

## Enrichment and Characterization of 2 Subgroups of Committed Osteogenic Cells within the Mouse Endosteal Bone Marrow with Expression Levels of CD24

Ching-Fang Chang<sup>1,2</sup>, Ke-Hsun Hsu<sup>1,2</sup>, Chia-Ning Shen<sup>1,2,3</sup>, Chung-Leung Li<sup>2,3\*</sup> and Jean Lu<sup>1,2</sup>

*1Institute of biology and medical specialty, National Yang-Ming University, Taipei, Taiwan 2Genomics centre, domain Sinica, Taipei, Taiwan 3Institute of natural science and Biotechnology, school of Life Sciences, National Taiwan Ocean University, Keelung, Taiwan, E-mail: jeanlu@gate.sinica.edu.tw*

### ABSTRACT

Primary osteogenic cells are acknowledged to reside at intervals the CD45- CD31- Ter119- Sca-1- cell fraction, notably within the CD51+ universe. However, careful determination of the frequency of osteogenic cells at intervals this Sca-1- cell population remains nonetheless to be determined. additionally, it's not clear that alternative cell surface markers is wont to more sub-fractionate this Sca-1- CD51+ osteogenic cell population and to outline their organic process stages. during this report, each Sca-1- CD24<sup>med</sup> and Sca-1- CD24<sup>lo</sup> cells are shown to be 2 tiny subsets of the Sca-1- CD51+ cell fraction. These 2 cell fractions show delicate distinction within the expression level of osteogenic marker genes like *Osx* and *Opn*, and in vitro proliferate rate. of these observations counsel that they'll be at totally different organic process stages of osteogenesis. The Sca-1- CD24<sup>med</sup> cell fraction is enriched for the a lot of mature osteolineage cells than the Sca-1- CD24<sup>lo</sup> counterpart. In distinction, most of the Sca-1- CD24<sup>hi</sup> and Sca-1+CD24<sup>lo</sup> cells don't contain CFU-ALP nor categorical osteogenic factor markers. The high proliferation ability and osteo-adipogenic differentiation potentials ensure that the Sca-1+CD24<sup>lo</sup> cells ar the multipotential mesenchymal stromal cells. The determination of individual stromal cell subpopulations can cause a much better understanding within the hierarchal organization of those osteolineage cells.

Bone may be a extremely organized tissue, comprised of a calcified animal tissue matrix and specific bone cells, as well as bone progenitors, osteoblasts, and osteocytes. Osteoblasts ar derived from potent marrow stromal cells (MSCs) through a series of proliferation and differentiation steps before expressing recognizable specific osteoblasts marker genes. though abundant has been learned regarding the cellular identity and differentiation potential of MSCs, very little is understood regarding the hierarchal relationship between cells of the osteogenic lineage and people with alternative connected cell lineages at intervals the bone marrow.

For analysis of stromal cell subpopulations, cells were incubated on ice for fifteen min with every of following antibodies combining with above-named mixture of antibodies. throughout organic process, 3 consecutive steps are recognized: proliferation, matrix maturation, and step-down. Conceptually, ancestor cells would lose their proliferation ability once these cells progress through the osteogenic differentiation.

In this report, supported the results of flow cytometric analysis of stromal cell subpopulations with Sca-1 and CD24, (ii) in vitro enlargement capability and differentiation assays (iii) primary adherent CFU assay, and (iv) osteogenic marker factor identification in primary cells, we've got known there ar one single MS population (Sca-

1+CD24-/lo) and 2 subsets of committed osteogenic cells (Sca-1- CD24med and Sca-1- CD24-/lo) within the mouse endosteal bone (Figure 5). Thus, we've got incontestible that CD24 substance may be a new cell surface marker for the enrichment and identification of committed osteogenic cell subpopulations within the endosteal bone marrow. solely only a few of the Sca1- CD51+ cells, that ar Sca-1- CD24med or Sca-1- CD24-/lo committed to osteolineage. each Sca-1- CD24med or Sca-1- CD24-/lo cells extremely

enriched CFU-ALP, expressed osteogenic genes, and each of them cannot differentiation into adipocyte.

The hypothesis of hierarchal organization of osteogenic lineage were planned that in osteogenesis, high proliferative Sca-1+CD24-/lo MSCs would lose their proliferation and adipocyte differentiation capability, so differentiate into Sca-1- CD24-/lo early stage of osteogenic cells.

**Keywords:** Osteolineage cells; Osteogenic subpopulation; Fluorescence-activated cell sorting; CD24; alkaline phosphatase