Research Article

PPARγ2 Regulates a Molecular Signature of Marrow Mesenchymal Stem Cells

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Bone formation and hematopoiesis are anatomically juxtaposed and share common regulatory mechanisms. Bone marrow mesenchymal stromal/stem cells (MSC) contain a compartment that provides progeny with bone forming osteoblasts and fat laden adipocytes as well as fibroblasts, chondrocytes, and muscle cells. In addition, marrow MSC provide an environment for support of hematopoiesis, including the development of bone resorbing osteoclasts. The PPARγ2 nuclear receptor is an adipocyte-specific transcription factor that controls marrow MSC lineage allocation toward adipocytes and osteoblasts. Increased expression of PPARγ2 with aging correlates with changes in the MSC status in respect to both their intrinsic differentiation potential and production of signaling molecules that contribute to the formation of a specific marrow micro-environment. Here, we investigated the effect of PPARγ2 on MSC molecular signature in respect to the expression of gene markers associated exclusively with stem cell phenotype, as well as genes involved in the formation of a stem cell supporting marrow environment. We found that PPARγ2 is a powerful modulator of stem cell-related gene expression. In general, PPARγ2 affects the expression of genes specific for the maintenance of stem cell phenotype, including LIF, LIF receptor, Kit ligand, SDF-1, Rex-1/Zfp42, and Oct-4. Moreover, the antidiabetic PPARγ agonist TZD rosiglitazone specifically affects the expression of "stemness" genes, including ABCG2, Egfr, and CD44. Our data indicate that aging and anti-diabetic TZD therapy may affect mesenchymal stem cell phenotype through modulation of PPARγ2 activity. These observations may have important therapeutic consequences and indicate a need for more detailed studies of PPARγ2 role in stem cell biology.

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1. INTRODUCTION

PPARγ, an essential regulator of lipid, glucose, and insulin metabolism [1], is expressed in bone marrow mesenchymal stem cells (MSC). PPARγ is expressed in mice and humans in two isoforms, PPARγ1 and PPARγ2, which originate from up to seven different transcripts due to alternative promoter usage and alternative splicing [2–5]. PPARγ2 differs from PPARγ1 by 30 additional amino acids on its N-terminus, which constitute AF-1 domain of ligand-independent gene-activating function [6]. While PPARγ1 is expressed in a variety of cell types, including osteoblasts, PPARγ2 is expressed in cells of adipocyte lineage and serves as an essential regulator of adipocyte differentiation and function [7, 8].

Osteoblasts and adipocytes are derived from a marrow mesenchymal cell compartment which also serves as a source of progenitors for marrow fibroblasts and cartilage cells and functions as hematopoiesis-supporting stroma [9, 10]. Commitment of marrow MSC toward adipocyte and osteoblast lineage occurs by a stochastic mechanism, in which lineage-specific transcription factors (such as Runx2 for osteoblasts and PPARγ2 for adipocytes) representing intrinsic determinants of this process are activated [8, 11]. Embryonic stem cells with a null mutation in PPARγ spontaneously differentiate to osteoblasts and are unable to differentiate to adipocytes [12]. In marrow MSC, PPARγ2 acts as a dominant negative regulator of osteoblast differentiation [8, 13]. Using a model of marrow MSC differentiation (U-33/γ2 cells), we have previously demonstrated that activation of the PPARγ2 isoform by the highly specific agonist and antidiabetic thiazolidinedione (TZD), rosiglitazone, converted cells of osteoblast lineage to terminally differentiated adipocytes
| Gene symbol | Probe IDa | FCb | Gene description | Biological processc |
|-------------|-----------|-----|------------------|---------------------|
| Cd3g        | 1419178_at| 1.5 | CD3 antigen, gamma polypeptide | Immune and hematopoietic system, cell surface receptor linked signal transduction |
| Cd3e        | 1445748_at| 1.5 | CD3 antigen, epsilon polypeptide | Cell surface receptor linked signal transduction, positive regulation of T cell proliferation and T cell receptor signaling pathway |
| Cd4         | 1419696_at| 1.5 | CD4 antigen | Immune response, cell adhesion, cell surface receptor linked signal transduction, positive regulation of T cell activation |
| Cd7         | 1419711_at| 1.5 | CD7 antigen | Immune response, myeloid cells antigen |
| Cd8a        | 1451673_at| 1.7 | CD8 antigen, alpha chain | Immune response, cell surface receptor linked signal transduction, cellular defense response, cytotoxic T cell differentiation |
| Cd19        | 1450570_at| 1.9 | CD19 antigen | Lymphocyte progenitors |
| Cd24a       | 1416034_at| 9.8 | CD24a antigen | Cell surface antigen expressed in T and B lymphocytes, macrophages, dendritic endothelial, and epithelial cells |
| Cd33        | 1450513_at| 1.5 | CD33 antigen | Myeloid cells antigen, cell adhesion |
| Cd37        | 1419206_at| 1.7 | CD37 antigen | B and T cell antigen |
| Cd96        | 1419226_at| 1.5 | CD96 antigen | T-cell activation, cell adhesion |
| Cd207       | 1425243_at| 1.5 | CD207 antigen | Specific for Langerhans cell precursors |
| Cd209b      | 1426157_at| 1.7 | CD209b antigen | Dendritic cell-specific, positive regulation of tumor necrosis factor-alpha biosynthesis, positive regulation of phagocytosis |
| Cd209c      | 1421562_at| 1.9 | CD209c antigen | Dendritic cell specific |
| Cxcl9       | 1418652_at| 1.6 | Chemokine (C-X-C motif) ligand 9 | Inflammatory response, immune response |
| Cxcl13      | 1448859_at| 2.0 | Chemokine (C-X-C motif) ligand 13 | Chemotaxis, inflammatory response, immune response, lymph node development |
| Cxcl16      | 1418718_at| 1.7 | Chemokine (C-X-C motif) ligand 16 | Chemotaxis, keratinocytes, released into the wound after injury |
| Fgf4        | 1450282_at| 1.8 | Fibroblast growth factor 4 | Trophoblast proliferation and differentiation, regulation of progression through cell cycle, stem cell maintenance, embryonic limb and hindlimb morphogenesis, odontogenesis, negative regulation of apoptosis |
| Gata4       | 1441364_at| 1.6 | GATA binding protein 4 | Embryonic development, regulation of transcription, heart development, embryonic gut morphogenesis |
| Gjb1        | 1448766_at| 1.6 | Gap junction membrane channel protein beta 1 | Cell communication, cell-cell signaling |
| Kit/CD117   | 1452514_at| 1.6 | Kit oncogene | Germ cell development, transmembrane receptor protein tyrosine kinase signaling pathway, cell proliferation, cytokine and chemokine mediated signaling pathway, hematopoiesis, cell differentiation |
| Gene symbol | Probe ID<sup>a</sup> | FC<sup>b</sup> | Gene description | Biological process<sup>c</sup> |
|-------------|---------------------|---------------|------------------|-------------------------------|
| Kdr         | 1449379_at          | 1.6           | Kinase insert domain protein receptor | Angiogenesis, vasculogenesis, transmembrane receptor protein tyrosine kinase signaling pathway, development, cell migration, hemopoiesis, cell differentiation, cell fate commitment, endothelial cell differentiation |
| Nkx2-5      | 1449566_at          | 1.9           | NK2 transcription factor related, locus 5 | Regulation of transcription, embryonic heart tube development |
| Psca        | 1451258_at          | 1.5           | Prostate stem cell antigen | |
| Pou3f2      | 1450831_at          | 1.7           | POU domain, class 3, transcription factor 2 | Positive regulation of cell proliferation, regulation of transcription |
| Pou5f1/Oct-4 | 1417945_at      | 1.5           | POU domain, class 5, transcription factor 1 | Germ-line stem cell maintenance, expressed in mouse totipotent embryonic stem and germ cells, regulation of transcription |
| Sox10       | 1451689_a_at        | 2.3           | SRY-box containing gene 10 | Regulation of transcription, cell differentiation and maturation |
| Thy1/CD90   | 1423135_at          | 1.5           | Thymus cell antigen 1, theta | MSC specific marker |
| Utf1        | 1416899_at          | 1.5           | Undifferentiated embryonic cell transcription factor 1 | Regulation of transcription |
| Col4a3bp    | 1420384_at          | −1.6          | Procollagen, type IV, alpha 3 binding protein | Goodpasture antigen binding protein |
| Egr2/Krox20 | 1427683_at          | −3.9          | Early growth response 2 | Schwann cell differentiation, myelination, rhythmic behavior, regulates osteocalcin expression |
| Falz        | 1427310_at          | −3.2          | Fetal Alzheimer antigen | Negative regulation of transcription |
| H2-K1       | 1426324_at          | −4.2          | Histocompatibility 2, K1, K region | Immune response, antigen presentation, endogenous antigen via MHC class I |
| Lif         | 1421207_at          | −8.7          | Leukemia inhibitory factor (transient downregulation during cell growth) | Embryonic stem cell maintenance, immune response, tyrosine phosphorylation of Stat3 protein, muscle morphogenesis, neuron development |
| Lifr        | 1425107_a_at        | −5.8          | Leukemia inhibitory factor receptor | Positive regulation of cell proliferation |
| TNFRSF11b/OPG | 1449033_aat     | −34.6         | Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) | Apoptosis, signal transduction, negative regulation of osteoclastogenesis |
| Zfp42/Rex-1 | 1451244_a_at        | −1.9          | Zinc finger protein 42 | The putative human stem cell marker, Rex-1 (Zfp42): structural classification and expression in normal human epithelial and carcinoma cell cultures |

