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Molecular Mediators of Mesenchymal Stem Cell Biology

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Abstract

Mesenchymal stem cells (MSCs) have the ability to self-renew and differentiate into multiple lineages making them an appropriate candidate for stem cell therapy. In spite of achieving considerable success in preclinical models, limited success has been achieved in clinical settings with MSCs. A major impediment that is faced is low survival of MSCs in injured tissues following implantation. In order to enhance the reparative properties of MSCs, it is vital to understand the molecular signals that regulate MSC survival and self-renewal. This review assimilates information that characterizes MSCs and mentions their utilization in myocardial infarction therapy. Additionally, our attempt herein is to gather pertinent published information regarding the role of canonical Wnt and BMP signaling in regulating the potential of MSCs to self-renew, proliferate, differentiate, and survive.

I. Introduction

Stem cell therapy is an exciting new field that shows a lot of promise. The efficacy of mesenchymal stem cells (MSCs) as treatment for regeneration and/or repair is currently being clinically tested. However, preclinical models of MSC-directed wound repair show disparate degrees of effectiveness. The greatest barrier faced in these models is the low levels of engraftment of the transplanted cells. Therefore, increasing MSC maintenance in the wound would increase their reparative capabilities. Understanding MSC biology would allow for clinicians to harness the potential these cells have to offer. Several published reports have looked at molecular mediators of MSC biology and this review aims to assemble such data. Particularly, data pertaining to the canonical Wnt and bone morphogenetic protein (BMP) signaling cascades, which have been implicated in regulating MSC differentiation, proliferation, and survival, will be discussed. Signals which amplify the MSC pool, that is, enhance their self-renewal, are largely unknown. This review will also discuss the effects of secreted Frizzled-related protein 2 (sFRP2) on self-renewal and ultimately try to elucidate important molecules involved in MSC biology.

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II. Mesenchymal Stem Cells

Stem cells are defined by their ability to self-renew and differentiate into multiple cell types (Fuchs and Segre, 2000). MSCs, traditionally defined as bone marrow-derived fibroblast-like cells, can also be isolated from adipose tissue (Madonna *et al.*, 2009), umbilical cord blood (Harris, 2008; Zhang *et al.*, 2011), and fetal tissues (Pozzobon *et al.*, 2010). MSCs can differentiate into osteogenic, chondrogenic, and adipogenic lineages and give rise to any tissue derived from mesenchyme including bone, muscle, fibroblast, tendon, ligament, and adipose tissue (Pittenger *et al.*, 1999; Tropel *et al.*, 2004). Murine MSCs have been characterized to be negative for CD11b, CD14, CD31, CD34, and CD45 but positive for CD44, CD29, CD73, CD105, CD106, CD166, and Stem Cell Antigen 1 (SCA1) (Baddoo *et al.*, 2003; Rombouts and Ploemacher, 2003; Short *et al.*, 2003). However, the minimal criteria for antigenic identification is shown in Fig. 2.1, as murine MSCs must be lineage negative (Lin⁻), CD45⁻, CD44⁺, and SCA-1⁺.

III. Differentiation of MSCs

The trilineage *in vitro* differentiation of MSCs can be confirmed in different manners. Molecular markers of adipogenic (i.e., peroxisome proliferator-activated receptor gamma, PPAR- γ), chondrogenic (i.e., Collagen XI or Runx2), and osteogenic (i.e., Osteocalcin or Runx2) differentiation may be quantified (Takada *et al.*, 2009). Staining techniques exist for the visual confirmation of lineage commitment. Adipogenesis can be verified with Oil Red-O staining (Delorme and Charbord, 2007). Matrix calcification, which is present following osteogenesis, can be visualized with Alizarin Red or Von Kossa stains (Delorme and Charbord, 2007). Finally, glycosaminoglycans can be stained with Alcian blue (Denker *et al.*, 1999) or dimethylmethylene blue (Farndale *et al.*, 1986) as confirmation of chondrogenic differentiation. Several cytokines, growth factors, adhesion molecules, and extracellular matrix components have been identified as cues that signal MSCs to differentiate (Kratchmarova *et al.*, 2005; Mannello *et al.*, 2006). Included among these are canonical Wnt and BMP signaling which will be discussed in more detail in the following sections.

