Periosteal Skeletal Stem and Progenitor Cells in Bone Regeneration

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Abstract

Purpose of review:

The periosteum, the outer layer of bone, is a major source of skeletal stem/progenitor cells (SSPCs) for bone repair. Here, we discuss recent findings on the characterization, role, and regulation of periosteal SSPCs (pSSPCs) during bone regeneration.

Recent findings:

Several markers have been described for pSSPCs but lack tissue specificity. *In vivo* lineage tracing and transcriptomics analyses have improved our understanding of SSPC functions during bone regeneration. Bone injury activates pSSPCs that migrate, proliferate, and have the unique potential to form both bone and cartilage. The injury response of pSSPCs is controlled by many signaling pathways including BMP, FGF, Notch and Wnt, their metabolic state, and their interactions with the blood clot, nerve fibers, blood vessels and macrophages in the fracture environment.

Summary:

Periosteal SSPCs are essential for bone regeneration. Despite recent advances, further studies are required to elucidate pSSPC heterogeneity and plasticity that make them a central component of the fracture healing process and a prime target for clinical applications.

Keywords: periosteum – skeletal stem/progenitor cells – bone regeneration – In vivo lineage tracing -

Introduction

The periosteum is a thin fibrous membrane covering the outer surface of bones. It is organized in 2 layers: the outer fibrous layer, mostly composed of fibroblasts and extracellular matrix and the inner cambium layer, composed of osteoblasts and skeletal stem/progenitor cells (SSPCs). The periosteum is highly vascularized and innervated by sensory and sympathetic fibers, and hosts resident and osteal macrophages (osteomacs) [1–3]. In long bones, the periosteum is derived from the limb mesenchyme that forms the cartilage template and the surrounding perichondrium during development [4, 5]. The

periosteum plays crucial roles in bone physiology during development, growth and remodeling. Periosteal cells directly contribute to cortical bone formation and control bone growth by secreting paracrine factors such as osteocrin [6, 7]. Extrinsic factors such as mechanical loading stimulate bone formation in the periosteum during bone growth and remodeling [8–10].

The capacity of the periosteum to form bone after a fracture was first described in 1845 by Watson [11]. The periosteum displays a remarkable ability to regenerate bone and is indispensable to achieve bone healing [12]. Periosteum removal or damage at the time of fracture can cause altered healing and non-union in experimental models [13–17]. The periosteum is a key source of SSPCs for bone repair in addition to SSPCs localized within the bone marrow compartment and the adjacent skeletal muscle [18–25]. Following a bone injury, SSPCs are activated during the inflammatory phase of repair and differentiate into osteoblasts and chondrocytes. SSPC differentiation is regionalized in the fracture callus. Osteogenesis leading to intramembranous ossification occurs primarily at the periphery of the callus while chondrogenesis leading to endochondral ossification occurs in the center of the callus. The mechanical environment also influences cell differentiation as fracture stability favors intramembranous ossification while fracture instability favors endochondral ossification. The role of periosteal SSPCs in coordinating these events is still poorly understood.

In this review, we summarize recent findings on the characterization of periosteal skeletal stem/progenitor cells (pSSPCs) and their contribution to bone repair. We describe the current knowledge on pSSPC molecular regulation and interactions with the fracture environment following bone injury. Finally, we discuss the role of pSSPCs in bone repair defects and the relevance of pSSPCs in cell-based therapies.

Isolation and characterization of periosteal stem and progenitor cells

Studying periosteum and pSSPCs is challenging due to the inaccessibility and thinness of the periosteum. Human pSSPCs have been isolated by scrapping or peeling away the periosteum from the cortex, followed by enzymatic digestion or explant culture [26]. In animal models, similar techniques have been employed. In addition, protocols have been developed to directly isolate cells by enzymatic digestion of the bone surface or by whole bone explant culture [27–30]. Isolated pSSPCs are adherent to plastic and can be cultured *in vitro* for several passages.