<sup>a</sup> Affymetrix probe ID  
<sup>b</sup> fold change  
<sup>c</sup> gene ontology [28]
and irreversibly suppressed both the osteoblast phenotype and the osteoblast-specific gene expression [8]. The expression of PPARγ2 in marrow MSC increases with aging [14]. Moreover, bone marrow derived from old animals produces unknown PPARγ activator(s) that stimulates adipocyte differentiation and suppresses osteoblast differentiation [14]. These changes cause alterations in the milieu of intrinsic and extrinsic signals that determine MSC lineage allocation. For instance, this contributes to the preferential MSC differentiation toward adipocytes and decreased differentiation toward osteoblasts that leads to the development of senile osteopenia.

PPARγ plays an important role in the maintenance of bone homeostasis as demonstrated in several animal models of either bone accrual or bone loss depending on the status of PPARγ activity [12, 15–19]. A decrease in PPARγ activity resulted in increased bone mass due to increased osteoblast number [12, 18], whereas increased PPARγ activity

| Gene symbol | Probe ID a | FC b | Gene Description | Biological Process c |
|-------------|------------|------|-------------------|---------------------|
| Abcg2       | 1422906_at | −3.1 | ATP-binding cassette, subfamily G, member 2 | Stem cell marker, drug resistance |
| Cd9         | 1416066_at | −3.2 | CD9 antigen       | Stromal cell and adipose stem cell surface marker, tetraspan protein |
| Cd47        | 1419554_at | −2.4 | CD47 antigen (Rh-related antigen, | Hematopoietic cells, membrane glycoprotein, the same as integrin-associated protein (IAP) and ovarian tumor marker OA3 |
| Cd81        | 1416330_at | −1.6 | CD 81 antigen     | Cell adhesion, fertilization |
| Egfr        | 1424932_at | −1.8 | Epidermal growth factor receptor | Active in early events of stem cells recruitment and differentiation |
| Gja7        | 1449094_at | −3.8 | Gap junction membrane channel protein alpha 7 | Cell communication, synaptic transmission, heart development, visual perception, cell development, cardiac muscle development |
| Il6st       | 1437303_at | −2.9 | Interleukin 6 signal transducer | Signal transduction, positive regulation of cell proliferation, regulation of Notch signaling pathway |
| Lims1       | 1418231_at | −2.5 | LIM and senescent cell antigen-like domains 1 | Cell-matrix adhesion, establishment and/or maintenance of cell polarity, cell-cell adhesion, embryonic development |
| Cd36        | 1423166_at | 178.8 | CD36 antigen      | Fatty acid transporter associated with adipogenesis |
| Cd200 (Ox2) | 1448788_at | 2.4  | Cd200 antigen     | Cell surface antigen of thymocytes, B cells, T cells, neurons, kidney glomeruli, tonsil follicles, the syncytiotrophoblast and endothelial cells |
| Cd5         | 1418353_at | 1.6  | CD5 antigen       | B lymphocytes antigen |
| Cd63        | 1455777x_at | 1.9  | Cd63 antigen      | Melanoma antigen |
| Vegfa       | 1451959_a_at | 1.5  | Vascular endothelial growth factor A | Regulation of progression through cell cycle, angiogenesis, development, cell proliferation, cell differentiation |
| Vegfb       | 1451803_a_at | 2.6  | Vascular endothelial growth factor A | Regulation of progression through cell cycle, angiogenesis, development, cell proliferation, cell differentiation |