IV. Self-Renewal

The self-renewal process allows a stem cell to perform symmetric cell division to give rise to two nondifferentiated daughter cells. For self-renewal to occur, stem cells must proliferate in such a way that apoptosis and differentiation are avoided (Satija *et al.*, 2007; Schofield, 1983). Few reports have documented the self-renewal capability of MSCs (Song *et al.*, 2006), and therefore, the signaling involved in this process is largely unknown. On the other hand, self-renewal has been widely documented in the hematopoietic stem cell (HSC) field, and the Wnt, Notch, and BMP signaling cascades are accepted as mediators of the maintenance of a non-differentiated HSC pool (McReynolds *et al.*, 2007; Reya and Clevers, 2005; Suzuki and Chiba, 2005). Canonical Wnt signaling together with Notch and BMP signaling have also been shown to modulate stem cell self-renewal in the intestinal stem cell niche (Haramis *et al.*, 2004; He *et al.*, 2004; van Es *et al.*, 2005). Additionally, Wnt signaling has been shown to direct self-renewal and differentiation of Islet1-expressing precursor cells in neo- and postnatal hearts (Klaus *et al.*, 2007; Qyang *et al.*, 2007; reviewed in reference

Klaus and Birchmeier, 2008). Building on this knowledge, our laboratory has recently demonstrated that inhibition of BMP and Wnt by sFRP2 increases MSC self-renewal.

V. MSC Therapy

Although preclinical studies have recognized significant benefits of stem cell therapy, its translation for clinical application is still in its infancy. MSCs have been able to repair infarcted myocardium, bone, and soft tissue (Horwitz *et al.*, 1999; Orlic *et al.*, 2001). MSCs have been reported to induce angiogenesis and secrete paracrine and mitogenic growth factors (Iyer and Rojas, 2008; Kinnaird *et al.*, 2004; Silva *et al.*, 2005; Tang *et al.*, 2004). Two major clinical approaches are utilized for stem cell therapy: endogenous mobilization of progenitor stem cells and exogenous transplantation of culture-expanded stem cells. Presently, there are around 100 clinical trials that involve exogenous human MSCs (<http://clinicaltrials.gov/ct2/home>; U.S. National Library of Medicine, accessed on 04/28/11). They are at various stages and target a wide variety of pathological conditions such as Crohn's disease and graft versus host disease (GVHD), cardiovascular disease and myocardial infarction (MI), brain and spinal cord injury, ischemic stroke, diabetes, cartilage and bone injury (Phinney and Prockop, 2007). Mixed results have been obtained from these trials, and strategies to enhance MSC engraftment and survival in the regenerating tissues are being developed.

MSC therapy has demonstrated a few promising results. One particular trial documented that intracoronary administration of MSC following MI initially showed significant improvement in one parameter assessing left ventricular function; however, this difference was no longer significant when analyzed after 18 months (Meyer *et al.*, 2006). A phase II clinical trial documented a 2-year reduction in the mortality rate in the GVHD patients treated with MSCs (Le Blanc *et al.*, 2008). Clinically, MSCs homed to the site of injury when injected intravenously into irradiated osteogenesis imperfecta patients, engrafting into bones, skin, and marrow stroma, ultimately stimulating growth (Horwitz *et al.*, 1999, 2002). There have been no adverse events identified in the past and ongoing trials, this being the most advantageous outcome from these trials.

VI. Immunomodulatory Properties

The majority of the clinical trials, approximately 44%, are utilizing MSCs for their immunomodulatory properties (<http://clinicaltrials.gov/ct2/home>; U.S. National Library of Medicine, accessed on 04/28/11). In conditions like GVHD, Crohn's disease, primary Sjogren's syndrome, organ transplantation and rejection, systemic sclerosis, type I diabetes, systemic lupus erythematosus, multiple sclerosis, neuroblastoma, and non-malignant red blood cell disorders, MSCs are being transplanted as treatment by themselves or as adjunct therapy.

The biology behind the effects of MSCs on the immune system is mostly unknown; however, a few experimental models have elucidated some of the key molecular players involved in the anti-inflammatory role of MSCs. One of these models is a rat renal transplantation model where MSC injections increased overall survival of the recipient

animals due, in part, to a decrease in interleukin-1 α (IL-1), tumor necrosis factor- α (TNF- α), and transforming growth factor (TGF)- β 1 (Zhang *et al.*, 2007). The authors of this work remind us that there are still unknowns, as they note that adjunct immunosuppression (cyclosporine A) treatment that inhibits IL-2 signaling further increases the survival of the rats and further diminishes the levels of the inflammatory cytokines (Laupacis *et al.*, 1982).

Other models have shown a similar effect of MSCs on the downregulation of the immune function. Such is a rodent model of interstitial lung disease, where bleomycin (a cytotoxic glycopeptide antibiotic; Mitchell *et al.*, 1989) treatment induces pulmonary fibrosis and concomitant inflammation (Iyer and Rojas, 2008). Following induction of the disease, Ortiz *et al.*, 2007 treated the mice with MSCs and demonstrated a decrease in the levels of two important proinflammatory cytokines: TNF- α and IL-1 α . This group demonstrated the anti-inflammatory capacity of MSCs in this setting due to the expression of interleukin-1 receptor antagonist (IL-1RN). These results were confirmed by *in vitro* assays in which MSC-conditioned media decreased the proliferation of an IL-1 responsive T-cell population (Ortiz *et al.*, 2007).

