as a positive control for the expression of osteogenic markers. The expression levels of each gene analyzed are shown relative to GAPDH. Filled bars: pretreated cells; white bars, CT; gray bars: OB, diagonal filled bars: SAOS-2 cells. * One way ANOVA followed by Tukey post-hoc test $P>0.05$.