Chapter 4

Gene Expression Regulation underlying Osteo-, Adipo-, and Chondro-Genic Lineage Commitment of Human Mesenchymal Stem Cells

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ABSTRACT

Human mesenchymal stem cells have a high potential in regenerative medicine. They can be isolated from a variety of adult tissues, including bone marrow, and can be differentiated into multiple cell types of the mesodermal lineage, including adipocytes, osteocytes, and chondrocytes. Stem cell differentiation is controlled by a process of interacting lineage-specific and multipotent genes. In this chapter, the authors use full genome microarrays to explore gene expression profiles in the process of Osteo-, Adipo-, and Chondro-Genic lineage commitment of human mesenchymal stem cells.

INTRODUCTION

Human Mesenchymal Stem Cells (hMSCs) can be obtained in relatively large numbers from a variety of connective tissues sources including adipose tissue, umbilical cord and bone marrow (De Bari, et al., 2003; Pittenger, et al., 1999; Zuk, et al., 2002). The cells are multipotent cells and can differentiate *in vivo* into a variety of mesenchymal tissues, including bone, muscle, cartilage, and fat. Although they lack specific markers, upon *in vitro* culturing they can be identified by the expression

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of surface molecules such as CD105 and CD73, while they are negative for the hematopoietic markers CD34, CD45, and CD14 (Chamberlain, Fox, Ashton, & Middleton, 2007). They have the ability to expand many-fold *in vitro* while maintaining their growth potential and multipotency (Bouchez, et al., 2011), giving rise to cultures ranging from narrow spindle shaped to large polygonal cells (Javazon, Beggs, & Flake, 2004). Also *in vitro* they have the ability to differentiate into osteoblasts, chondrocytes, and adipocytes (Dezawa, et al., 2005; Pittenger, et al., 1999). The fact that these cells can be differentiated into several different cell types, in combination with their immune-modulatory properties, make MSCs a promising source of stem cells for tissue repair and gene therapy.

With aging of the population, degenerative diseases such as osteoporosis and arthritis will have an increasing impact. The increase in marrow adipogenesis associated with osteoporosis and age-related osteopenia is well known clinically, and classical *in vitro* and *in vivo* studies strongly support an inverse relationship between the commitment of bone marrow-derived mesenchymal stem cells to the adipoctye and osteoblast lineage pathways (Nuttall & Gimble, 2004). Restoration of damaged bone and cartilage or of an unbalanced cell fate by stimulating hMSCs to differentiate into a specific lineage, provides a novel and attractive therapeutic approach. This interest in developing new therapies with cells that can repair non-hematopoietic tissues is currently of high interest and the first successful clinical trials with MSCs claimed to improve osteogenesis in children with osteogenesis imperfecta (Horwitz, et al., 2001). Currently hMSCs are being employed in clinical trials in heart disease, Crohn's disease, cartilage repair, stroke, spinal cord injury, and several other diseases (Giordano, Galderisi, & Marino, 2007; Körbling & Estrov, 2003; Prockop & Olson, 2007) with positive results. In addition, implanted cell–host interaction needs to be addressed carefully (Shi, et al., 2012), which requires detailed knowledge of the pathways involved in hMSC differentiation, as key factor for understanding normal development and disease processes.

This study aims to apply a high-throughput screening of gene expression regulation underlying lineage commitment in hMSCs to understand tissue development and to identify key genes involved in lineage-specific differentiation. hMSCs were induced to differentiate *in vitro* into three distinct lineages, i.e. bone, cartilage and fat, by applying different culture conditions. The percentage of differentiated cells was determined for each differentiation condition. Analysis of the multiple gene expression data sets, obtained upon specific treatments and time points during the course of lineage-specific differentiation, was used to confirm and understand hMSC fate.

MATERIALS AND METHODS

Culture and Differentiation of Human Mesenchymal Stem Cells

Human Mesenchymal Stem Cells (hMSCs), harvested from normal human bone marrow, were purchased from Lonza (Walkersville, MD) at passage 2. Cells were tested by the manufacturer and were found to be positive by flow cytometry for expression of CD105, CD166, CD29, and CD44, and negative for CD14, CD34, and CD45. We confirmed multipotency of all donor batches based on *in vitro* osteo-, chondro- and adipogenic differentiation capacity. The cells were expanded for no more than 5 passages in 'Mesenchymal Stem Cell Growth Medium' (MSCGM; Lonza, Walkersville, MD) at 37ºC in a humidified atmosphere containing 7.5% CO_2. Studies were performed with hMSCs from multiple donors, including 5F0138 and 1F1061.

Osteogenesis Data Set: For osteogenic differentiation, 2.0 x 10^4 cells per cm^2 were seeded

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