Studies using an experimental autoimmune encephalomyelitis model demonstrated that MSCs were able to suppress T-cell activation *in vitro* and *in vivo*, and this effect was partially reversible by the addition of IL-2 (Zappia *et al.*, 2005). In this case, the authors suggest that the limited expression of MHC class II molecules as well as lack of costimulatory molecules, such as CD80, CD86, and CD40, on MSCs may be the reason behind the observed suppression. A more detailed study on secreted factors in MSC-conditioned media demonstrated that IL-10, TGF- β 1, and prostaglandin E2 (PGE2) were not responsible for the T-cell inhibition (Shi *et al.*, 2000).

In summary, the roles of MSCs in immune suppression have been partially described and the molecular mechanism behind this capacity remains elusive. Further studies on the effects of IL-1RN on T-cells might give insight into how MSCs inhibit inflammation and prevent T-cell activation.

VII. MI Therapy

Treatment for myocardial repair and ventricular dysfunction is the second most common application for MSCs in the clinic (U.S. National Library of Medicine). Recently, Osiris Therapeutics published the safety of utilizing MSCs in the setting of MI. Moreover, they demonstrated that MSC transplantation, compared to placebo control, increased left ventricular ejection fraction and reversed adverse remodeling (Hare *et al.*, 2009). Although the data was only available after a 6-month follow-up, all MSC-treated patients had a statistical improvement in heart function.

In spite of having considerable potential, the clinical application of MSC-based therapy in the context of myocardial repair faces many challenges. Those challenges include poor tissue engraftment, low potency, and low survival of transplanted MSCs. In clinical trials, in spite of MSCs positively affecting myocyte regeneration, poor short-term survival with modest improvement in heart function together with inconsistent outcomes were identified

(Lunde *et al.*, 2006; Schachinger *et al.*, 2006; Tendera *et al.*, 2009). In the light of these observations, it is evident that the therapeutic effects of MSCs could only be seen if transplanted MSCs could exert the long-term benefits to the patients. It is therefore very important to understand the biology and the tissue repair mechanism of MSCs in order to overcome the limitations of cell engraftment and survival.

VIII. Molecular Mediators of MSC Biology

A. Wnt pathway

Figure 2.2 depicts a simplified model of the canonical cascade through which secreted Wnt glycoproteins initiate signal transduction upon receptor binding. In the absence of Wnt signaling, β -catenin is in a complex with Axin, APC, Dsh, GSK3- β , and CK-1 (Reya and Clevers, 2005). The latter two phosphorylate β -catenin, marking it for ubiquitination and subsequent proteosomal degradation. Occupancy of the transmembrane receptors LRP5/6 and Frizzled by Wnt family members block the kinase activity of the β -catenin destruction complex, allowing the accumulation of β -catenin in the cytoplasm and its translocation to the nucleus. Within the nucleus, this signaling molecule engages TCF/LEF DNA binding proteins to help drive expression of target genes (Moon *et al.*, 2004).

Inhibition of the Wnt pathway can occur at different stages of the cascade. For example, Dickkopf-1 (Dkk-1) antagonizes the signaling cascade when it binds LRP5/6, forms a ternary complex with Kremen, and promotes endocytosis and degradation of the receptor (Mao *et al.*, 2001, 2002). The sFRP family members are capable to act as inhibitors by binding to and sequestering the Wnt ligand from its receptor (Kawano and Kypta, 2003; Uren *et al.*, 2000).

The noncanonical and canonical Wnt signaling pathways are involved during embryonic development (Eisenberg and Eisenberg, 2006; Pandur *et al.*, 2002). Wnt plays a role in cellular proliferation (Kioussi *et al.*, 2002), differentiation, and self-renewal (Reya and Clevers, 2005; Wang and Wynshaw-Boris, 2004). More on the specific role of the Wnt cascade on MSC biology is included in subsequent sections.

B. Bone morphogenetic pathway

Members of the TGF superfamily include TGF- β , activins, and BMPs. These members play important and distinct roles in directing differentiation of stem cells during development and in the adult tissue (Korchynskyi and ten Dijke, 2002; Shi *et al.*, 2000). As seen in Fig. 2.2, this superfamily signals through activation of distinct types of serine/threonine kinase receptors and subsequent activation of signaling molecules called Smads. Upon ligand binding to type II receptors, type I receptors are cross-phosphorylated by the activated type II receptor. The now activated type I receptor phosphorylates its receptor-associated Smad protein (pSMAD). Phosphorylated R-Smads must form a complex with Smad 4 (co-SMAD) to translocate to the nucleus and exert a transcriptional effect on the cell (Miyazono *et al.*, 2005). The specificity of receptor activation and R-Smad activation is such that BMP activity uses only Smads 1, 5, and 8 as signaling molecules and thus directs transcription of

target genes responsive to these Smads only. The role of BMP signaling in MSC biology will be expanded upon in the following sections.