a Affymetrix probe ID  
b fold change  
c gene ontology [28]
| Gene symbol | Probe ID | PR versus P | P versus V | Gene description | Biological process |
|-------------|----------|-------------|------------|------------------|--------------------|
| Akp2        | 1423611_at | -11.5       | -2.0       | Alkaline phosphatase | Marker of osteoblasts |
| Cd2bp2      | 1417224_at | -1.9        | -1.5       | CD2 antigen binding protein 2 | T cell activation |
| Cd29 (lgb1) | 1426918_at | -2.1        | -1.5       | Integrin beta 1 (fibronectin receptor beta) | Regulation of progression through cell cycle, G1/S transition of mitotic cell cycle, cell adhesion, cell-matrix adhesion, integrin-mediated signaling pathway, development, positive regulation of cell proliferation, negative regulation of cell differentiation |
| Cd44        | 1423760_at | -3.9        | -5.6       | CD44 antigen | Cell surface glycoprotein, cell adhesion, stem cells, implicated in tumor growth and dissemination |
| Cd105 (Eng) | 1432176_at | -2.3        | -2.0       | Endoglin | Angiogenesis, cell adhesion, heart development, regulation of transforming growth factor beta receptor signaling pathway |
| Cd109       | 1425658_at | -2.8        | -5.2       | CD109 antigen | Membrane glycoprotein, elevated expression in variety of cancers |
| H2-D1       | 1451934_at | -3.2        | -3.0       | Histocompatibility 2, D region locus 1 | Immune response, detected on surface of MSC and adipocyte stem cells at low levels and reduced with passage |
| H2-K1       | 1427746_at | -1.6        | -1.5       | Histocompatibility 2, K1, K region | Immune response, antigen presentation |
| Mki67       | 1426817_at | -4.3        | -5.9       | Antigen identified by monoclonal antibody Ki 67 | Meiosis, cell proliferation |
| Pcna        | 1417947_at | -2.4        | -1.7       | Proliferating cell nuclear antigen | DNA replication |
| S100b       | 1434342_at | -4.2        | -2.7       | S100 protein, beta polypeptide, neural | Marker of differentiated neural cells |
| Spred1      | 1460116_s_at | -1.9       | -2.1       | Sprouty protein with EVH-1 domain 1, related sequence | Inhibition of MAP kinases, activated in hematopoietic cells, involved in mesoderm organization, inhibit Ras pathway (G protein) |
| Spred2      | 1434403_at | -2.3        | -1.7       | Sprouty protein with EVH-1 domain 2, related sequence | As above |
| Stag1       | 1434189_at | -1.5        | -1.7       | Stromal antigen 1 | Key mediator of p53-dependent apoptotic pathway, cell cycle, chromosome segregation, mitosis, and cell division |
| Stag2       | 1421849_at | -1.6        | -1.6       | Stromal antigen 2 | As above |
Table 2: Continued.

| Gene symbol | Probe IDa | PR versus P | FCb | P versus V | Gene description | Biological processc |
|-------------|-----------|-------------|-----|------------|------------------|---------------------|
| Cd1d1       | 1449130_at| 4.9         | 5.1 |            | CD1d1 antigen    | MHC class I-like glycoprotein, development and function of natural killer T lymphocytes |
| Cd151       | 1451232_at| 1.9         | 1.5 |            | CD151 antigen    | PPARγ positively regulates it in squamous cell carcinoma, implicated in tumor invasiveness |
| Fabp4       | 1424155_at| 69.6        | 1.7 |            | Fatty acid binding protein 4 | Marker of differentiated adipocytes |

a Affymetrix probe ID  
b fold change  
c gene ontology [28]

due to TZD administration led to the bone loss [15–17, 19]. TZD-induced bone loss was accompanied with changes in the cellular composition of the bone marrow, such as decreased numbers of osteoblasts and increased numbers of adipocytes, and changes in the MSC phenotype characterized by a loss of MSC plasticity. These changes are characteristics for aging bone marrow [20]. Recently, several human studies have demonstrated that TZD use is associated with decreased bone mineral density and an increased risk of fractures in postmenopausal diabetic women [21–23]. This prompted US Food and Drug Administration to issue a warning of possible adverse effects of TZD on human bone.

The development of high throughput analysis of gene expression using microarrays has advanced studies on genes and signaling pathways controlled by a single gene product. The transcriptional role of PPARγ in either differentiated cells or functional tissues has been studied using DNA microarrays, mostly to determine its role in the physiology during disease and as a result of therapeutic treatment with TZDs of these target tissues [24–26]. None of these studies, however, were designed to test for the effect of the PPARγ isoform on the molecular signature of MSC. Using a model of marrow MSC differentiation under the control of the PPARγ transcription factor, we found that both the presence of PPARγ and its activation with the antidiabetic TZD, rosiglitazone, resulted in gene expression changes for multiple genes that characterize the stem cell phenotype and their phenotypic lineages. Even though our model was originally developed to study the mechanisms by which PPARγ suppressed osteoblastogenesis and promoted adipogenesis, our studies suggest that PPARγ2 has a profound effect on the expression of signature genes for cell "stemness."

2. MATERIAL AND METHODS

2.1. Cell cultures and RNA isolation

Murine marrow-derived U-33 (previously referred to as UAMS-33) cells represent a clonal cell line spontaneously immortalized in the long term bone marrow culture conditions. To study the effect of PPARγ2 on marrow mesenchymal stem cell differentiation, U-33 cells were stably transfected with either PPARγ2 expression construct (referred to as U-33/γ2 cells) or an empty vector control (referred to as U-33/c cells) as described previously [8]. Several independent clones were retrieved after transfection and carefully analyzed for their phenotype. Clone 28.6, representing U-33/γ2 cells, and clone yc2, representing U-33/c cells, were used in the experiments presented in this manuscript. Cells were maintained in aMEM supplemented with 10% FBS heat-inactivated (HyClone, Logan, UT), 0.5 mg/ml G418 for positive selection of transfected cells, 100 μ/ml penicillin, 100 μ/ml streptomycin, and 0.25 μ/ml amphotericin (sigma) at 37°C in a humidified atmosphere containing 5% CO2. Media and additives were purchased from Life Technologies (Gaithersburg, MD).

