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Resource

The Transcriptional and Functional Properties of Mouse Epiblast Stem Cells Resemble the Anterior Primitive Streak

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SUMMARY

Mouse epiblast stem cells (EpiSCs) can be derived from a wide range of developmental stages. To characterize and compare EpiSCs with different origins, we derived a series of EpiSC lines from pregastrula stage to late-bud-stage mouse embryos. We found that the transcriptomes of these cells are hierarchically distinct from those of the embryonic stem cells, induced pluripotent stem cells (iPSCs), and epiblast/ectoderm. The EpiSCs display globally similar gene expression profiles irrespective of the original developmental stage of the source tissue. They are developmentally similar to the ectoderm of the late-gastrula-stage embryo and behave like anterior primitive streak cells when differentiated in vitro and in vivo. The EpiSC lines that we derived can also be categorized based on a correlation between gene expression signature and predisposition to differentiate into particular germ-layer derivatives. Our findings therefore highlight distinct identifying characteristics of EpiSCs and provide a foundation for further examination of EpiSC properties and potential.

INTRODUCTION

Epiblast stem cells (EpiSCs) are self-renewing multipotent stem cells that can be derived from the epiblast of postimplantation mouse embryos (Brons et al., 2007; Tesar et al., 2007). In contrast to mouse embryonic stem cells (mESCs), EpiSCs are inefficient in contributing to the tissue of chimeras following blastocyst injection, show less active expression of "naive" pluripotency-related genes, and exhibit increased Oct4 occupancy of the proximal enhancer of *Pou5f1* (Tesar et al., 2007). Like human ESCs, EpiSCs are dependent on FGF/ERK and Activin(Nodal)/Smad signaling activities for derivation and maintenance (Vallier et al., 2009a, 2009b, 2009c).

EpiSCs derived from E5.5 to E6.5 epiblast display the gene expression characteristics of gastrula-stage epiblast/ectoderm (epi/ect) (Brons et al., 2007; Hayashi et al., 2011; Iwafuchi-Doi et al., 2012; Tesar et al., 2007). EpiSCs show monoallelic expression of imprinted genes (Sun et al., 2012) and display X-inactivation in XX lines (Bao et al., 2009; Guo et al., 2009; Hayashi et al., 2008). The presence of histone methylation marks in the *Stella* (*Dppa3*), *Otx2, Rex1*, and *Fbxo15* loci (Bao et al., 2009; Hayashi and Surani, 2009; Tesar et al., 2007) further suggests that EpiSCs are distinct from mESCs. EpiSCs are multipotent and can generate a multitude of germ-layer-derived tissues in teratomas and during in vitro differentiation. Although there are variations in the neural potential among EpiSC lines (Bernemann et al., 2011), they readily differentiate into neuronal lineages after removal of supporting growth factors (Iwafuchi-Doi et al., 2012; Najm et al., 2011b). When grafted into gastrula-stage mouse embryo, EpiSCs were incorporated into the derivatives of all three germ layers (Huang et al., 2012), revealing that the EpiSCs may be the in vitro equivalent of gastrula-stage epiblast cells. However, not all cells display similar lineage potential. A subset of cells (0.1%–0.5% of the colony) exhibit occupancy of the distal enhancer of *Pou5f1* gene that is characteristic of the naive pluripotency state and are able to contribute to chimeras like the ESCs (Han et al., 2010). Also, some cells in the EpiSC colonies express *Stella* and are competent to produce primordial germ cells in vitro (Hayashi et al., 2008; Hayashi and Surani, 2009). These cells therefore may retain the potency of germ cell formation and are functionally similar to the epiblast at (or before) E6.25–E6.5 in vivo when germ cells are specified (Ohinata et al., 2009).

Recently, it has been shown that EpiSCs can be derived from mouse embryos of a wide range of developmental stages from E3.5 blastocysts (Najm et al., 2011a) and from epi/ect of E6.5–E8.0 presomite-stage embryos (Osorno et al., 2012). It is not clear if EpiSCs derived from these cellular sources display similar or different states of pluripotency and lineage characteristics. In this study, we examined the molecular properties and the differentiation potential of EpiSCs generated from embryos