C. Canonical Wnt and BMP signaling and MSC differentiation

MSCs possess an extensive potential to self-renew and differentiate into multiple lineages (Beresford, 1989; Caplan, 1991). MSCs can be induced to give rise to osteoblasts, chondrocytes, adipocytes, and myoblasts under suitable culture conditions (Caplan, 1991; Prockop, 1997). The ability to self-renew and differentiate into multiple lineages, easy isolation, and accessibility make MSCs a suitable candidate for therapeutic purposes. To enhance the beneficial effects of MSCs, it is necessary to identify the molecular mechanisms that regulate the self-renewal and differentiation of MSCs (Caplan, 2000). In this section, we will discuss the involvement of Wnt and BMP signaling in MSC differentiation.

1. Osteogenesis—The canonical Wnt signaling is involved in the lineage specification of MSCs (Etheridge *et al.*, 2004). Typically, for osteogenic differentiation in culture conditions, MSCs are treated with ascorbic acid, β -glycerophosphate, and dexamethasone in fetal bovine serum (FBS)-containing medium (Jaiswal *et al.*, 1997; Pittenger *et al.*, 1999) that results in the increase in calcium deposition and alkaline phosphatase activity. The effect of canonical Wnt signaling on osteogenesis is context dependent. It varies from differences in the level of Wnt, type of cell, the species, type and timing of stimulus, and other experimental conditions. Enhanced Wnt signaling either by the addition of high levels of exogenous Wnt3a or through overexpression of LRP5 or stabilization of the mutant form of β -catenin augments osteogenesis in human MSCs (De Boer *et al.*, 2004a; Gong *et al.*, 2001; Qiu *et al.*, 2007). On the other hand, some reports have identified that enhanced activity of Wnt signaling via exogenous addition of Wnt3a or lithium, which is a GSK3 β inhibitor, diminishes osteogenesis in dexamethasone-induced human MSCs (de Boer *et al.*, 2004b). In murine pluripotent mesenchymal and osteoprogenitor cells, canonical Wnt signaling promotes the osteoblastogenesis via upregulating RUNX2, Dlx5, or osterix (Bennett *et al.*, 2005; Gaur *et al.*, 2005). It is interesting to observe that the stage of targeted cells also determine the effect of canonical Wnt signaling on osteogenesis in some cases. For example, Wnt signaling enhances the differentiation of MSCs that are committed to osteogenic lineage, on the other hand, inhibiting the terminal differentiation of mature osteoblasts (Eijken *et al.*, 2008; Kahler and Westendorf, 2003; Kahler *et al.*, 2006, 2008).

The BMP cascade, as the name implies, is more clearly implicated in osteogenic commitment of MSCs. For example, adenoviral expression of BMP2, 6, and 9 significantly induced alkaline phosphatase activity in pluripotent C3H10T1/2 cells (Cheng *et al.*, 2003). Similarly the preosteoblastic C2C12 cell line had increased alkaline phosphatase activity in the presence of BMP2, 4, 6, 7, and 9 (Cheng *et al.*, 2003). This data translated to the human MSC field as adenoviral gene expression of BMP2 increased their osteogenic commitment as demonstrated by increased expression of Runx2 and Type I collagen and alkaline phosphatase activity (Koch *et al.*, 2005). Human MSCs derived from osteoporotic patients responded beneficially to BMP2 and 7 as they increased alkaline phosphatase activity and total calcium production (Pountos *et al.*, 2010).

2. Chondrogenesis—MSCs are treated with TGF- β for chondrogenic differentiation induction because it has been shown to be a key regulator of early stages of MSC chondrogenesis (Tuli *et al.*, 2003). The chondrogenic differentiation results in the generation of cartilage-specific highly sulfated proteoglycans and type II collagen (Hwang *et al.*, 2007). The expression of β -catenin is different in different stages of chondrogenesis, indicating a differential role of Wnt signaling during various stages of chondrogenesis (Ling *et al.*, 2009). Canonical Wnt signaling has also been shown to regulate chondrocyte differentiation of MSCs in a Sox9-dependent manner (Yano *et al.*, 2005). A dual role of Wnt signaling has been observed in the process of chondrogenesis, which depends on the particular Wnt ligand and also on the developmental stage. In the chicken limb, during the process of chondrogenesis, Wnt4a promotes the maturation of chondrocytes, whereas Wnt5a negatively affects chondrocyte maturation (Hartmann and Tabin, 2000). The observed opposing effect could be due to the involvement of distinct pathways. The activating affect of Wnt4a on chondrogenesis was possibly due to activation of β -catenin and Wnt receptors FZD1 and 7 (Hartmann and Tabin, 2000). Trabecular bone-derived MSCs induced for TGF- β -mediated chondrogenesis involve a cross talk between mitogen-activated protein kinase and Wnt signaling (Tuli *et al.*, 2003). In the process, the canonical Wnt signaling regulates N-cadherin expression during cellular condensation and chondrogenesis. Recently, Maruyama *et al.* (2010) indicated that Wnt pathway regulates the fate of MSC lineage specification during skeletal development by modulating the balance of the fibroblast growth factor and BMP pathways.