Cells were propagated for one passage and then seeded at the density of 3 × 10^5 cells/cm^2. After 48 hours of growth, when cultures achieved approximately 80% confluency, cells were treated with either 1 μm rosiglitazone or the same volume of vehicle (DMSO) for 2, 24, and 72 hours, followed by RNA isolation using RNeasy kit (QIAGEN Inc., Valencia, CA). The replicate experiment was performed independently on a fresh batch of cells. Two replicates were used for microarray analysis. The factorial design of experiment was 2 × 3 × 2 which corresponded to two cell lines (with and without PPARγ2), three time points (2, 24, 72 hours), and two treatment regiments (rosiglitazone and vehicle).

2.2. Microarray experiments

RNA quality was assessed using the Agilent Model 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Five micrograms of total RNA were processed for use on the microarray by using the Affymetrix GeneChip one-cycle target labeling kit (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer’s recommended protocols. The resultant biotinylated cRNA was fragmented then hybridized to the GeneChip Mouse Genome 430 2.0 Array (45,000 probe sets used to analyze over 39,000 mouse transcripts and variants from over 34,000 well-characterized mouse genes; Affymetrix, Inc.). The arrays were washed, stained,
| Gene symbol | Probe IDa | FCb | Gene description | Biological processc |
|-------------|-----------|-----|------------------|---------------------|
| Actc1       | 1415927_at | −1.5 | Actin, alpha, cardiac | Cytoskeleton organization and biogenesis, muscle development, regulation of heart and muscle contraction |
| Actg2       | 1422340_at | −4.7 | Actin, gamma 2, smooth muscle, enteric | Cytoskeleton organization and biogenesis, muscle development |
| Cd97        | 1418394_at | 2.3  | CD97 antigen | Cell adhesion, signal transduction, G-protein coupled receptor protein signaling pathway, neuropeptide signaling pathway |
| Cd166 (ALCAM) | 1437466_at | 2.1  | Activated leukocyte cell adhesion molecule | Cell adhesion, axon guidance, motor axon guidance |
| Cxcl1       | 1419209_at | −2.7 | Chemokine (C-X-C motif) ligand 1 | Regulation of progression through cell cycle, inflammatory response, immune response |
| Cxcl4       | 1448995_at | −2.1 | Chemokine (C-X-C motif) ligand 4 | Chemotaxis, immune response, negative regulation of angiogenesis, cytokine, and chemokine mediated signaling pathway, platelet activation, negative regulation of megakaryocyte differentiation |
| Cxcl12 (SDF-1) | 1417574_at | −2.4 | Chemokine (C-X-C motif) ligand 12 (stem cell differentiation factor) | Patternning of blood vessels, ameboidal cell migration, chemotaxis, immune response, germ cell development and migration, brain development, motor axon guidance, T cell proliferation, induction of positive chemotaxis |
| Cxcl16      | 1456428_at | −1.7 | Chemokine (C-X-C motif) ligand 15 | Chemotaxis, inflammatory response, immune response, signal transduction, hematopoiesis, neutrophil chemotaxis |
| Foxa1       | 1418496_at | −1.5 | Forkhead box A1 | Regulation of transcription, lung development, epithelial cell differentiation, branching morphogenesis of a tube |
| Kitl        | 1415854_at | −4.1 | Kit ligand | Cell adhesion, germ cell development, positive regulation of peptidyl-tyrosine phosphorylation, cytokine product associated with MSC/stromal cells, stem cell factor |
| Ntf3        | 1450803_at | −1.5 | Neurotrophin 3 | Neuromuscular synaptic transmission, glial cell fate determination, axon guidance, brain and peripheral nervous system development, epidermis development, mechanoreceptor differentiation, regulation of neuron apoptosis |
Table 3: Continued.

| Gene symbol | Probe IDa | FCb | Gene description | Biological processc |
|-------------|-----------|-----|------------------|---------------------|
| Pdgfα       | 1421916_at | −2.1 1.6 | Platelet derived growth factor receptor, alpha polypeptide | Protein amino acid phosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway, morphogenesis, organ morphogenesis, extracellular matrix organization and biogenesis, male genitalia development, odontogenesis |
| Tnfsf11 (RANKL) | 1419083_at | −1.6 9.2 | Tumor necrosis factor (ligand) superfamily, member 11 | Positive regulation of osteoclast differentiation and bone resorption, immune response, lymph node development |
| Snai2       | 1418673_at | −6.4 1.9 | Snail homolog 2 (Drosophila) | Development of human melanocytes, regulation of transcription, DNA dependent, development, response to radiation, regulation of survival gene product activity |
| Vegfc       | 1419417_at | −5.6 11.5 | Vascular endothelial growth factor C | Regulation of progression through cell cycle, angiogenesis, positive regulation of neuroblast proliferation, development, positive regulation of cell proliferation, organ morphogenesis |

a Affymetrix probe ID  
b fold change  
c gene ontology [28]

and scanned using the Affymetrix Model 450 Fluidics Station and Affymetrix Model 3000 scanner using the manufacturer’s recommended protocols by the University of Iowa DNA Core Facility. Raw gene expression measurements were generated using the microarray suite (MAS) version 5.0 software (Affymetrix, Inc.). Statistical assessment of differential gene expression is described in Lecka-Czernik et al. [27].