SSPCs were first described in the bone marrow compartment and defined as mesenchymal stem cells based on stem/progenitor marker expression, multipotency and self-renewal capacity [31, 32]. The periosteum contains cells displaying SSPC criteria. Analysis of cell surface markers showed that pSSPCs are negative for hematopoietic and endothelial markers and express canonical mesenchymal

markers such as CD90, CD105, CD51, CD29 and Sca1 in mice and CD90, CD105, CD73 in human [20, 27, 33–35]. In 2015, Chan *et al.* identified a population of mouse skeletal stem cells from whole digested bone based on a combination of cell surface markers ($CD45^-TER119^-TIE2^-CD51^+THY1^-6C3^-CD105^-$) [36]. The SSPC populations defined by these markers were also identified in freshly isolated and primary periosteal cells from mouse and human bones [24, 25, 37, 38]. Periosteal SSPCs exhibit multipotency *in vitro* with tri-lineage differentiation capacity towards osteogenesis, chondrogenesis and adipogenesis [34, 39]. The multipotency and differentiation potential of pSSPCs was confirmed by *in vivo* ectopic transplantation experiments [40, 41]. A key feature of stem cells is their ability to self-renew, and both mouse and human pSSPCs display self-renewal potential in serial CFU or ectopic transplantation assays [22, 42].

Fate of periosteal stem and progenitor cells during bone repair

The direct contribution of periosteum to bone regeneration is well established. The periosteal response to bone fracture is characterized by periosteum thickening near the site of injury within few days after fracture. Activated periosteal SSPCs proliferate and migrate at the fracture site, and participate in both intramembranous and endochondral ossification [18, 20, 25]. This ability of pSSPCs to form cartilage and bone after fracture is unique to the periosteum as SSPCs from bone marrow mostly contribute to bone and SSPCs from skeletal muscle contribute to cartilage [18, 20–23]. It remains unclear how cell fate decision of pSSPCs towards osteogenesis or chondrogenesis is regulated. Using single-cell RNAseq (scRNAseq) analyses of primary periosteal cells at steady state and 3 days post-fracture, Julien *et al.* described the steps of pSSPC activation towards the chondrogenic lineage [25]. After bone injury, periosteal SSPCs transition from a stem/progenitor state to a fibrogenic state, marked by an active extracellular matrix production and the expression of cell migration related genes. Following this transient activation step, cells undergo chondrogenic differentiation and proliferate. A recent study by Van Gastel et al. showed that pSSPCs can be directed towards chondrogenesis after fracture by blocking vascular ingrowth to prevent nutrient availability [43]. Activated pSSPCs with sufficient lipid intake can maintain sufficient levels of fatty acid oxidation and differentiate into osteoblasts. However, in regions with low lipid availability, reduced fatty oxidation leads to FOXO activation, SOX9 expression and chondrogenic differentiation [43]. Further studies are needed to determine how cell fate decisions in the periosteum are controlled by environmental factors, and whether distinct cell populations in the periosteum respond differently to signals in the fracture environment to undergo either osteogenesis or chondrogenesis.

Periosteal SSPCs orchestrate another crucial step of bone repair via endochondral ossification. In the center of the callus, a site of active replacement of cartilage by bone, transdifferentiation of hypertrophic chondrocytes into osteoblasts appears to be an important source of newly formed osteoblasts [44, 45].

Through lineage tracing, Julien *et al.* showed that periosteal SSPCs exhibit a strong potential to participate in this transdifferentiation process required for bone bridging [37]. Finally, during the late stages of repair, pSSPCs can self-renew to reconstitute a new periosteum at the periphery of the callus. The *in vivo* self-renewal capacity of pSSPCs during bone repair was revealed using periosteum graft and serial fractures, showing that the periosteum maintains a pool of pSSPCs able to contribute to a subsequent injury [20].

Genetic lineage tracing of pSSPCs during bone repair

Additional *in vivo* approaches, based on the Cre/LoxP system for genetic lineage tracing, have been instrumental to understand the endogenous role and fate of pSSPCs during bone regeneration (Table 1). Due to their mesenchymal origin, pSSPCs in long bones can be tracked from developmental stages using the limb bud mesenchymal marker PRX1. The non-inducible *Prrx1*^{Cre} model labels not only SSPCs in the periosteum but also in bone marrow and skeletal muscle [20, 21]. In adult bone, pSSPCs still express *Prrx1* but the inducible *Prrx1*^{CreERT} model does not allow efficient labeling of pSSPCs [20, 37, 46]. The platelet-derived growth factor α (PDGFRα) has been described as a marker of mesenchymal cells from various tissue origins. Studies using the *Pdgfra*^{CreERT} model showed that PDGFRα marks pSSPCs with osteochondral potential during bone healing but is not restricted to the periosteum [24, 25, 47–49].

