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Pericyte/myofibroblast phenotype of osteoprogenitor cell

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Introduction

The osteoblast lineage arises from a mesenchymal progenitor cell that also has the ability to generate a diversity of other lineages including adipocytes, chondrocytes, myocytes and cells that support hematopoiesis^{1,2}. In order to better understand the developmental and homeostatic processes in bone it is imperative to define strictly the stages within the osteoprogenitor lineage maturation. The development of experimental models in which green fluorescent protein (GFP) reporter genes are expressed under the control of osteoblast lineage promoters has provided valuable information on the progression of the lineage at relatively mature stages of development. However, they have not provided information on the identity of mesenchymal progenitor cells prior to entering the osteogenic pathway. We propose that the step of recruitment of multipotent progenitors into the osteogenic pathway is a critical stage on the control of bone mass and could be an attractive target for pharmacological intervention. Unfortunately, at this moment we do not possess good strategies to confidently identify and isolate these early progenitors. Based on the successful development of osteoblast lineage directed GFP markers, we now aim to identify a definitive marker specific for progenitor cells prior to entering the osteogenic pathway.

Defining the gene expression profiles of osteoblast lineage cells

We have generated GFP expressing transgenic mice containing a 3.6kb (pOBCol3.6GFPtpz) and a 2.3 kb

(pOBCol2.3GFPemd) rat type I collagen (Col1a1) promoter fragment³. The pattern of fluorescence evaluated in calvarial bone cell cultures derived from the transgenic mice has shown that pOBCol3.6GFPtpz positive cells first appear in the preosteoblast stage prior to nodule formation and continue to show a strong signal in cells within bone nodules. In contrast, pOBCol2.3GFPemd fluorescence first appears in nodules undergoing mineralization and therefore identifies a mature matrix producing osteoblast. These findings suggest that different forms of the Col1a1GFP transgenes are marking different subpopulations of cells during differentiation of skeletal osteoprogenitors. One of the advantages of the generation of GFP markers of differentiation is the ability to characterize and isolate these cells as a relatively homogeneous population from a heterogeneous cell population using flow cytometry⁴. To better define the populations identified by GFP markers we used fluorescent activated cell sorting (FACS) to isolate GFP+ or GFP- cells from primary calvarial culture for microarray studies⁵. Seven-day old cultures were harvested to obtain low-level pOBCol3.6GFP+ and GFP- cells (at the preosteoblast level) and 17-day-old cultures to separate pOBCol2.3GFP+ and GFP- cells (at the osteoblast level). RNA profiling of subpopulations of cells demonstrates regulatory changes that would not be appreciated and could be misleading when compared to results based on the total cell population. Although there are many examples of genes that are up-regulated in the total unsorted population that are also up-regulated in the isolated cell population (i.e., osteocalcin, bone sialoprotein, dentin matrix protein), there are also numerous examples of genes that are highly expressed in the unsorted mature stage of the culture (unsorted, day 17 culture) but are expressed at very low levels in the mature osteoblasts that are isolated based on the pOBCol2.3 expression. Examples include insulin-like growth factor-1, Sfrp1, Sfrp2, Ptger4, follistatin and others⁵.

We have also analyzed comparatively the expression pattern of pOBCol3.6GFP+ cells with respect to the pOBCol3.6GFP. None of the genes showed increased expression, while only 69 genes were expressed at lower levels in the pOBCol3.6GFP+

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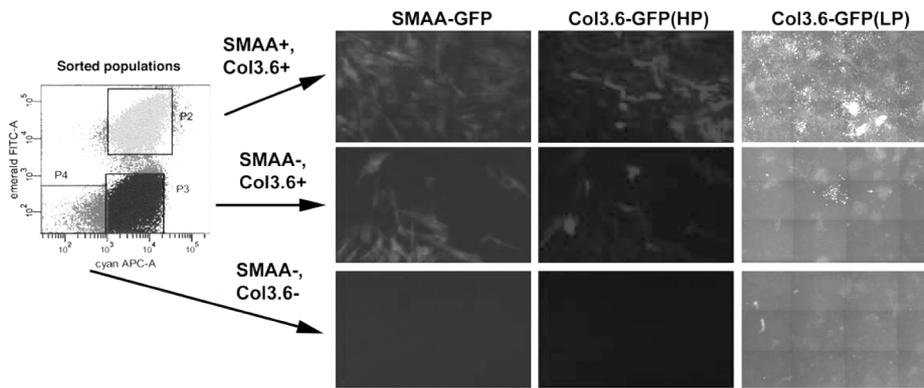


Figure 1. Bone marrow stromal cell cultures were sorted on day 7 into the following groups: SMAA+/Col3.6+ (P2), SMAA-/Col3.6+ (P3) and SMAA-/Col3.6- (P4) and replated. After induction to osteogenic differentiation SMAA+/Col3.6+ yielded a high proportion of cells that achieved a strong signal of Col3.6GFPcyan associated with mineralized nodules (middle panel). Scan of culture dish at low power detects large areas of osteogenic differentiation in this group (right panel). SMAA-/Col3.6+ population showed limited ability of osteogenic differentiation, while SMAA-/Col3.6- did not show any osteogenic colonies (lower panel).

population. We find it very indicative that the population of genes with lower expression included 11 of them associated with the myofibroblastic phenotype (Table 1), and another group of genes characteristic of macrophage/dendritic/osteoclast lineage cells. These data led to the proposal that myofibroblastic cell could behave as osteoprogenitors⁵.

Myofibroblastic markers of osteoprogenitor cells

It is intriguing that cells with mesenchymal progenitor properties have been found associated to a wide variety of tissues, indicating a common population of cells residing in these tissues. In recent studies it has been shown that when placed *in vitro*, retinal pericytes can generate mineralizing nodules⁶, chondrocytes and adipocytes⁷.

To identify and isolate this population of cells we have obtained transgenic mice expressing GFP under the control of smooth muscle alpha-actin (SMAA) promoter. This transgene (SMAA-GFP) directs the GFP expression towards smooth muscle cells and pericytes⁸. Cell populations derived from a bone marrow stromal cell culture of dual SMAA-GFP(Green)/pOBCol3.6cyan(blue) transgenic mice were isolated by FACS following a 7-day culture period that allowed subsequent activation of SMAA-GFP and Col3.6GFP promoter. Osteogenic differentiation of individual population was examined. As indicated in Figure 1, the most prominent osteogenic differentiation was detected in cells that were positive for both SMAA-GFP and pOB3.6cyan (SMAA+/Col3.6+), indicating the ability of SMAA to select a population of osteoprogenitors from this heterogeneous cell culture model.

To achieve success in cell-based therapeutical approaches mesenchymal progenitor cells must be identified, isolated, maintained in an undifferentiated state, expanded and eventually genetically manipulated. In future work, we plan to

Name	3,6 GFP ^{neg}	3,6 GFP ^{pos}
Actin, alpha 1, skeletal muscle	1,573	420
Actin, alpha, cardiac	1,108	14
Clusterin	1,569	505
Myoglobin	243	76
Matrix metalloproteinase 3, stromelysin-1	5,381	1,088
Myosin, heavy polypeptide 3	127	12
MLCIF/MLC3F myosin light chain	2,068	344
Myosin light chain, alkali, cardiac atria	1,406	311
Troponin C, cardiac/slow skeletal	929	136
Troponin T2, cardiac	294	104

Table 1. Gene expression during osteoprogenitor commitment.

apply our experience in maintaining primary bone marrow stromal cells in an undifferentiated state to highly purified SMAA-GFP positive cells from bone marrow, adipose and other easily accessible non-osseous tissues.

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