Although BMP2 was implicated in directing osteogenesis of multipotential murine C3H10T1/2 cells, it was also implicated in their chondrogenic lineage commitment (Alcian blue stained cartilage-like matrix and type II collagen; Denker *et al.*, 1999). BMP2-directed chondrogenesis in these cells is presumably due to the induced expression of the cell cycle inhibitory protein/differentiation factor p21/WAF1 (Carlberg *et al.*, 2001). Although involved in noncanonical BMP signaling, BMP13 has been implicated in the chondrogenesis of these cells (Nochi *et al.*, 2004).

3. Adipogenesis—In cell culture conditions, MSCs are induced toward adipogenic differentiation by treatment with dexamethasone, insulin, isobutyl methyl xanthine, and indomethacin in complete media containing serum (Janderova *et al.*, 2003). The differentiation leads to the formation of the lipid vacuoles which are detected by Oil Red-O staining. The commitment of pluripotent MSCs toward adipogenic lineage leads to the formation of preadipocytes before terminally differentiating into mature adipocytes (Bowers and Lane, 2007). Inhibition of Wnt signaling is necessary for the adipogenic differentiation of MSCs (Moldes *et al.*, 2003). The canonical Wnt signaling has been shown to inhibit the expression of adipogenic transcription factor PPAR γ in MSCs (Rawadi *et al.*, 2003). Overexpression of Axin2 or dominant-negative TCF4 leads to the inhibition of Wnt signaling which drives MSCs toward adipogenic differentiation (Ross *et al.*, 2000). On the other hand, canonical Wnt1 and Wnt10b and an activated mutant β -catenin averts adipocyte differentiation via reducing the expression of adipogenic transcription factors C/EBP α and PPAR γ (Bennett *et al.*, 2005; Ross *et al.*, 2000). It has been demonstrated that in addition to regulating MSC proliferation, cyclin D1 and c-Myc directly inactivate PPAR γ and C/EBP α ,

respectively (Fu *et al.*, 2005; Tetsu and McCormick, 1999). One can speculate that cyclin D1 and c-Myc might be involved in regulating the inhibitory effects of Wnt signaling on adipogenic transcription factors. Additionally, Wnt10b has also been demonstrated to act as a molecular switch from adipogenesis toward osteogenesis of bipotential mesenchymal precursors (Bennett *et al.*, 2005).

Two separate cell lines (growth-arrested 10T1/2 and preadipocyte cell line, A33 cells, derived from 10T1/2 cells) were utilized to demonstrate the involvement of BMP4 in adipocyte development (Bowers and Lane, 2007). This cascade is deemed necessary for this as disruption of BMP4 signaling by noggin blocks the preadipocyte phenotype (Bowers *et al.*, 2006). BMP2 was also implicated in adipogenesis, and overexpression of constitutively active BMP receptor 1A or 1B induced commitment (Huang *et al.*, 2009).

D. Involvement of Wnt on survival and proliferation of MSCs

Many studies have suggested the role of canonical Wnt signaling in self-renewal and maintenance. Exogenous application of Wnt3a in cell culture enhanced MSC proliferation due to both increased proliferation and inhibition of apoptosis (Boland *et al.*, 2004; Cho *et al.*, 2006). The effect of Wnt3a on MSC proliferation is possibly due to its effect on cell cycle regulators, cyclin D1 and c-Myc (Baek *et al.*, 2003). Additionally, the overexpression of LRP5, a coreceptor involved in Wnt signaling, has been demonstrated to enhance proliferation of MSCs (Baksh *et al.*, 2007). On the other hand, canonical Wnt signaling has also been accounted for the inhibition of human MSC proliferation (Qiu *et al.*, 2007). In addition, inhibition of Wnt signaling through Dkk-1 and LRP inhibitors was necessary for human MSCs to reenter cell cycle and proliferate (Gregory *et al.*, 2003). Our studies also indicated that Wnt inhibition is a key factor required for better proliferation, engraftment, and survival of MSCs (Alfaro *et al.*, 2008). One study has reported inhibition and stimulation of human MSC proliferation by Wnt3a is dose dependent (De Boer *et al.*, 2004a). The differences in the findings about the role of Wnt signaling in MSC survival and proliferation may arise from differences in culture conditions, dose and type of Wnt ligands, and handling of the cells. These studies also indicate that Wnt signaling regulation of MSC biology (with specific regard to self-renewal) is a very complex mechanism and further studies are required to understand this mystery.