3. RESULTS AND DISCUSSION

An essential role of PPARγ2 in the regulation of marrow MSC lineage allocation, together with the evidence of its increased activity in MSC with aging [14], prompted us to study the effect of PPARγ2 on the expression of stem cell gene markers. Two aspects were examined: the effect of the presence of PPARγ2 in U-33 stem cells and the effect of PPARγ2 activation with rosiglitazone on stem cell phenotype. Here we used a model of marrow MSC differentiation under the exclusive control of a single protein, PPARγ2. This system allows for relatively unambiguous studies of the unique effects of PPARγ2 isofrom on MSC phenotype. The model of PPARγ2-dependent MSC differentiation consists of two cell lines derived from the same parental cell line (U-33 cells), which either express the PPARγ2 protein (U-33/γ2 cells) or do not express the PPARγ2 protein (U-33/c cells) [8, 29]. To assess the effects of the presence of PPARγ2 on the phenotype of U-33 cells in nontreated conditions, we compared gene expression in U-33/γ2 and U-33/c cells maintained in basal growth conditions (this is referred to as the “P versus V” analysis). This comparison provides information about PPARγ2 activities, which are either ligand independent or acquired as a result of activation with natural ligands present in the growth media or endogenously produced by tested cells. The results of “P versus V” analysis may provide information on a role of PPARγ2 in a continuum of changes that occur in stem cells during aging. To assess an effect of rosiglitazone on the expression of stem cell-related genes, we compared gene expression in U-33/γ2 cells treated with rosiglitazone and nontreated U-33/γ2 cells (this is referred to as the “PR versus P” analysis). This analysis provides important information on the effects of rosiglitazone on the stem cell phenotype. Finally, comparison of the results of both analyzes provides information on differences between endogenous and artificially induced PPARγ2 activities in respect to stem cell gene expression.

To avoid differences in the cell phenotype due to different rates of cell growth, we chose the 72-hour time point for the analysis of gene expression (see Section 2). In basal growth conditions at this time point, cell cultures of U-33/γ2 and U-33/c were in state of confluence, cells acquired fibroblast-like appearance and cell cultures were indistinguishable morphologically from each other. In contrast, U-33/γ2 cells
| Gene symbol | Probe ID<sup>a</sup> | Gene description |
|-------------|----------------------|-----------------|
| Afp         | 1416645_at           | Alpha fetoprotein |
| Cd34        | 1416072_at           | CD34 antigen |
| Cd3z        | 1438392_at           | CD3 antigen, zeta polypeptide |
| Cd5l        | 1449193_at           | CD5 antigen like |
| Cd6         | 1451910_at           | CD6 antigen |
| Cd8b1       | 1448569_at           | CD8 antigen, beta chain 1 |
| Cd22        | 1419769_at           | CD22 antigen |
| Cd53        | 1439589_at           | CD53 antigen |
| Cd86        | 1420404_at           | CD86 antigen |
| Cd164       | 1431527_at           | CD164 antigen |
| Cd209e      | 1420582_at           | Cd209e antigen |
| Cdh15       | 1418602_at           | Protocadherin 15 |
| Cer1        | 1450257_at           | Cerberus 1 homolog |
| Col6a2      | 1452250_at           | Procollagen, type VI, alpha 2 |
| Erbb2ip     | 1439080_at           | Erbb2 interacting protein |
| Erbb3       | 1452482_at           | V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian) |
| Fabp7       | 1450779_at           | Fatty acid binding protein 7, brain |
| Fzd9        | 1427529_at           | Frizzled homolog 9 |
| Gata2       | 1450333_at           | GATA binding protein 2 |
| Gcg         | 1425952_at           | Glucagon |
| Gcm2        | 1420455_at           | Glial cells missing homolog 2 |
| Gfap        | 1440142_at           | Glial fibrillary acidic protein |
| Gjb3        | 1416715_at           | Gap junction membrane channel protein beta 3 |
| Gjb4        | 1422179_at           | Gap junction membrane channel protein beta 4 |
| Ina         | 1418178_at           | Internexin neuronal intermediate filament protein, alpha |
| Ins1        | 1422447_at           | Insulin 1 |
| Isl1        | 1444129_at           | ISL1 transcription factor, LIM/homeodomain (islet 1) |
| Krt1-14     | 1460347_at           | Keratin complex 1, acidic, gene 14 |
| Krt1-17     | 1423227_at           | Keratin complex 1, acidic, gene 17 |
| Krt2-8      | 1435989_at           | Keratin complex 2, basic, gene 8 |
| Mbp         | 1454651_at           | Myelin basic protein |
| Mtap1b      | 1450397_at           | Microtubule-associated protein 1 B |
| Myh11       | 1448962_at           | Myosin, heavy polypeptide 11, smooth muscle |
| Ncam1       | 1439556_at           | Neural cell adhesion molecule 1 |
| Ncam2       | 1425301_at           | Neural cell adhesion molecule 2 |
| Nes         | 1453997_at           | Nestin |
| Ngfr        | 1421241_at           | Nerve growth factor receptor (TNFR superfamily, member 16) |
| Nkx2-2      | 1421112_at           | NK2 transcription factor related, locus 2 (Drosophila) |
| Numb        | 1425368_at           | Numb gene homolog (Drosophila) |
| Oligl1      | 1416149_at           | Oligodendrocyte transcription factor 1 |
| Pax6        | 1456342_at           | Paired box gene 6 |
| Pou3f3      | 1422331_at           | POU domain, class 3, transcription factor 3 |
| Pou6f1      | 1420749_at           | POU domain, class 6, transcription factor 1 |
| Prox1       | 1457432_at           | Prospero-related homeobox 1 |
| Ptprc       | 1440165_at           | Protein tyrosine phosphatase, receptor type, C |
| Slc1a2      | 1451627_at           | Solute carrier family 1 (glial high affinity glutamate transporter), member 2 |
| Slc1a6      | 1418933_at           | Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6 |
| Sox1        | 1422205_at           | SRY-box containing gene 1 |
| Sox2        | 1416967_at           | SRY-box containing gene 2 |
| Syn1        | 1453467_at           | Synapsin 1 |
| Tubb3       | 1415978_at           | Tubulin, beta 3 |
| Zif110      | 1450998_at           | Zinc finger protein 110 |

<sup>a</sup> Affymetrix probe ID
treated for 72 hours with rosiglitazone acquired adipocyte phenotype typified by large fat droplets. A morphological appearance of U-33/c cells treated with rosiglitazone was indistinguishable from nontreated U-33/c cells as well as non-treated U-33/y2 cells.

There are no known exclusive markers for MSC. However, based on extensive work with MSCs and other stem cell populations, several proteins have emerged as candidate markers associated with a stem cell phenotype. These entities include ATP-binding cassette g2 (Abcg2), cell surface antigen CD44, stem cell factor or kit ligand (SCF/Kitl), epidermal growth factor receptor (Egrf), early growth response factor 2 (Egr2), leukemia inhibitory factor (Lif), leukemia inhibitory factor receptor (Lifr), and stromal-derived factor/CXC-chemokine ligand 12 (SDF-1/CXCL12). Based on the available published information for stem cell gene expression for the analysis, we arbitrarily chose 135 genes that represent markers of either early or lineage committed stem cells [9, 30–34]. The analysis showed that the expression of 38% of analyzed genes was not affected by activation state of PPARγ2 (see Table 4), the expression of 28% genes was exclusively affected by the presence of PPARγ2 ("P versus V" analysis) (see Table 1(a)), and the expression of 10% genes was exclusively affected by rosiglitazone-activated PPARγ2 ("PR versus P" analysis) (see Table 1(b)). The genes whose expression was affected by both rosiglitazone-activated and non-activated PPARγ2 constituted 24% of the total genes studied; their expression was affected in equal proportion either similarly (see Table 2) or in the opposite direction in these two conditions (see Table 3).

