Stem Cell Research 30 (2018) 100–112

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Isolation of primitive mouse extraembryonic endoderm (pXEN) stem cell lines

Yixiang Zhong, Taewoong Choi, Minjae Kim, Kyoung Hwa Jung, Young Gyu Chai, Bert Binas*

Department of Molecular & Life Science, College of Science and Technology, Hanyang University (ERICA Campus), 55 Hanyangdaehak-ro, Sangrok-gu, Ansan-si, Gyeonggi-do 15588, Republic of Korea.

ARTICLE INFO

Keywords:
Stem cells
Blastocyst
Mice
Rats
Extraembryonic endoderm

ABSTRACT

Mouse blastocysts contain the committed precursors of the extraembryonic endoderm (ExEn), which express the key transcription factor Oct4, depend on LIF/LIF-like factor-driven Jak/Stat signaling, and initially exhibit lineage plasticity. Previously described rat blastocyst-derived ExEn precursor-like cell lines (XENP cells/HypoSCs) also show these features, but equivalent mouse blastocyst-derived cell lines are lacking. We now present mouse blastocyst-derived cell lines, named primitive XEN (pXEN) cells, which share these and additional characteristics with the XENP cells/HypoSCs, but not with previously known mouse blastocyst-derived XEN cell lines. Otherwise, pXEN cells are highly similar to XEN cells by morphology, lineage-intrinsic differentiation potential, and multi-gene expression profile, although the pXEN cell profile correlates better with the blastocyst stage. Finally, we show that pXEN cells easily convert into XEN-like cells but not vice versa. The findings indicate that (i) pXEN cells are more representative than XEN cells of the blastocyst stage; (ii) mouse pXEN, rather than XEN, cells are homologs of rat XENP cells/HypoSCs, which we propose to call rat pXEN cells.

Key resources table

Reagent or resource	Source	Identifier
Antibodies		
Oct4	Santa Cruz	Cat# sc-5279 RRID:AB_628051
Gata4	Santa Cruz	Cat# sc-9053 RRID:AB_2247396
Lifr	Bioss	Cat# bs-1458R RRID:AB_10857440
Ssea1	DSHB	Cat# MC-480 (SSEA-1) RRID:AB_528475
SM22	Santa Cruz	Cat# sc-53932 RRID:AB_1129519
VEcad (VE-cadherin)	Bioss	Cat# bs-0878R RRID:AB_10858193
Ecad (E-cadherin)	Bioss	Cat# bs-10009R RRID: Not found
Vim (Vimentin)	DSHB	Cat# H5 RRID:AB_2216409
Donkey anti-rb IgG 488	Abcam	Cat# ab150073 RRID:AB_2636877
Donkey anti-ms IgG 594	Jacksonimmuno	Cat# 715-585-151 RRID:AB_2340855
Donkey anti-ms IgM 594	Jacksonimmuno	Cat# 715-585-140 RRID:AB_2340853
Mouse IgG	Invitrogen	Cat# 10400C RRID:AB_2532980
Rabbit IgG	Invitrogen	Cat# 10500C RRID:AB_2532981
Chemicals, peptides, and recombinant proteins		
Chemically defined lipid concentrate	Gibco	11905-031
Fatty acid-free BSA	GenDEPOT	A0100-010
Mitomycin C	Tocris	3258
FBS	MP Biomedicals	2916754
Stattic	Tocris	2798
Imatinib	LC Laboratories	I-5508
CHIR99021	LC Laboratories	C-6556
Human TGF-β1	PeproTech	100-21
Fibronectin	Sigma	F1141

Abbreviations: ExEn, extraembryonic endoderm; LIF, leukemia inhibitory factor; MEF, mouse embryo fibroblasts; PDGF, platelet-derived growth factor; PrE, primitive endoderm; pXEN cells, primitive (mouse) ExEn stem cells; XEN cells, (mouse) ExEn stem cells; XENP cells, (rat) ExEn stem cells

* Corresponding author.
E-mail address: bbinas@hanyang.ac.kr (B. Binas).

https://doi.org/10.1016/j.scr.2018.05.008
Received 21 November 2017; Received in revised form 16 March 2018; Accepted 16 May 2018
Available online 18 May 2018

LIF	ORF Genetics	01-A1140
PDGF	eBioscience	14-8501-80
EGF	Miltenyi Biotec	130-093-825
Trypsin/EDTA	Welgene	LS 015-01
DMEM	Sigma	D6046
MCDB-201	Sigma	M6770
ITS	Gibco	41400
LA-BSA	Sigma	L9530
Ascorbic acid	Sigma	A8960
Aragose	Invitrogen	16500
DAPI	Sigma	D9542
Deposited data		
Raw and analyzed data	This paper	GEO: GSE106158
Published gene expression profiling data downloaded from GEO database, see Suppl Table 6	–	–
UCSC (*Mus musculus* UCSC) reference genome: mm10	–	http://hgdownload.soe.ucsc.edu/goldenPath/mm10/bigZips/
Experimental models: cell lines		
see Suppl. Table 2	–	–
Experimental models: organisms/strains		
Mouse	DBL, Incheon, South Korea	ICR
Oligonucleotides		
For cell line authentication, see Suppl Table 3.	–	–
For lineage marker analysis, see Suppl Table 5.	–	–
Software and algorithms		
Trimmomatic (v 0.36)	(Bolger et al., 2014)	http://www.usadellab.org/cms/?page=trimmomatic
STAR (v 2.5.2b) aligner software	(Dobin et al., 2013)	https://github.com/alexdobin/STAR
DESeq2 (v 1.14.1)	(Love et al., 2014)	http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html
Prism GraphPad 6.0c	GraphPad Software	https://www.graphpad.com

