Transcription factor mediated differentiation of human iPSCs to oogonia-like and ovarian granulosa-like cells

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Abstract

An *in vitro* model of the human ovary would greatly benefit the study of female reproduction. Ovarian development requires the combination of germ cells and several types of somatic cells. Among these, granulosa cells play a key role in follicle formation and support for oogenesis. Whereas reliable protocols exist for generating human primordial germ cell-like cells (hPGCLCs) from human induced pluripotent stem cells (hiPSCs), methods for generating later-stage germ cells (such as oogonia) or ovarian granulosa cells have been elusive. Here we report that overexpression of transcription factors (TFs) can direct the differentiation of hiPSCs to these crucial cell types.

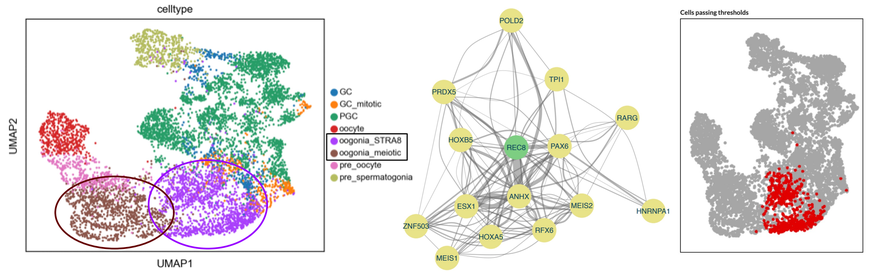
First, we elucidate the regulatory effects of several granulosa-related TFs and establish that overexpression of NR5A1 and either RUNX1 or RUNX2 is sufficient to generate granulosa-like cells. Our granulosa-like cells have transcriptomes similar to human fetal ovarian cells and recapitulate key ovarian phenotypes including follicle formation and steroidogenesis. When aggregated with hPGCLCs, our cells form ovary-like organoids (ovaroids) and support hPGCLC development from the premigratory to the gonadal stage as measured by induction of DAZL expression.

In parallel, we examine the effects of TF overexpression on human germ cell specification and maturation. We show that the TFs DLX5, HHEX, and FIGLA increase the efficiency of hPGCLC specification from hiPSCs when overexpressed individually, and in particular, that DLX5 can substitute for BMP signaling during this differentiation. Additionally, we find that the combination of LHX8, SOHLH1, and ZNF281 can differentiate hiPSCs to DDX4-positive oogonia-like cells after four days of overexpression. We characterize these TF-based germ cells via gene expression analyses and demonstrate their broad similarity to *in vivo* and *in vitro* germ cells.

Together, these results increase our understanding of regulatory factors involved in germ cell and ovarian development. Our new protocols for generating oogonia-like and granulosa-like cells will also provide unique opportunities for studying human ovarian biology *in vitro*, and may enable the development of therapies for female reproductive health.

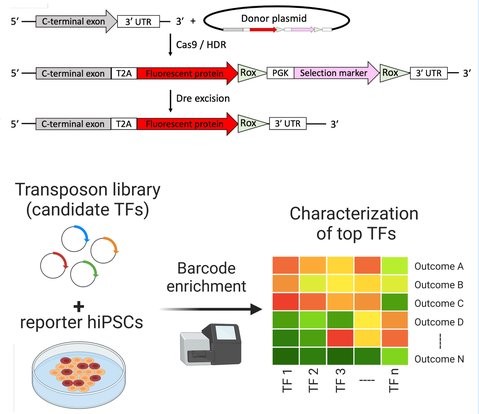
Transcription factors control cellular identity

- Cellular identity is based on gene expression
- TFs can alter cellular identity
  - Previous examples: iPSCs, neurons, Sertoli cells
- We can computationally predict which TFs induce a desired cell type[1]



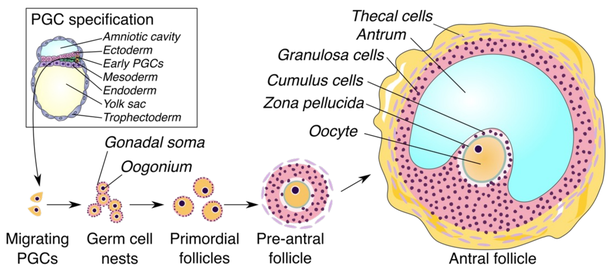
Our method for screening TFs

- Engineer iPSC lines with reporter alleles for cell types of interest
- Integrate a barcoded library of TF cDNA expression plasmids
- Induce TF expression, sort reporter-positive cells, and identify enriched barcodes
- Validate top TFs for ability to induce the cell type of interest