Debnath and colleagues combined the markers described by Chan *et al.* with the marker Cathepsin K (CTSK) and identified a population of pSSPCs involved in intramembranous ossification [22]. CTSK$^+$ pSSPCs, labelled by the non-inducible *Ctsk*^{Cre} model, display self-renewing capacity and osteochondral potential in calvarial or femoral injury models. scRNAseq analysis of sorted CTSK$^+$ pSSPCs showed heterogeneity within this population, with clusters expressing chondrogenic genes (*Col2a1, Sox9*), osteogenic markers (*Kera, Alpl*),, stemness markers (*Ly6a, Cd34*), and *Acta2* [22]. CTSK$^+$ and CD200$^+$ periosteal cells were also detected in human periosteum [22].

The protein alpha-Smooth Muscle Actin (αSMA), encoded by the *Acta2* gene, labels cells in the periosteum [24, 50]. αSMA$^+$ periosteal cells, traced using the *Acta2*^{CreERT}mouse line, are an heterogenous population of osteochondroprogenitors, with self-renewing potential and required for efficient bone healing [24]. Ortinau and colleagues described a sub-population of αSMA+ cells, using the pIpC inducible *Mx1*^{Cre} line [51]. αSMA$^+$ Mx1$^+$ pSSPCs display self-renewal potential and are required for callus formation after tibial and calvarial injury [51].

Several other markers have been identified, such as the Wnt signaling downstream target AXIN2, the receptor PDGFRβ and HOX11 [52–54]. Markers, such as Leptin receptor and Nestin, used to

characterize bone marrow self-renewing SSPCs also label cells in the periosteum [42, 55]. Tournaire *et al*. reported that Nestin-GFP+ periosteal cells in Nestin-GFP transgenic mice correspond to unipotent osteoprogenitors, with limited contribution to callus formation and no stemness properties. Lineage tracing in the Nes^{CreERT} model revealed an osteogenic and self-renewing potential of $Nestin^{+}$ periosteal cells [42, 55]. GLI1 labels populations of SSPCs, including in the periosteum, forming chondrocytes and osteoblasts after fracture [56, 57]. Osteochondrogenic markers such as SOX9 ($Sox9^{CreERT}$ model) and Osterix (Osx^{Cre} model) both label populations of periosteal progenitors contributing to cartilage and bone formation after fracture [53, 58].

Overall, periosteal SSPCs populations defined by these various CRE models exhibit similar proprieties, such as the expression of stem/progenitor markers, osteochondral fate after fracture and self-renewing capacity. It remains unclear how these populations overlap and differ in their identity and potential, and whether the periosteum encompasses several populations of SSPCs. Moreover, no marker currently used to characterize pSSPC is fully specific to the periosteum as most of them are expressed also by cells localized in the bone marrow, skeletal muscle or by stromal cells in other tissues [21, 25, 49, 59]. Thus, marker expression is not sufficient to specifically trace periosteum-derived SSPCs and exclude the contribution of traced cells from other bone compartments during bone repair. Specifying the tissue of origin and using protocols to isolate periosteal cells without contamination from other tissues remain essential to identify pSSPCs and investigate their role in bone repair.

Molecular regulation of periosteal stem and progenitor cells

During bone repair, pSSPC proliferation, migration and differentiation are governed by several signaling pathways including Bone Morphogenic Protein (BMP), Wnt, Notch, Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor β (TGFβ) and Hedgehog (HH) signaling (Figure 1). Bone Morphogenetic protein (BMP) signaling is one of the earliest pathway upregulated after fracture [25]. Increased BMP ligand and receptor expression as well as activated downstream SMAD effectors are observed in the activated periosteum at day 3 post-fracture [25, 60]. The role of BMP signaling during the early stage of bone healing was evaluated by inactivating *Bmpr1a* using the $Pdgfra^{CreERT}$ model. *Bmpr1a*-deficient periosteal SSPCs display reduced proliferation, migration and osteochondrogenic differentiation after fracture [25]. While endogenous BMP2 is required for bone healing, BMP4 and BMP7 are dispensable [61–63]. BMP2 plays a role in pSSPC fate decision, and *Bmp2* gene inactivation prevents periosteal activation and bone healing [64–66].

