In vitro differentiation of hESCs into ovarian follicle-like-cells

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Mammalian Germ Cell Development

Premeiotic Development
- Specification
- Migration & Colonization

Postmeiotic Development
- Meiosis
- Differentiation
  - Spermatocytes
  - Oocytes

Epiblast → PGCs → Gonia → *Sperm → Mature Oocyte
Building in vitro model to study human germ cell development

+ Human cell-based/organoid system

hESCs

In vitro differentiation
Creating an in vitro system to study human primordial germ cells

1. Stably integration of germ cell reporter into hESCs
   - VASA 2.5 kb promoter
   - eGFP
   - 3'UTR (1 kb)

2. Adherent differentiation and FACS isolation for VASA:GFP+ cells

3. Molecular and functional characterization of VASA:GFP cells
   - Responsiveness to BMPs
   - Early germ cell expression profile
   - Epigenetic status
   - Embryonic germ cell formation

VASA:GFP cells can grow on MEFs

**a**
Replated VASA:GFP+ colonies

**b**
GFP+ cells replated on MEF 7 days after FACS

**c**
Alkaline Phosphatase Assays

- hESCs
- GFP+

BMPs differentiation + 7th day replated on MEF

Kee et al. *Nature*, 2009
DAZL and pluripotent marker expressions in human fetal ovary are almost mutually exclusive
Transcriptions of pluripotent markers were down-regulated by expressing DAZL in hESCs.
Overexpression of DAZL decreases OCT4 protein level.
DAZL mutation found in POI patient is defective in regulating target genes
DAZL regulate exit of pluripotency and entry into meiosis
In vitro differentiation of hESCs to oocyte/follicles

Undifferentiated hESCs (XX lines)

Express intrinsic factors that promote germ cell development & meiosis

Meiotic germ cells

Adding extrinsic factors that promote folliculogenesis

Ovarian follicle
BOULE distributions in human fetal ovary

Human fetal ovary

DAPI  BOULE  VASA  MERGED

W12  W16  W20

% BOULE + GERM CELLS
DAZL and BOULE induce meiosis in hESCs
Meiotic markers PRDM9, γH2AX, SYCP3 appeared in differentiated nuclei *in vitro*
Meiotic markers SYCP3, MLH1 appeared in differentiated nuclei *in vitro*
Examples of *in vitro* derivations of organoids

**ARTICLE**

Cerebral organoids model human brain development and microcephaly

Madeline A. Lancaster, Magdalena Renner, Carol-Anne Martin, Daniel Wemel, Louise S. Bicknell, Matthew E. Hurley, Tessa Homfray, Josef M. Peminger, Andrew P. Jackson & Jürgen A. Knoblich

Self-organizing optic-cup morphogenesis in three-dimensional culture

Motosugu Eiraku, Nozomi Takači, Hiroki Ishibashi, Masako Kawada, Eiko Sakakura, Satoru Okuda, Kiyotoshi Sekiguchi, Taiji Aizachi & Yoshiki Sasai

Balanced organogenesis requires the orchestration of multiple cellular interactions to create the collective cell behaviours that progressively shape developing tissues. It is currently unclear how individual, localized parts are able to coordinate with each other to develop a whole organ shape. Here we report the dynamic, autonomous formation of the optic cup (retinal primordium) structure from a three-dimensional culture of mouse embryonic stem cell aggregates. Embryonic-stem-cell-derived retinal epithelium spontaneously formed hemispherical epithelial vesicles that became patterned along their proximal–distal axis. Whereas the proximal portion differentiated into mechanically rigid pigment epithelium, the flexible distal portion progressively folded inward to form a shape reminiscent of the embryonic optic cup, exhibited interkinetic nuclear migration and generated stratified neural retinal tissue, as seen in vivo. We demonstrate that optic-cup morphogenesis in this simple cell culture depends on an intrinsic self-organizing program involving stepwise and domain-specific regulation of local epithelial properties.
*In vitro* differentiation of hESCs to oocyte/follicles

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Express intrinsic factors that promote germ cell development & meiosis

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Adding extrinsic factors that promote folliculogenesis

Ovarian follicle
Regulation of oogenesis by extrinsic factors

Matzuk et al, Science, 2002
GDF9 & BMP15 induce appearance of follicle-like-cells (FLCs)

(a) Timeline:
- **D0**: hESCs on matrigel + BMP4, BMP8a (1hr) + Lentiviral expression of DAZL+ BOULE (24hr)
- **D2**: Selection with blasticidin
- **D6**: +BMP15, GDF9
- **D9**: FLCs appearance

(b) Images of hESCs on matrigel

(c) Images showing the expression of DAZL+ BOULE

(d) Images showing the appearance of FLCs
GDF9 & BMP15 induce appearance of follicle-like-cells (FLCs)-HSF6 line

a

Stereo view

b

Phase contrast view
GDF9 & BMP15 induce appearance of follicle-like-cells (FLCs) \textit{in vitro}
FLC transcriptomes are similar to ovarian primordial follicles
FLCs secret estradiols

Adding IVM medium and collecting supernatent for 6 days

Estradiol

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ZP2 expression in FLCs
Granulosa cell marker AMH expression around FLCs
Oocyte specific marker NOBOX expressed in FLCs
Transplantation of FLCs into kidney capsule

Day: D0  D2  D6  D9
hESCs on matrigel
+ BMP4, BMP8a (1hr)
+ Lentiviral expression of DAZL+ BOULE (24hr)
Selection with blasticidin
+ BMP15, GDF9
FLCs appearance

D12
hanging drop culture (48hr)

D14
mouse kidney capsule transplantation

D60
harvest transplants
Appearance of primary follicle in transplant

Mouse kidney

Induced transplant (Magnified)
NOBOX expression in the transplanted FLCs
AMH expression in transplanted FLCs
**In vitro** differentiation of hESCs to oocyte/follicles

Undifferentiated hESCs (XX lines)

- Express intrinsic factors that promote germ cell development & meiosis

- Meiotic germ cells

- Adding extrinsic factors that promote folliculogenesis

- Follicle-like cells
Studying mutations associated with infertility

AMHR; POI
*Hum Reprod*, 2016

NOBOX; POI
*Hum Reprod*, 2017

BRDT; acephalic spermatozoa
*Oncotarget*, 2017

Li et al., *Human reproduction*, 2017
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