Comparison of the two cell lines indicates that a majority of analyzed genes are up-regulated in U-33/y2 versus U-33/c cells (see Tables 1(a) and 3). Most of these genes are characteristic for stem cells of hematopoietic and neural lineages while some of them are expected to be up regulated in hematopoiesis supporting stromal cells (e.g. Kitl, RANKL (Table 1(a)), and the CXCL family (Tables 1(a) and 3)).

These interesting observations have at least two reasonable interpretations. The first interpretation suggests that observed differences are a reflection of different phenotypes of the two individual parental cells from which each of the two clones originated. Hence, differences in gene expression between both cell lines are PPARγ2-independent. The second possibility suggests that these differences are PPARγ2-dependent and result from either PPARγ2 ligand-independent activity or activity acquired from endogenous ligand. Several lines of evidence suggest a correlation between the adipocyte-like phenotype of marrow stroma cells and support for hematopoiesis [35, 36]. Hematopoiesis depends heavily on the microenvironment provided by mesenchymal cell compartment in the marrow and the ability of these cells to produce growth factors and cytokines that act in a paracrine fashion to influence the differentiation of hematopoietic progenitors. In the long term bone marrow cultures, an in vitro system of hematopoietic cell differentiation, stroma cell support for myelopoiesis, is provided by cultures consisting mostly of adipocytes [35, 37]. Similarly, in vivo studies in a model of SAMP6 mice that are characterized by senile osteopenia due to a diminished number of osteoblasts and increased myelopoiesis, correlates positively with an increased number of marrow adipocytes [38]. Interestingly, U-33/y2 cells support osteoclastogenesis much better than U-33/c cells (unpublished observation), in part due to relatively higher RANKL (9-fold in “P versus V”, Table 3) and lower OPG (34.6-fold in “P versus V”); Table 1(a)) expression. Another important regulator of bone marrow hematopoiesis, including osteoclastogenesis, is represented by the chemokine CXCL12 or SDF-1 [39, 40]. Growing experimental evidence indicates that CXCL12 and its receptor CXCR4 axis is not only required for hematopoietic stem cell signaling but also has a crucial role in the formation of multiple organ systems during embryogenesis as well as adult nonhematopoietic tissue regeneration and tumorigenesis [39]. According to our analysis, an expression of CXCL12, but not CXCR4, is up regulated in U-33/y2 cells (“P versus V”) and suppressed by PPARγ2-activated with rosiglitazone (“PR versus P”) (see Table 3). Thus, it is conceivable that mesenchymal cells which express PPARγ2 acquire the adipocyte-like phenotype typified by the production of number of cytokines and support hematopoietic stem cell differentiation.

While PPARγ2 has a positive effect on the stromal phenotype supporting hematopoiesis, it has a negative effect on the expression of “stemness” genes. The expression of LIF cytokine and its receptor, a regulatory system required for the stem cell self renewal, is significantly suppressed in U-33/y2 cells as compared to U-33/c cells (see Table 1(a)). Interestingly, activation of PPARγ2 with rosiglitazone did not affect the expression of these genes. The presence of PPARγ2 in U-33/y2 cells suppresses the expression of Egr2/Krox20, a stem cell-specific transcription factor with a role in the development of nervous system and endochondral bone formation [41]. Egr2/Krox20 also regulates osteoblast differentiation and osteocalcin expression [42]. Again, rosiglitazone does not affect Egr2/Krox20 gene expression (see Table 1(a)). PPARγ2 cellular presence also affects expression of Zfp42 transcription factor, which is a marker of human and murine embryonic stem (ES) cells. Expression of Zfp42 is down regulated during ES cell differentiation [43]. An artificial knockdown of Zfp42 with RNAi resulted in spontaneous differentiation of ES cells toward endoderm and mesoderm lineages, whereas its overexpression led to the loss of self-renewal capacity of ES cells [44].

The expression of ABCG2, a well recognized stem cell marker [45], was down-regulated in “PR versus P” (−3.1 fold) (see Table 1(b)) and slightly in “P versus V” (−1.3 fold, P < .01) conditions (not shown). ABCG2 represents an ATP-binding cassette (ABC) transporter which serves to efflux certain xenobiotics (including anticancer drugs) that can lead to the development of multidrug resistance syndrome. This is a significant obstacle in cancer treatment [46]. This gene is also considered to be a marker of primitive pluripotent stem cells, termed “side population,” which were identified based on their ability to exclude Hoechst dye [45]. The ability to exclude a variety of substances may comprise a mechanism that protects stem cells from exogeneous and endogeneous toxins. Finding that ABCG2 expression is down regulated by PPARγ2, especially after activation with
rosiglitazone, implicates PPARγ as a negative regulator of stem cell phenotype as well as a negative regulator of multidrug resistance. Similarly, Egfr a marker of early stem cells is down regulated by PPARγ when activated with rosiglitazone [47].

Interestingly, however, the expressions of Oct-4 (POU5f1) and FGF4, well recognized embryonic stem cell markers highly expressed in the totipotent and pluripotent ES cells [48, 49] are up regulated in U-33/y2 cells compared to U-33/c cells and are not affected in U-33/y2 cells treated with rosiglitazone (see Table 1(a)).

Another interesting grouping consists of genes whose expression is differentially regulated by both activated and non-activated PPARγ (see Table 2). A number of genes implicated in early stem cell maintenance and recruitment, among them CD44, H2-D1, PCNA, CD109, Spred1 and 2, and Stag1 and 2, are down regulated in U-33/y2 cells in both basal conditions and upon rosiglitazone treatment.