Notch signaling also plays a role in the early steps of bone healing before pSSPCs commit to the chondrogenic or osteogenic lineage. Notch inactivation in *Prx1*-derived cells, using $Prx1^{Cre}$ mice, causes bone non-union but Notch inactivation in chondrocytes using $Acan^{Cre}$ mice, or osteoblasts, using

Col1.1^{Cre} mice, does not impact healing [67]. The crosstalk between Notch and Wnt signaling pathways is essential for the progression of the bone healing process. Whereas Notch signaling is active early to promote pSSPC activation and proliferation, the expression of Wnt pathway and target genes peaks between 5 and 10 days post-fracture [68, 69]. Inhibition of Notch signaling by Wnt signaling marks the end of the pSSPC activation phase and the initiation of osteogenic differentiation [67, 70, 71]. Wnt regulates the differentiation of SSPCs by promoting osteogenic differentiation over chondrogenesis through SOX9 repression [72]. Wnt overactivation, in Sostdc1 knock-out mice causes premature activation of periosteal cells and overmineralized callus formation [73]. The factors regulating Wnt expression and activation remain poorly understood.

FGFs are expressed not only during pSSPCs activation (FGF2, FGF5, FGF9), but also during cartilage formation and maturation (FGF16, FGF18), and during ossification (FGF1, FGF17), suggesting their involvement through all stages of repair [74]. FGF2 promotes the proliferation and chondrogenic differentiation of pSSPCs through BMP2 signaling [75, 76]. By over activating FGF receptor 3 (FGFR3) signaling in *Prx1*^{Cre} mice, Julien et al showed that FGFR3 is crucial for the differentiation of periosteum-derived chondrocytes and their ability to support cartilage-to-bone transformation during endochondral ossification [37].

In addition, PDGF-BB, TGFβ and Hedgehog are required for pSSPC proliferation and differentiation [57, 77–81]. PDGF-BB stimulates pSSPCs migration and angiotropism while inhibiting apoptosis [42, 82]. Cyclooxygenase 2 (COX2) is essential for the initiation of the periosteal response to cortical bone injury by modulating several key pathways such as HIF, PI3K-Akt and Wnt [83–85]. In sum, many signaling pathways and growth factors must act in coordination to control pSSPC activation and subsequent steps of differentiation. Whether several pSSPCs subpopulations respond to distinct molecular signals and whether regulatory networks specific to pSSPCs define their unique osteochondral potential after fracture remains to be investigated.

Influence of the fracture environment on periosteal stem and progenitor cells

Bone fracture creates a major perturbation of bone tissue homeostasis with the rupture of blood vessels and nerve fibers initiating an acute inflammatory response shortly after fracture. This complex multicellular environment influences the activation and fate of pSSPCs (Figure 1). The disruption of blood vessels causes immediate bleeding and subsequent blood clot formation. The blood clot is then progressively degraded by the action of enzymes such as plasminogen (Plg) [86, 87]. Plg knock-out mice display impaired bone healing, with reduced callus, cartilage, and bone formation. Plg plays a paracrine role in the activation of pSSPCs. By cleaving the inactivated form of the matrix associated growth factor Cyr61 secreted by pSSPCs, Plg can stimulate pSSPC proliferation and migration [87].

Periosteum is essential for the revascularization of the fracture site as periosteum removal reduces blood vessel density [43]. Vascular disruption at the fracture site also causes hypoxia in the first days after injury [88]. The hypoxic environment stimulates HIF1α expression by pSSPCs and the secretion of the proangiogenic factors VEGF and TSP-4 required for angiogenesis and bone healing [27, 89–91]. More research is needed to elucidate the role of the periosteum and the interplay between blood vessels and pSSPCs during fracture revascularization.

