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Different Concentrations of FGF Ligands, FGF2 or FGF8 Determine Distinct States of WNT-Induced Presomitic Mesoderm

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ABSTRACT

Presomitic mesoderm (PSM) cells are the precursors of the somites, which flank both sides of the neural tube and give rise to the musculo-skeletal system shaping the vertebrate body. WNT and FGF signaling control the formation of both the PSM and the somites and show a graded distribution with highest levels in the posterior PSM. We have used reporters for the mesoderm/PSM control genes *T, Tbx6*, and *Msgn1* to investigate the differentiation of mouse ESCs from the naïve state via EpiSCs to PSM cells. Here we show that the activation of WNT signaling by CHIR99021 (CH) in combination with FGF ligand induces embryo-like PSM at high efficiency. By varying the FGF ligand concentration, the state of PSM cells formed can be altered. High FGF concentration supports posterior PSM formation, whereas low FGF generates anterior/differentiating PSM, in line with in vivo data. Furthermore, the level of *Msgn1* expression depends on the FGF ligand concentration. We also show that Activin/Nodal signaling inhibits CH-mediated PSM induction in EpiSCs, without affecting *T*-expression. Inversely, Activin/Nodal inhibition enhances PSM induction by WNT/high FGF signaling. The ability to generate PSM cells of either posterior or anterior PSM identity with high efficiency in vitro will promote the investigation of the gene regulatory networks controlling the formation of nascent PSM cells and their switch to differentiating/somitic paraxial mesoderm. STEM CELLS *2016;34:1790–1800*

SIGNIFICANCE STATEMENT

Our study adds a new dimension to the current understanding of the Wnt-mediated PSM induction of EpiSCs by deciphering the importance of FGF concentration in the lineage commitment toward different presomitic mesoderm (PSM) states. We believe that this study thereby provides valuable information for directed differentiation of ESCs. Furthermore, this method of differentiation toward PSM cell states can be a valuable method for studying the dynamics of gene regulation during PSM formation and musculoskeletal disorders that arise during early development.

INTRODUCTION

Presomitic mesoderm (PSM) is generated as mesenchymal, unsegmented tissue by the primitive streak, followed by the tail bud, located at the caudal end of the elongating amniote embryo. PSM is the precursor of the segmental somites, epithelial spheres residing along both sides of the neural tube, which give rise to the axial skeleton and skeletal muscles, tendons, dorsal dermis, and adipose tissue. A new pair of epithelial somites buds off from the anterior PSM at the same rate (about every 2 hours in mouse embryos) as the posterior PSM is replenished with new mesenchymal cells derived from the primitive streak or the tail bud (reviewed in [1]). The process of PSM segmentation, termed somitogenesis involves the oscillatory transcriptional activity of a set of so-called cyclic genes. They are components and targets of three molecular oscillators composed of the wingless-type MMTV integration site family (WNT), fibroblast growth factor (FGF), and Notch signaling pathways, which are integrated in a network called the segmentation clock [2].

The differentiation status of the cells in the PSM is thought to be controlled by a combinatorial gradient system in the PSM, involving the FGF [3–5] and WNT/β-catenin