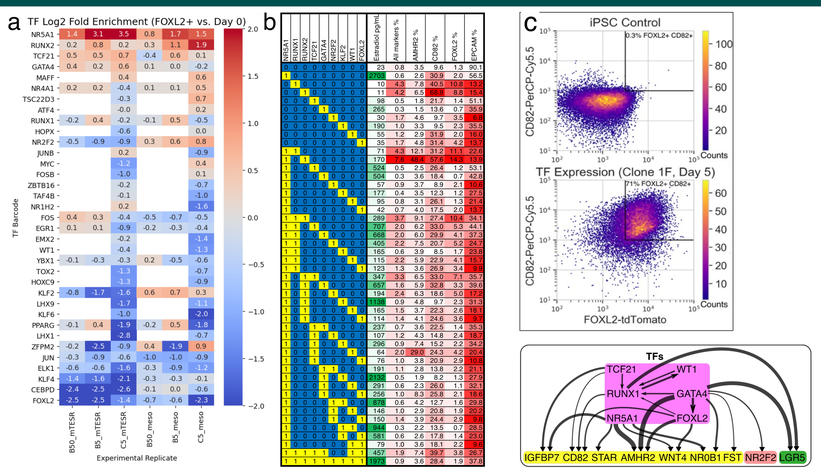
Ovarian development *in vivo*

- Ovarian follicles are formed from germ cells and ovarian somatic cells



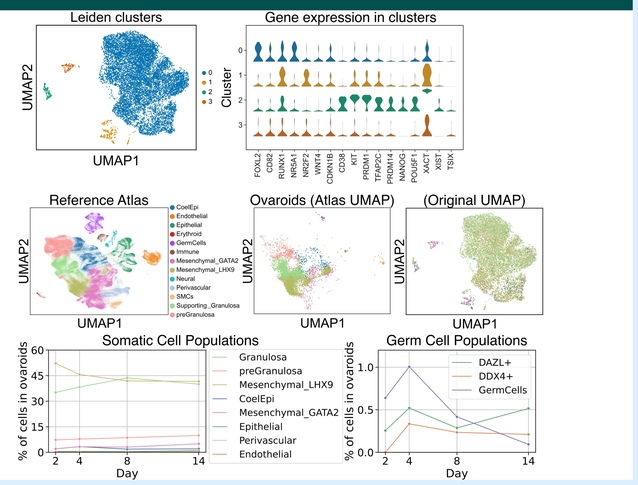
- In mice, ovarian follicles can be created *in vitro* from iPSCs, and used to grow oocytes[2]

Screening TFs to make granulosa-like cells



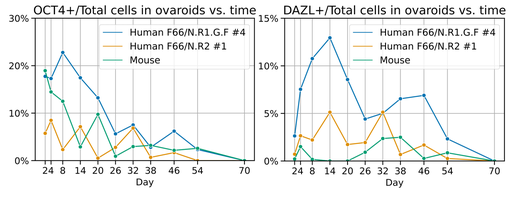
Gene expression in granulosa-like cells

	Gonad_wk7_M	Gonad_wk7_F	primary_GC	primordial_GC	**FOXL2_tdT+**	KGN	COV434	iPSC
Gonad / Granulosa								
ALDH1A2	6.1	5.8	1.3	1.7	5.1	0.9	0.3	0.2
AMHR2	6.3	6.6	2.5	2.5	4.1	3.8	0.1	0.1
CD82	2.5	2.4	2.8	4.9	4.5	6.6	1.7	3.2
CDH2	5.9	5.6	2.6	0.9	6.5	7.0	1.9	5.5
COL1A1	7.9	8.0	1.2	0.9	8.9	11.7	1.4	4.9
CXCL12	7.2	6.3	0.4	0.4	5.8	1.5	0.2	5.9
FOXL2	0.5	1.8	3.3	4.2	3.7	5.3	0.0	1.1
FSHR	1.0	0.7	0.6	0.4	0.4	3.1	0.0	0.0
IGF2	8.6	9.3	0.8	1.0	11.2	0.3	5.2	2.1
IGFBP7	6.0	4.6	6.3	2.3	8.8	11.3	9.5	1.2
KRT19	3.8	4.4	1.4	2.6	7.1	5.6	0.0	6.7
LHX9	6.8	6.9	0.0	0.1	0.3	3.0	0.4	0.0
MYRF	6.0	5.9	0.9	1.1	3.4	6.5	1.0	1.4
NR2F2	7.9	8.2	1.9	1.2	6.5	6.0	7.5	0.2
NR5A1	4.5	4.0	0.7	0.0	11.3	5.7	0.1	1.6
RSPO1	4.2	4.5	0.0	0.0	0.8	0.1	0.1	0.1
STAR	6.2	6.1	3.7	4.0	7.8	7.1	0.1	1.6
TCF21	4.5	4.0	0.9	0.8	9.4	0.0	0.0	1.0
VIM	8.2	8.7	9.0	9.3	10.1	12.7	3.6	5.7
WNT4	3.2	2.5	0.0	0.0	2.4	0.1	0.3	0.3
WT1	8.1	8.2	2.2	2.3	0.3	0.0	7.9	0.0
Adrenal								
NTRK2	3.7	4.4	0.7	0.8	0.8	3.4	2.7	0.1
OSR2	1.8	3.4	0.4	1.0	0.7	1.2	2.2	0.4
Pluripotent								
SOX2	0.1	0.3	0.1	0.1	1.3	0.0	0.9	7.8
NANOG	4.3	2.9	0.4	0.1	0.6	0.3	0.1	8.2
POU5F1	2.4	2.7	0.2	0.1	4.2	0.1	0.6	11.1