During the inflammatory phase of bone repair, immune cells are recruited at the injury site. The periosteum becomes invaded with osteomacs and activated macrophages [3]. Macrophages play a critical role in bone healing, and their depletion, using the Mafia inducible system, reduces periosteal callus formation [92]. Macrophages remove necrotic tissue at the fracture site and secrete factors involved in the recruitment and activation of pSSPCs. Gao *et al* showed that TRAP+ periosteal macrophages secrete PDGF-BB, that binds to PDGFRβ expressed by pSSPCs to activate the Pi3K-Akt-CREB pathway and stimulate Periostin expression [42]. Periostin is a critical regulator of pSSPC response to injury and self-renewal [20]. Periosteal SSPCs are responsive to different chemokines. Chemokine ligand 2 (CCL2 / MCP-1) is expressed in the periosteum during the first 3 days following fracture [93]. Inactivation or inhibition of CCL2 and his receptor CCR2 delays fracture healing [93, 94]. The CCL5-CCR5 axis is necessary to induce the migration of murine and human pSSPCs [51]. *In vitro* osteogenic induction of periosteal cells can modulate macrophage polarization and promote M2 phenotype by secreting chemokines suggesting a crosstalk between macrophages and pSSPCs [95, 96].

The disruption of nerve fibers in the periosteum triggers rapid nerve sprouting from both sympathetic and sensory fibers in the first day post-bone injury. Nerve sprouting is concomitant with NGF expression in periosteal cells and macrophages, and occurs prior to revascularization [97]. Nerves subsequently regulate pSSPC activation by releasing neuropeptide calcitonin gene related peptide (CGRP), that binds to the CALCRL-RAMP1 receptor and stimulates *Osterix* expression [98]. Overall, the perturbations in the micro-environment of the periosteum following bone injury generates unique cell-cell interactions and specific signals. This environment can vary depending on the type of injury, the mechanical stimuli and additional interactions with the adjacent skeletal muscle, bone marrow and systemic factors. How changes in this complex tissue environment influence the fate of pSSPCs remains to be explored.

Clinical applications of periosteal stem and progenitor cells for bone repair

As the periosteum is an essential actor of bone healing, pSSPC deficiencies can have direct consequences on repair. Metabolic dysregulation in mice with induced type 1 diabetes reduces callus formation, correlated with decreased pSSPC proliferation and osteogenic differentiation [99]. Mice with diet-induced obesity (DIO) and subsequent type 2 diabetes also exhibit impaired fracture healing [100,

101]. Periosteal SSPCs isolated from DIO mice show reduced osteochondral and adipogenic differentiation potential *in vitro* [100]. Aging is a long-known factor affecting bone repair in human and animal models partially due to a reduction of pSSPC potential and number [102, 103]. Two reports indicate abnormal extracellular matrix deposition and proliferation of periosteal cells isolated from 1-year-old mice and reduced chondrogenic potential when isolated from 2-year-old mice [104, 105]. Aging is frequently linked to osteoporosis [106]. Mice with estrogen or glucocorticoid-induced osteoporosis display an abnormal periosteal response to scratch injury, with reduced cartilage formation and maturation [107]. Overall, dysfunctions of pSSPCs are still poorly described, but could be of major interest to understand bone healing impairment.

Periosteum flap or allograft are frequently used in orthopedic surgery to promote bone repair with convincing results in animal models and in human [108–110]. Periosteal SSPCs are therefore considered for cell-based therapies. Transplanted pSSPCs can improve bone healing in aged mice, genetically induced pseudarthrosis and critical size defects [37, 108, 111]. Bone tissue engineering aims to replace autograft approaches, by using cultured SSPCs embedded in a 3D matrix containing growth factors. The choice of the optimal cell source is key for successful bone tissue engineering and the periosteum rise as a promising source of cells. Compared to other cell sources, such as bone marrow, adipose, or dental pulp derived cells, pSSPCs display higher clonogenicity, proliferation, osteogenic and chondrogenic differentiation [20, 112, 113]. Moreover, the potential of pSSPCs can be increased depending on the harvesting site and by using pre-treatment with BMP2 or FGF2 [38, 114, 115]. The development of periosteum-like matrix, that mimics the structural organization and cellular composition of the periosteum is also explored with encouraging results [116]. Growth factors can be added to the scaffold in order to stimulate endogenous pSSPCs or promote angiogenesis of the grafted bioengineered tissue (VEGF) [113, 117]. To exploit pSSPCs as an alternative source of cells for orthopedic cell-based therapies, a better understanding of their identity and the factors regulating their fate is needed.