The last category represents gene markers specific for terminally-differentiated cells. Consistent with the proadipocytic and antiosteoblastic activities of PPARγ2 activated with rosiglitazone, the expression of the gene encoding FABP4 increases, whereas an expression of the gene underlying alkaline phosphatase decreases. Markers of the neuronal phenotype are either decreased (S100b, Table 2) or not affected (nestin and NCAMs, Table 4), and the expression of CD34, a bona fide marker for cells of hematopoietic lineage, is not affected (see Table 4). However, the expression patterns of gene markers characteristic for embryonic stem cells and a large number of markers that are associated with a nonmesenchymal phenotype, including markers of different hematopoietic and neuronal lineages, indicates that marrow mesenchymal U-33 cells possess a mixed phenotype with some characteristics of early primitive pluripotent stem cells and lineage oriented mesenchymal cells.

In conclusion, PPARγ2 is a powerful modulator of the stem cell phenotype and its activation with antidiabetic TZDs affect the expression of “stemness” genes. It is unclear at this time whether, and to what extent, PPARγ2 is expressed in MSCs with aging [14] and a loss of marrow MSC plasticity or ability to convert between phenotypes as a result of aging and TZD therapy [20], suggest that aging and TZD therapy may affect stem cell phenotype through modulation of PPARγ2 activity. These observations may also have important therapeutic consequences and indicate a need for more detailed studies of PPARγ2 role in stem cell biology.

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REFERENCES

[1] E. D. Rosen and B. M. Spiegelman, “PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth,” Journal of Biological Chemistry, vol. 276, no. 41, pp. 37731–37734, 2001.
[2] Y. Zhu, C. Qi, J. R. Korenberg, et al., “Structural organization of mouse peroxisome proliferator-activated receptor γ (mPPARγ) gene: alternative promoter use and different splicing yield two mPPARγ isoforms,” Proceedings of the National Academy of Sciences of the United States of America, vol. 92, no. 17, pp. 7921–7925, 1995.
[3] L. Fajas, D. Auboeuf, E. Raspé, et al., “The organization, promoter analysis, and expression of the human PPARγ gene,” Journal of Biological Chemistry, vol. 272, no. 30, pp. 18779–18789, 1997.
[4] L. Fajas, J.-C. Fruchart, and J. Auwerx, “PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter,” FEBS Letters, vol. 438, no. 1-2, pp. 55–60, 1998.
[5] Y. Chen, A. R. Jimenez, and J. D. Medh, “Identification and regulation of novel PPARγ splice variants in human THP-1 macrophages,” Biochimica et Biophysica Acta, vol. 1759, no. 1-2, pp. 32–43, 2006.
[6] D. Yamashita, T. Yamaguchi, M. Shimizu, N. Nakata, F. Hirao, and T. Osumi, “The transactivating function of peroxisome proliferator-activated receptor γ is negatively regulated by SUMO conjugation in the amino-terminal domain,” Genes to Cells, vol. 9, no. 11, pp. 1017–1029, 2004.
[7] D. Ren, T. N. Collingwood, E. J. Rebar, A. P. Wolfe, and H. S. Camp, “PPARγ knockdown by engineered transcription factors: exogenous PPARγ2 but not PPARγ1 reactivates adipogenesis,” Genes and Development, vol. 16, no. 1, pp. 27–32, 2002.
[8] B. Lecka-Czernik, I. Gubrij, E. J. Moerman, et al., “Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARγ2,” Journal of Cellular Biochemistry, vol. 74, no. 3, pp. 357–371, 1999.
[9] Y. Jiang, B. N. Jahagirdar, R. L. Reinhardt, et al., “Pluripotency of mesenchymal stem cells derived from adult marrow,” Nature, vol. 418, no. 6893, pp. 41–49, 2002.
[10] D. Bianco, M. Riminucci, S. Gronthos, and P. G. Robey, “Bone marrow stromal stem cells: nature, biology, and potential applications,” Stem Cells, vol. 19, no. 3, pp. 180–192, 2001.
[11] J. E. Aubin, “Regulation of osteoblast formation and function,” Reviews in Endocrine and Metabolic Disorders, vol. 2, no. 1, pp. 81–94, 2001.
[12] T. Akune, S. Ohba, S. Kamekura, et al., “PPARγ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors,” Journal of Clinical Investigation, vol. 113, no. 6, pp. 846–855, 2004.
[13] M. J. Jeon, J. A. Kim, S. H. Kwon, et al., “Activation of peroxisome proliferator-activated receptor-γ inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts,” Journal of Biological Chemistry, vol. 278, no. 26, pp. 23270–23277, 2003.
[14] E. J. Moerman, K. Teng, D. A. Lipschitz, and B. Lecka-Czernik, “Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPARγ2 transcription factor and TGF-β/BMP signaling pathways,” Aging Cell, vol. 3, no. 6, pp. 379–389, 2004.
[15] S. O. Rzonca, L. J. Suva, D. Gaddy, D. C. Montague, and B. Lecka-Czernik, “Bone is a target for the antidiabetic compound rosiglitazone,” Endocrinology, vol. 145, no. 1, pp. 401–406, 2004.
[16] V. Sottile, K. Seuwen, and M. Kneissel, “Enhanced marrow adipogenesis and stromal resorption in estrogen-deprived rats treated with the PPARγ agonist BRL49653 (rosiglitazone),” *Calculated Tissue International*, vol. 75, no. 4, pp. 329–337, 2004.

[17] M. A. Sorocéanu, D. Miao, X.-Y. Bai, H. Su, D. Goltzman, and A. C. Karaplis, “Rosiglitazone impacts negatively on bone by promoting osteoblast/osteocyte apoptosis,” *Journal of Endocrinology*, vol. 183, no. 1, pp. 203–216, 2004.

[18] T.-A. Cock, J. Back, F. Elefteriou, et al., “Enhanced bone formation in lipidostrophic PPARγΔ/Δ mice relocates haematopoiesis to the spleen,” *EMBO Reports*, vol. 5, no. 10, pp. 1007–1012, 2004.

[19] A. A. Ali, R. S. Weinstein, S. A. Stewart, A. M. Parfitt, S. C. Manolagas, and R. L. Jilka, “Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation,” *Endocrinology*, vol. 146, no. 3, pp. 1226–1235, 2005.

[20] O. P. Lazarenko, S. O. Rzonca, W. R. Hogue, F. L. Swain, L. J. Suva, and B. Lecka-Czernik, “Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone,” *Endocrinology*, vol. 148, no. 6, pp. 2669–2680, 2007.