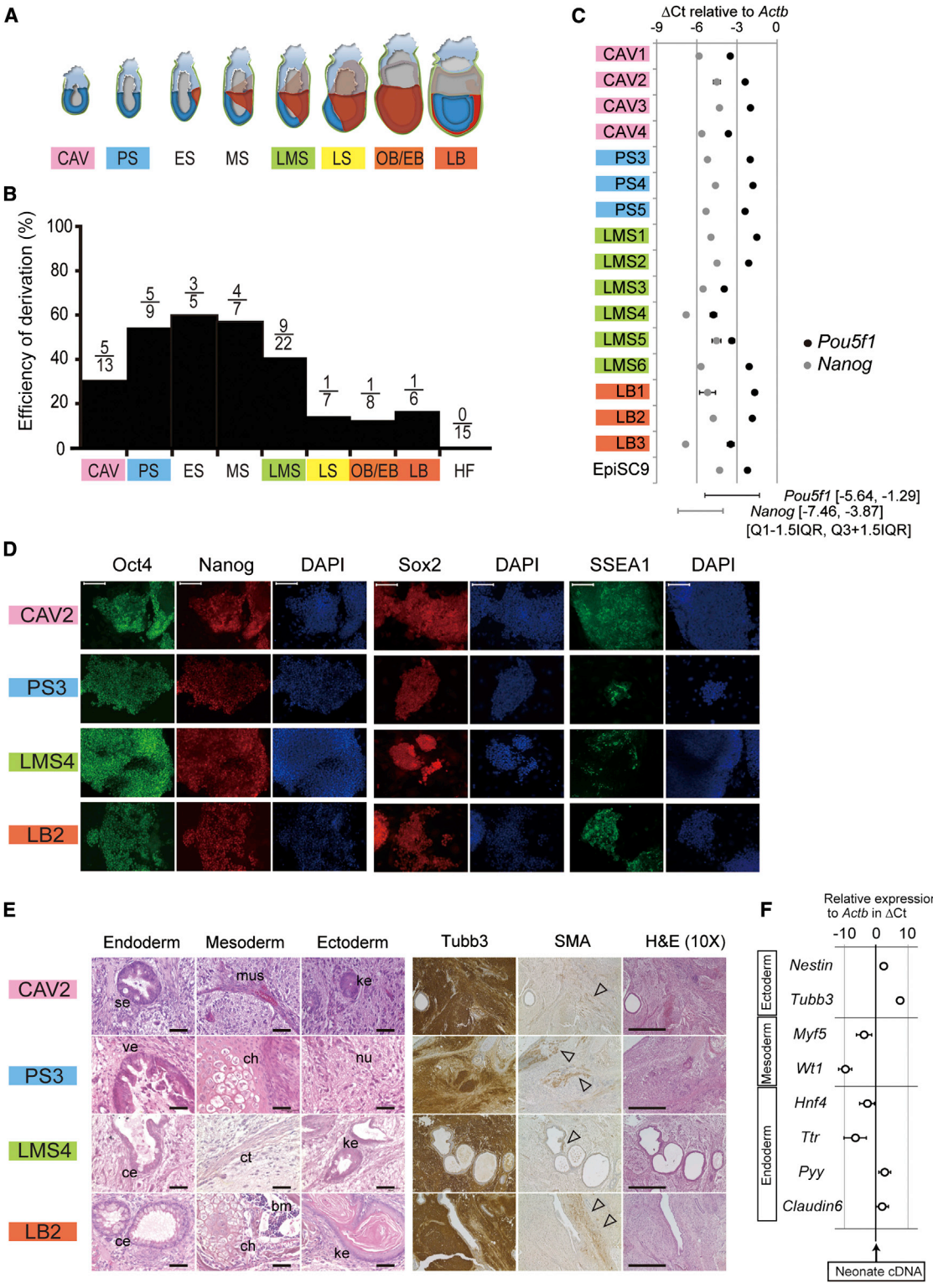

Figure 1. Derivation of EpiSCs from Mouse Embryos at Immediate Postimplantation Stages of Development

(A) Staging of embryos by the morphology of the epiblast and proamniotic cavity before gastrulation, the shape of the epibast/ectoderm (epi/ect, blue), formation of the mesoderm, the primitive streak, and the allantois (red), and the development of the extraembryonic mesoderm and the chorion (gray). See Supplemental Information for staging criteria. CAV, cavity; PS, prestreak; ES, early-streak; MS, midstreak; LMS, late midstreak; LS, late streak; OB/EB, no bud/early bud; LB, late bud; HF, head fold.

(B) The efficiency of EpiSC derivation from epi/ect of CAV- to HF-stage embryos. Fraction above the bar = number of established lines/number of explants.

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