Conclusions

The first studies describing periosteal SSPCs *in vitro* provided limited relevance for endogenous pSSPCs functions. *In vivo* lineage tracing, using transgenic mouse models, is a valuable tool to characterize pSSPCs in their periosteal niche and study their behavior in the complex fracture environment. Different subpopulations of pSSPCs have been identified using markers such as PRX1, CTSK, and αSMA. These pSSPCs share common features, including multipotency after fracture and self-renewal. It remains to be elucidated whether distinct sub-populations differ in their identity and potency or if they overlap and exhibit great plasticity based on their tissue localization and environmental signals in the fracture vicinity. Additionally, the absence of a specific marker to distinguish pSSPCs from the other SSPC sources remains a challenge. While SSPCs from the bone marrow and skeletal muscle are mostly

unipotent *in vivo*, pSSPCs are bi-potent differentiating into osteoblasts or chondrocytes. The origin of this bi-potentiality is unknown. The heterogeneity of pSSPCs populations with distinct osteochondral potential is one hypothesis. pSSPCs also evolve in a unique fracture environment at the interface of bone and skeletal muscle, exposing them to numerous biological and mechanical stimuli. Advances in single cell transcriptomics will provide new insights in the heterogeneity of pSSPCs and their responses to bone injury. The complementarity of *in vitro*, *in vivo* and transcriptomic approaches will enhance our understanding of pSSPCs and pave the way for new orthopedic cell-based therapies.

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Competing interests

Authors declare no competing interests.

Human and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Figures:

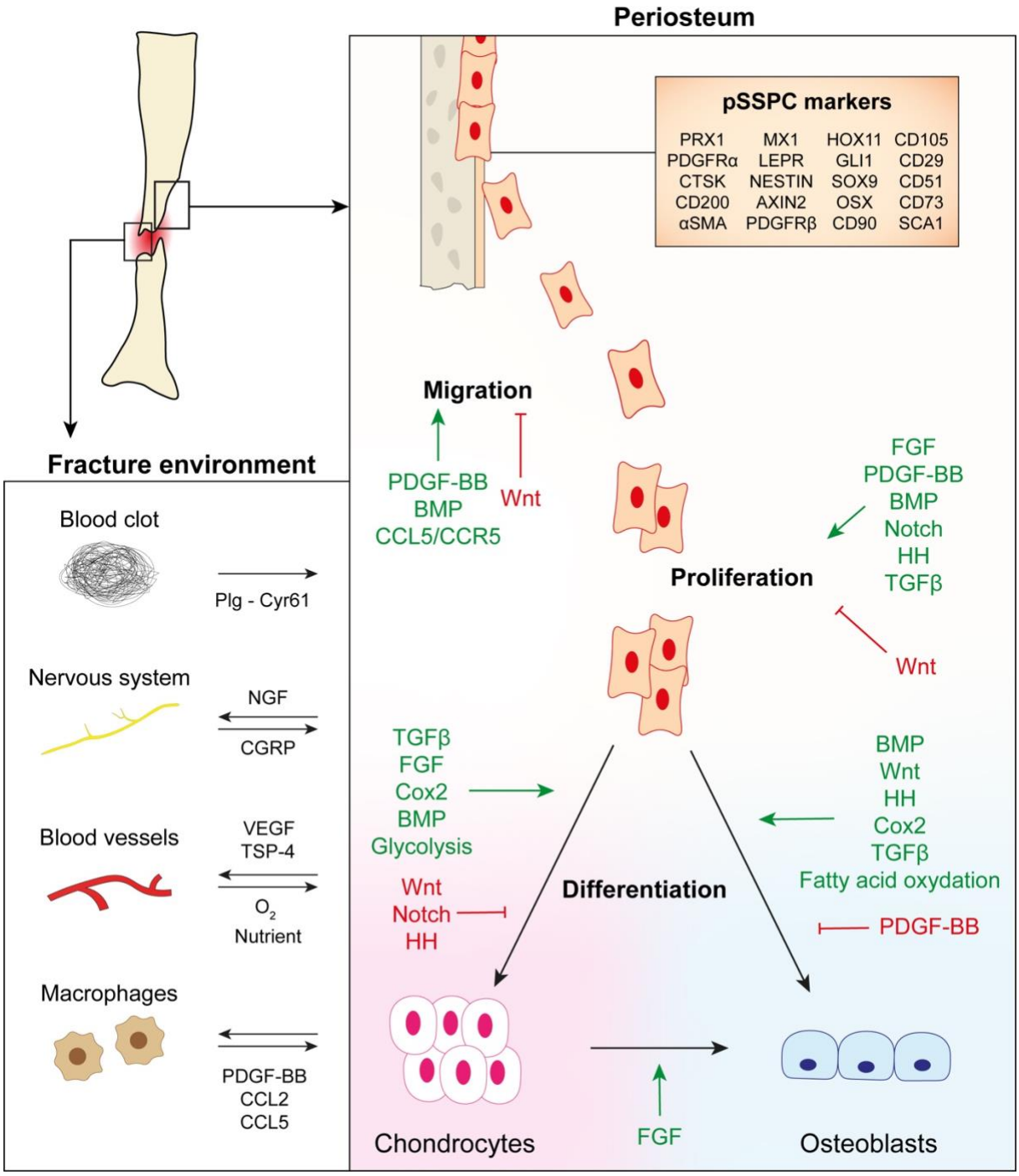

Figure 1: **Fate and regulation of periosteal stem/progenitor cells during bone repair**

After fracture, periosteal stem/progenitor cells (pSSPC), labelled by several markers (orange box) migrate to the site of injury, proliferate and differentiate into chondrocytes or osteoblasts. These steps are controlled by several signaling pathways (activation in green and inhibition in red). The fate of pSSPCs is also influenced by their interactions with the fracture environment, including the blood clot, nerve fibers, blood vessels and macrophages (left box).

Table 1.: Markers and mouse lines labeling pSSPCs

Markers	Mouse model	Injury model	Contribution	Comment	Used in
PRX1	*Prx1* ^{Cre}	Non stabilized tibial fracture	Cartilage / Bone	Labels all SSPCs	Duchamp et al. [20]
	Prx1 ^{CreERT}	Non stabilized ulna fracture	Cartilage / Bone	Low cell labelling	Kawanami et al. [46]
PDGFRα	*Pdgfra* ^{CreERT}	Non stabilized tibial fracture	Bone / Cartilage	Labels mesenchymal cells from various tissues	Julien et al. [25]
		Non stabilized forelimb fracture	Bone / Cartilage		Xu et al. [47]
CTSK	*Ctsk* ^{Cre}	Semi stabilized femoral fracture	Bone / Cartilage	Marks a subpopulation of pSSPCs and osteoclasts	Debnath et al. [22]
		Calvarial cortical defect	Bone		
αSMA	*αSMA* ^{CreERT}	Semi stabilized tibial fracture	Bone / Cartilage		Matthews et al. [24]
	αSMA-GFP	Calvarial cortical defect	Bone		Ortinau et al. [51]
MX1	*Mx1* ^{Cre}	Non stabilized tibial fracture	Bone / Cartilage	Requires pIpC injection to induce Cre recombination	Ortinau et al. [51]
		Tibial periosteal defect	Bone		
		Tibial cortical defect	Bone		
		Calvarial cortical defect	Bone		
Axin2	*Axin2* ^{CreERT}	Tibial cortical defect	Bone		Ransom et al. [52]
PDGFRβ	*Pdgfrb* ^{Cre}	Semi stabilized femoral fracture	Bone / Cartilage		Bohm et al. [53]
HOX11	*Hoxa11* ^{CreERT2}	Non stabilized ulnar fracture	Bone / Cartilage	Restricted to zeugopod bone	Pineault et al. [54]
GLI1	*Gli1* ^{CreERT1}	Semi stabilized femoral fracture	Bone / Cartilage	Marks bone marrow SSPCs	Shi et al. [56]
		Semi stabilized tibial fracture	Bone / Cartilage		Xia et al. [57]
Nestin	*Nes-GFP*	Semi stabilized tibial fracture	Bone	Marks bone marrow SSPCs	Tournaire et al. [55]
	Nes ^{CreERT}	Tibial cortical defect	Bone	Marks bone marrow SSPCs	Gao et al. [42]
LEPR	*Lepr* ^{Cre}	Tibial cortical defect	Bone	Marks bone marrow SSPCs	Gao et al. [42]
SOX9	*Sox9* ^{CreERT}	Semi stabilized femoral fracture	Bone / Cartilage		He et al. [58]
		Rib bone resection	Bone / Cartilage	Labelling is increased when induced after fracture	Kuwahara et al. [17]
OSX	*Osx* ^{Cre}	Semi stabilized tibial fracture	Bone / Cartilage	Labels osteogenic progenitors	Bohm et al. [53]

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