[21] A. V. Schwartz, D. E. Sellmeyer, E. Vittinghoff, et al., “Thiazolidinedione use and bone loss in older diabetic adults,” *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3349–3354, 2006.

[22] A. Grey, M. Bolland, G. Gamble, et al., “The peroxisome proliferator-activated receptor-γ agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial,” *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 4, pp. 1305–1310, 2007.

[23] S. E. Kahn, S. M. Haffner, M. A. Heise, et al., “Glycemic dura-

[24] M. A. Sorocéanu, D. Miao, X.-Y. Bai, H. Su, D. Goltzman, and A. C. Karaplis, “Rosiglitazone impacts negatively on bone by promoting osteoblast/osteocyte apoptosis,” *Journal of Endocrinology*, vol. 183, no. 1, pp. 203–216, 2004.

[25] T.-A. Cock, J. Back, F. Elefteriou, et al., “Enhanced bone formation in lipidostrophic PPARγΔ/Δ mice relocates haematopoiesis to the spleen,” *EMBO Reports*, vol. 5, no. 10, pp. 1007–1012, 2004.

[26] A. A. Ali, R. S. Weinstein, S. A. Stewart, A. M. Parfitt, S. C. Manolagas, and R. L. Jilka, “Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation,” *Endocrinology*, vol. 146, no. 3, pp. 1226–1235, 2005.

[27] O. P. Lazarenko, S. O. Rzonca, W. R. Hogue, F. L. Swain, L. J. Suva, and B. Lecka-Czernik, “Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone,” *Endocrinology*, vol. 148, no. 6, pp. 2669–2680, 2007.

[28] A. V. Schwartz, D. E. Sellmeyer, E. Vittinghoff, et al., “Thiazolidinedione use and bone loss in older diabetic adults,” *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3349–3354, 2006.

[29] A. Grey, M. Bolland, G. Gamble, et al., “The peroxisome proliferator-activated receptor-γ agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial,” *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 4, pp. 1305–1310, 2007.

[30] S. E. Kahn, S. M. Haffner, M. A. Heise, et al., “Glycemic dura-

[31] A. J. Katz, A. Tholpady, S. S. Tholpady, H. Shang, and R. C. Ogle, “Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells,” *Stem Cells*, vol. 23, no. 3, pp. 412–423, 2005.

[32] N. B. Ivanova, J. T. Dimos, C. Schaniel, J. A. Hackney, K. A. Moore, and I. R. Lemischka, “A stem cell molecular signature,” *Science*, vol. 298, no. 5593, pp. 601–604, 2002.

[33] M. Ramalho-Santos, S. Yoon, Y. Matsuzaki, R. C. Mulligan, and D. A. Melton, “Stemness”: transcriptional profiling of embryonic and adult stem cells, *Science*, vol. 298, no. 5593, pp. 597–600, 2002.

[34] Superarray Bioscience Corporation, “Oligo GEArray: Mouse Stem Cell Microarray,” 2006.

[35] M. Tavassoli, “Fatty involution of marrow and the role of adipose tissue in hematopoiesis,” in *Handbook of the Hematopoietic Microenvironment*, M. Tavassoli, Ed., pp. 157–187, Humana Press, Clifton, NJ, USA, 1989.

[36] J. M. Gimble, M.-A. Dorheim, Q. Cheng, et al., “Response of bone marrow stromal cells to adipogenic antagonists,” *Molecular and Cellular Biology*, vol. 9, no. 11, pp. 4587–4595, 1989.

[37] M. Tavassoli, “Marrow adipose cells and hemopoiesis: an inter-

[38] O. Kajkenova, B. Lecka-Czernik, I. Gubriš, et al., “Increased adipogenesis and myelopoiesis in the bone marrow of SMP6, a murine model of defective osteoblastogenesis and low turnover osteopenia,” *Journal of Bone and Mineral Research*, vol. 12, no. 11, pp. 1772–1779, 1997.

[39] M. Z. Ratajczak, E. Zuba-Surma, M. Kucia, R. Rea, W. Wojakowski, and J. Ratajczak, “The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigen-

[40] S. Gronthos and A. C. W. Zannettino, “The role of the chemokine CXCL12 in osteoclastogenesis,” *Trends in Endocrinology and Metabolism*, vol. 18, no. 3, pp. 108–113, 2007.

[41] O. Voiculescu, P. Charnay, and S. Schneider-Maunoury, “Expression pattern of a Krox-20/Cre knock-in allele in the developing hindbrain, bones, and peripheral nervous system,” *Genesis*, vol. 26, no. 2, pp. 123–126, 2000.

[42] N. Lederc, T. Noh, A. Khokhar, E. Smith, and B. Frenkel, “Glucocorticoids inhibit osteocalcin transcription in osteoblasts by suppressing Egr2/Krox20-binding enhancer,” *Arthritis and Rheumatism*, vol. 52, no. 3, pp. 929–939, 2005.

[43] N. P. Mongan, K. M. Martin, and L. J. Gudas, “The putative human stem cell marker, Rex-1 (Zfp42): structural classifica-

[44] J.-Z. Zhang, W. Gao, H.-B. Yang, B. Zhang, Z.-Y. Zhu, and Y.-F. Xue, “Screening for genes essential for mouse embryonic stem cell self-renewal using a subtractive RNA interference library,” *Stem Cells*, vol. 24, no. 12, pp. 2661–2668, 2006.

[45] S. Zhou, J. D. Schuetz, K. D. Bunting, et al., “The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype,” *Nature Medicine*, vol. 7, no. 9, pp. 1028–1034, 2001.

[46] C. Hirschmann-Jax, A. E. Foster, G. G. Wulf, et al., “A distinct "side population" of cells with high drug efflux capacity in human tumor cells,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 39, pp. 14228–14233, 2004.
[47] R. W. C. Wong, “Transgenic and knock-out mice for deciphering the roles of EGFR ligands,” *Cellular and Molecular Life Sciences*, vol. 60, no. 1, pp. 113–118, 2003.

[48] M. Pesce and H. R. Schöler, “Oct-4: gatekeeper in the beginnings of mammalian development,” *Stem Cells*, vol. 19, no. 4, pp. 271–278, 2001.

[49] D. G. Simmons and J. C. Cross, “Determinants of trophoblast lineage and cell subtype specification in the mouse placenta,” *Developmental Biology*, vol. 284, no. 1, pp. 12–24, 2005.