E. Effects of the BMP cascade on MSC survival and proliferation

BMPs are widely known to affect the differentiation of MSCs and therefore not much is documented on their roles on survival and proliferation, particularly on murine MSCs. Regardless, a few reports have emerged. Noncanonical BMP3 activated TGF- β signaling in C3H10T1/2 MSCs and 3T3-L1 preadipocytes to cause a threefold increase in their proliferation (Stewart *et al.*, 2010). Human adipose-derived MSCs treated with low-dose BMP4 are less apoptotic and more proliferative (Vicente Lopez *et al.*, 2010). Also, human MSCs derived from osteoporotic bones had increased proliferation after addition of high-dose BMP7 (Pountos *et al.*, 2010). In essence, the effects of BMPs (canonical and noncanonical) on MSC survival and proliferation are largely context dependent and no solid data were found on murine bone marrow-derived MSCs.

IX. Enhancing MSC Survival in the Wound

As was briefly touched upon, the survival of MSCs within the wound microenvironment is a limiting factor on their reparative capabilities (Freyman *et al.*, 2006; Hofmann *et al.*, 2005). Thus, several groups have tried to alter MSCs genetically to increase their survival. Although Wnt and BMP signaling have not been directly tied to this effect, several other molecules which might be involved in such cascades will be addressed briefly. Retroviral expression of the prosurvival gene Akt-1 decreased the apoptotic index of MSCs *in vitro*, an effect also observed *in vivo* following transplantation into infarcted rat myocardium (Jiang *et al.*, 2006; Mangi *et al.*, 2003). A similar effect was observed with retroviral overexpression of erythropoietin by MSCs: increased survival both *in vitro* and *in vivo* in a murine subcutaneous implantation model of matrigel-embedded MSCs (Copland *et al.*, 2008). Our group has shown that overexpression of sFRP2 by MSCs enhances their *in vitro* proliferative index and increases their engraftment in two separate *in vivo* wound models (Alfaro *et al.*, 2008).

X. Secreted Frizzled-Related Proteins

The sFRP family was originally identified by their high homology to the Frizzled Wnt receptor (Rattner *et al.*, 1997; Shirozu *et al.*, 1996). There are eight family members; five of these are mammalian (Bovolenta *et al.*, 2008). The function of the five mammalian members, sFRP1 to sFRP5, has been identified as Wnt inhibition (Chang *et al.*, 1999; Finch *et al.*, 1997; Schumann *et al.*, 2000). sFRPs have a N-terminal conserved cysteine-rich domain (CRD) which shares 30–50% similarity with Frizzled proteins (Melkonyan *et al.*, 1997). The C-terminal domain of sFRPs shares a sequence similarity with the axon guidance protein netrin (NTR). The NTR domain has also been found in a few complement proteins and in the tissue inhibitors of metalloproteases (Banyai and Pathy, 1999). sFRPs inhibit Wnt signaling via interacting through their CRD with Wnt ligands (Lin *et al.*, 1997), by interaction of NTR-like domain with the Wnt ligands (Uren *et al.*, 2000) or binding to itself and the Frizzled protein (Bafico *et al.*, 1999). The contradiction in the mode of binding of sFRPs to Wnt and other ligands might be due to different affinity of sFRPs towards their binding partners. sFRPs are secreted proteins; however, in cultured cells, they have been found to be associated with the cell and are released into the culture media upon addition of heparin (Finch *et al.*, 1997).

Further documented roles for the sFRP family members include cytoprotection and proliferation. sFRP2 seems to have a positive function in wound repair. This molecule was implicated in skeletal muscle repair in mice and has been identified as a key factor in MSC-based therapy for myocardial repair (Mirotsoy *et al.*, 2007; Zhao and Hoffman, 2004). Melkonyan *et al.*, 1997 identified sFRP2 as a prosurvival protein and showed that its overexpression in human breast adenocarcinoma cell line, MCF7, conferred an antiapoptotic effect. Recently, Sfrp2 was identified as one of the key paracrine factors released by Akt-overexpressing MSCs found to play a critical role in the survival of ischemic cardiac myocytes (Mirotsoy *et al.*, 2007). Sfrp2 increases the proliferative index of both murine and human MSCs (Alfaro *et al.*, 2008). Identifying a cytoprotective role for sFRP2 in the context of wound repair will provide insight into improving MSC tissue repair. Recently, a new

hypothesis for an alternative role for the sFRP family has emerged; the mammalian members may inhibit the BMP signaling cascade much like the nonmammalian Sizzled members (Lee *et al.*, 2006; Muraoka *et al.*, 2006).