1. Introduction

During preimplantation development, the initially indistinguishable cells of the morula divide and differentiate into the founder cells of the three lineages that form the conceptus: Trophectoderm (TE; precursor of the placental trophoblast), epiblast (precursor of fetus and amnion) and hypoblast (also called PrE; precursor of the ExEn). In the morula, the first lineage decision creates an outside TE and an inner cell mass (ICM). During the following blastocyst stage, the ICM differentiates into a salt-and-pepper pattern of reversibly committed ExEn and epiblast precursors, which rapidly resolves into two layers of irreversibly determined hypoblast and epiblast cells (Chazaud and Yamanaka, 2016; Rossant, 2016; Schrode et al., 2013).

Various immortal cell lines have been derived from mouse blastocysts, which capture the blastocyst cell types in more or less authentic form. Mouse embryonic stem (ES) cells (Evans and Kaufman, 1981; Martin, 1981) have been studied best and are considered as the equivalent of the pre-implantation epiblast; culture versions exist that favor either the early (embryonic day (E) 3.5) or late (E4.5) ICM stage (Martin Gonzalez et al., 2016). ES cell cultures are not necessarily homogeneous, but constitute equilibria and/or mixtures with a minority of developmentally related stages or lineages (Canham et al., 2010; Chen et al., 2013; Lo Nigro et al., 2017; Meek et al., 2013; Morgani and Brickman, 2015; Morgani et al., 2013).

Amongst the minority components, cells with characteristics close to the ExEn precursor stages are present and may play an important role in the LIF-stimulated self-renewal of mouse ES cells (Lo Nigro et al., 2017; Morgani and Brickman, 2015; Morgani et al., 2013). Very recent work indicates that such in vitro intermediates can be stabilized in a self-renewable format. Indeed, mouse ES cell-derived cell lines (termed naïve extraembryonic endodermal, or nEnd, cells) with characteristics close to the blastocyst-stage ExEn precursors have been presented (Anderson et al., 2017). However, blastocyst-derived stable mouse cell lines with distinctive characteristics of the committed ExEn precursor present in the ICM have not been reported. Instead, XEN cells have been derived that lack such characteristics (Kunath et al., 2005).

Interestingly, with respect to blastocyst-derived cell lines of the ExEn lineage, the situation in the rat appears reciprocal to that in the mouse. From rat blastocysts, we previously isolated ExEn-committed stem cell lines (Debeb et al., 2009; Lo Nigro et al., 2012) that show distinctive characteristics of blastocyst-stage ExEn precursors, which notably include the expression of the key transcription factor Oct4 (Chazaud et al., 2006; Kurimoto et al., 2006) and a requirement for LIF or a LIF-like factor, possibly IL-6 (Morgani and Brickman, 2015). On the other hand, rat cell lines of the ExEn lineage that lack these characteristics and hence would be more XEN-like have not been reported. Therefore, some workers view the above rat cell lines simply as a species variant of the XEN cells (Chuykin et al., 2013).

While the rat cell lines (under the name XENP cells) were initially cultured in partially differentiated form (Debeb et al., 2009), use of a low-serum medium led to the isolation of practically identical but more homogenous Hypoblast Stem Cells (HypoSCs) (Lo Nigro et al., 2012). Moreover, it was shown that reminiscent of the somatic lineage plasticity of the ExEn precursor of the early (E3.5) mouse blastocyst in vivo (Nichols et al., 2009; Yamanaka et al., 2010), the XENP cells/HypoSCs showed somatic lineage plasticity in vitro (Lo Nigro et al., 2012). Whether XEN cells possess similar plasticity is not known, but would seem less likely because lineage plasticity is lost in the late (E4.5) ICM (Nichols et al., 2009; Yamanaka et al., 2010). Here, we used the low-serum conditions to isolate from mouse blastocysts novel cell lines that – similar to XENP/HypoSCs and nEnd cell lines- show distinctive features of the blastocyst-stage ExEn precursor. Otherwise, and unlike nEnd cells, they appear most similar to XEN cells. We term these cell lines primitive XEN (pXEN) cells. Furthermore, as justified herein, we propose to rename the rat XENP cells and HypoSCs as rat pXEN cells, to establish a terminology that builds on the prevailing terminology and is consistent for mouse and rat.

2. Materials and methods

2.1. Cell line derivation and maintenance

The mouse cell lines used here were derived from E3.5 blastocysts of outbred ICR mice. Detection of a vaginal plug was considered as E0.5.

Derivation of mouse pXEN cells essentially followed the procedure previously described for rat HypoSCs (Lo Nigro et al., 2012), but dexamethasone was omitted and 1X chemically defined lipid concentrate and 800 μg/ml fatty acid-free BSA were added (condition A). Blastocysts were individually seeded into fibronectin-coated 24-well plates and medium was changed every other day. Once colonies emerged, the ICM remnant was removed with a capillary, and when the colonies covered ~50% of the well, the primary cultures were passaged at a 1:1