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RESEARCH ARTICLE

Retinoic acid induces mouse bone marrow-derived CD15^{b}, Oct4^{b} and CXCR4^{b} stem cells into male germ-like cells in a two-dimensional cell culture system

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Abstract

We have examined the effect of retinoic acid (RA) on differentiation of bone marrow-derived CD15^{b}, Oct4^{b} and CXCR4^{b} cells into male germ cells. Bone marrow stem cells (BMSCs) were isolated from the femur of 3–4-week-old male C57BL/6 mice. Magnetic-activated cell sorting (MACS) system was used to sort CD15^{b}, Oct4^{b} and CXCR4^{b} cells. RT-PCR was used to follow the expression of pluripotency markers. Sorted CD15^{b}, Oct4^{b} and CXCR4^{b} cells were cultured in an undifferentiated condition on a feeder layer of mitomycin C-inactivated C2C12. The embryoid-like bodies were differentiated into male germ cells by retinoic acid. To identify the expression of male germ specific markers, differentiated cells were analysed by means of reverse transcriptase polymerase chain reaction (RT-PCR) and immunofluorescence staining. RT-PCR and immunofluorescence show that bone marrow-derived CD15^{b}, Oct4^{b} and CXCR4^{b} cells express pluripotency markers, Oct4, Nanog, Rex-1, SOX-2 and AP. The purified CD15^{b}, Oct4^{b} and CXCR4^{b} formed structures like embryoid bodies when plated over a feeder layer; these bodies were alkaline phosphatase positive. When cells were induced by RA, bone marrow-derived CD15^{b}, Oct4^{b} and CXCR4^{b} were positive for Mvh, Dazl, Piwil2, Dppa3 and Stra8, that known molecular markers of male germ cells. Thus RA can induce differentiation of mouse bone marrow-derived CD15^{b}, Oct4^{b} and CXCR4^{b} cells into male germ cells in vitro. Negative results for the gene expression analysis of female germ cells markers, GDF9 and ZP3, confirmed this conclusion.

Keywords: bone marrow stem cells; differentiation; male germ cells; retinoic acid

Introduction

Germ line cells provide the continuity of life between generations. They are responsible for transmitting genetic information from generation to generation (Eguizabal et al., 2009). Germ cells range from the primordial germ cells (PGCs) during embryogenesis, to sperm and oocytes during gametogenesis. The PGCs are highly specified cell lineage, derived from a subset of cells in the epiblast, and undergo major reprogramming events during their development (Hübner et al., 2003). Recent amazing studies show successful results of differentiation of germ cells (sperm and oocyte) in different stages from embryonic stem cells (ESCs) (Hübner et al., 2003; Nayernia et al., 2006b; Hayashi and Saitou, 2013a). However, ethical concerns and immune rejection after cell transplantation limited the clinical application of ESCs (Kang et al., 2010). Fortunately, stem cells from adult bone marrow show embryonic stem cell-like properties, expressing pluripotency genes (Jiang et al., 2002; Gronthos et al., 2003; D'Ippolito et al., 2004; Shirazi et al., 2012).

Murine bone marrow contains a population of stem cells that expresses early developmental markers such as cluster of differentiation 15 (CD15), octamer-binding transcription factor4 (Oct4) and chemokine (C-X-C motif) receptor4

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