XI. Mediating MSC Self-Renewal

The mechanisms by which MSCs modulate Wnt and BMP signaling events during growth and/or lineage commitment are immensely complex. The molecular interplay between these cascades may modulate MSC self-renewal, a hypothesis based on the self-renewal mechanisms of other stem cells. Recent studies performed by our laboratory demonstrated that over-expression of the Wnt inhibitory protein, sFRP2, by MSCs (sFRP2-MSCs) increases *in vitro* proliferation (Alfaro *et al.*, 2008) and survival of MSCs by regulating both the BMP and Wnt signaling pathways (Alfaro *et al.*, 2010). The effects of sFRP2, as depicted in Fig. 2.3, indirectly affect MSC self-renewal.

Regardless of their mode of action, direct regeneration, or paracrine effects, MSCs could not impact the repair process until and unless a substantial amount of cells are generated via MSC self-renewal and proliferation within the wounded area. Although encouraging results have been observed in preclinical models (Hung *et al.*, 2007; Shake *et al.*, 2002; Shi *et al.*, 2007), low survival and poor engraftment significantly restrict the efficiency of MSCs for remedial purposes in clinical setting. Some clinical trials address the issue of long-term engraftment of MSCs. For example, the engraftment of donor MSCs in osteogenesis imperfecta patients never exceeded 1% 4–6 weeks following MSC infusion (Horwitz *et al.*, 2002). Enhanced survival of sFRP2-MSCs observed also *in vivo* in two distinct wound repair models still led to a modest degree of engraftment (Alfaro *et al.*, 2008). Achieving a critical mass of MSCs by improving their survival and self-renewal capacity should increase their numbers in the damaged tissue to ultimately increase their reparative abilities. Figure 2.3 demonstrates one molecule that positively affects MSC self-renewal leading to enhanced wound repair.

XII. Conclusions

The focus of this review was BM-derived murine MSCs, with some discussion of their clinical utilization. The data demonstrated gaps in the knowledge of MSC biology, that if better understood would allow clinicians to improve the potential of MSC-directed wound repair. Particularly, we focused on canonical Wnt and BMP signaling to demonstrate how activation and/or inhibition of these two well-understood cascades could affect MSC activity, including cellular differentiation, survival, and proliferation.

The data gathered demonstrate that the effects of both Wnt and BMP signaling on MSCs are variable. The source of the MSCs (tissue of isolation), the passage number, the quantity of ligand, and the timing are all examples of variables that affect the outcome of these cascades on the MSCs. In general, however, BMP and Wnt signaling are thought to drive senescence, differentiation, and apoptosis of MSCs. We and others have recently identified that sFRP2 is a MSC-derived factor that modulates both Wnt and BMP pathways to improve MSC-mediated cardiovascular and wound repair. sFRP2 increased proliferation, prevented

apoptosis, and decreased differentiation of MSCs. As a whole, these data suggest sFRP2 is a potent factor involved in MSC self-renewal. Increasing MSC self-renewal is critical to enhancing their therapeutic efficacy; therefore, other molecules that might have similar effects would be of interest to the field. The significance of this finding can be appreciated by looking at the impact self-renewal factors have had on other stem cell fields. Particularly, the *ex vivo* expansion of HSCs (CD34⁺) through addition of recombinant self-renewal factors is being actively pursued and has yielded important results in several clinical trials of a variety of human diseases (Kelly *et al.*, 2009). Manipulation of the MSC self-renewal factors, including sFRP2 or other modulators of both Wnt and BMP signaling, could profoundly control the extent of their senescence, differentiation, and apoptosis enhancing the regeneration of MSC-mediated therapy.

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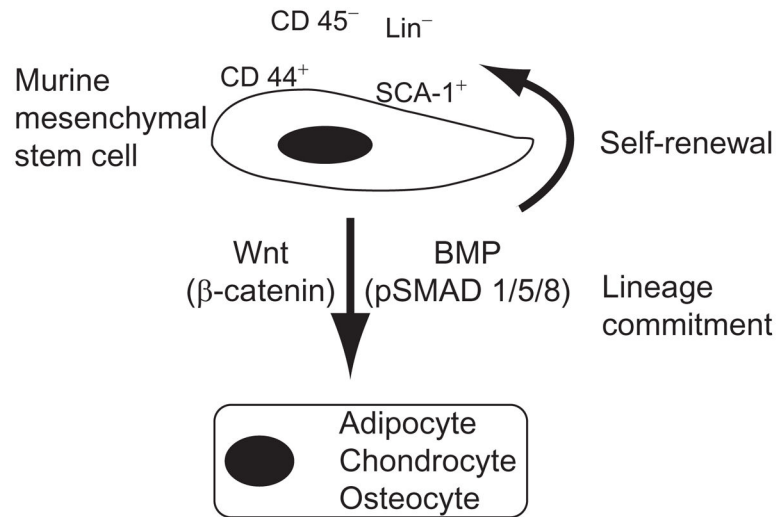


Figure 2.1. Definition and molecular mediators of murine mesenchymal stem cells. Murine MSCs are $CD44^{+}$ and $SCA-1^{+}$, but $CD45^{-}$ and Lin^{-} . As stem cells, they must give rise to self (self-renewal) and undergo trilineage differentiation toward the adipogenic, chondrogenic, and osteogenic lineages. The cascades involved in the lineage commitment are labeled with their effector molecules in parentheses.

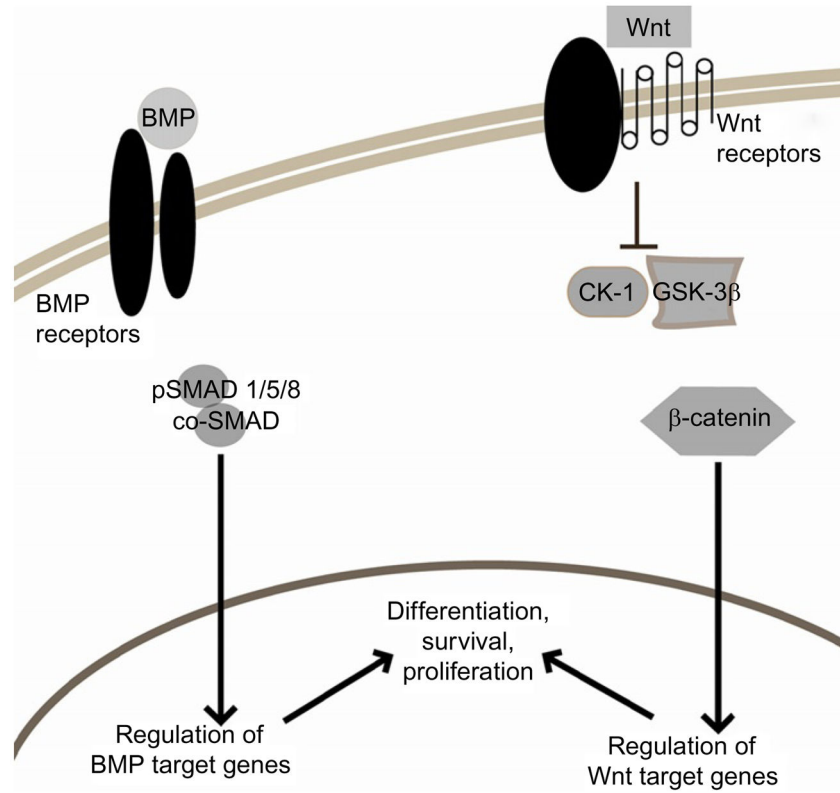


Figure 2.2.

Simplified schematic of BMP and Wnt signaling. Activation of BMP and Wnt signaling can lead to the regulation of specific target genes which affect MSC biology. Transcriptional control is exerted on the cell upon the nuclear translocation of signaling molecules: phosphorylated Smads (pSMAD 1/5/8) in the case of BMP signaling and β -catenin in the case of Wnt signaling.

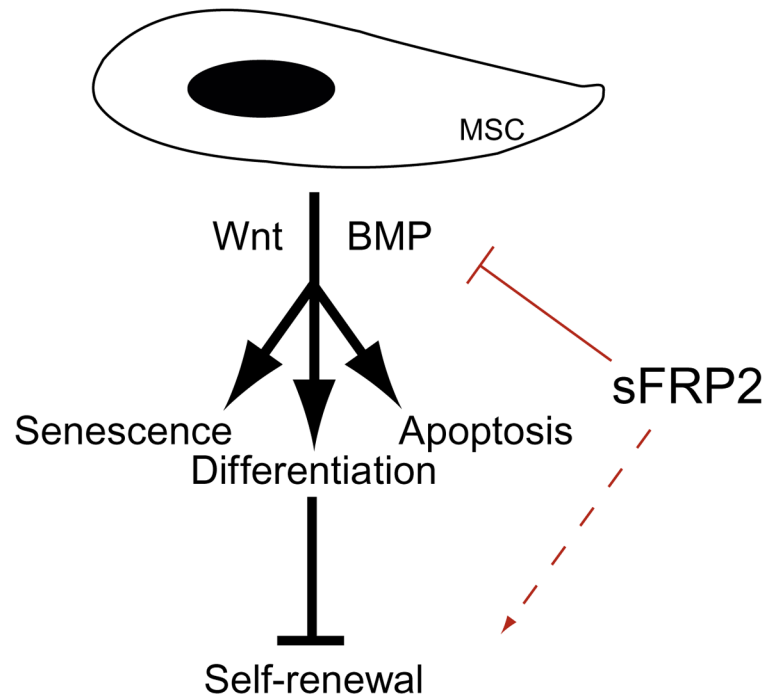


Figure 2.3. Model of the role of sFRP2 in MSC self-renewal. Self-renewal is evaded by senescence, lineage commitment, and apoptosis of MSCs; Wnt and BMP signaling drive these events. sFRP2 can inhibit these cascades to indirectly drive MSC self-renewal (discontinuous line).