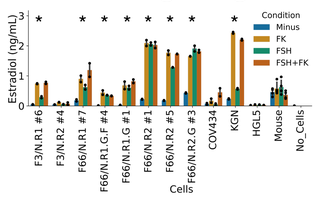


Functional validation of granulosa-like cells

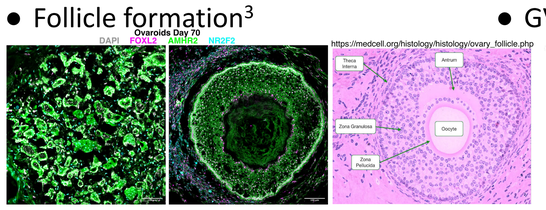
- Support PGCLC maturation[3]



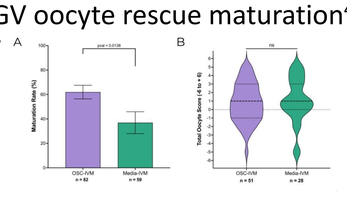
- Hormonal signaling[3]



- Follicle formation[3]

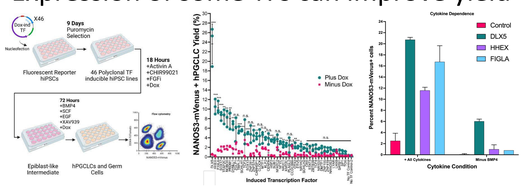


- GV oocyte rescue maturation[4,5]

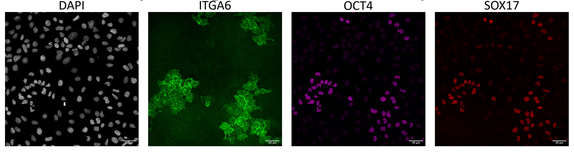


How to make germ cells

- iPSC -> posterior epiblast -> PGCLC
- Expression of some TFs can improve yield[6]

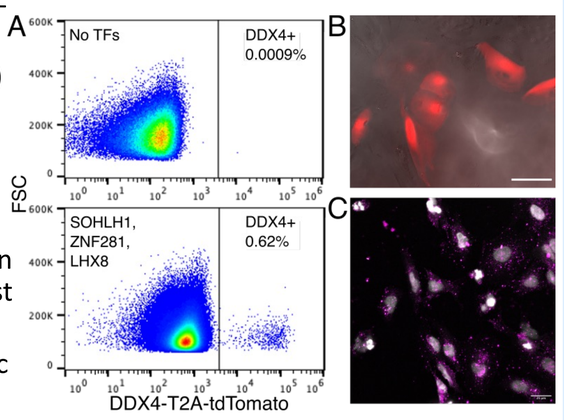


- PGCLCs express germ cell marker proteins



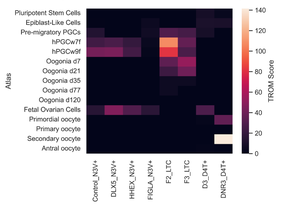
How to make **mature** germ cells

- PGCLCs correspond to pre-migratory germ cells
- We want oogonia (DDX4+)
- TFs can induce DDX4 expression[6]
- DDX4+ cells can be cultured on Matrigel
  - DDX4 protein expression is maintained for at least 28 days
  - DDX4 forms cytoplasmic puncta

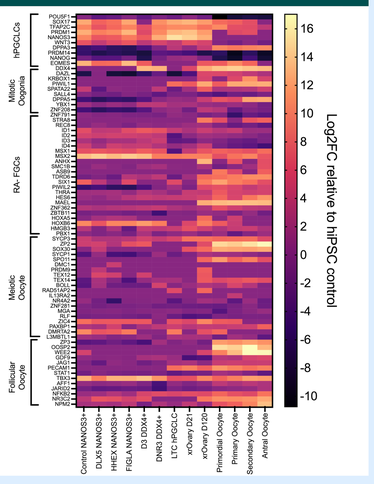


Gene expression in TF-induced germ cells

- TF-induced PGCLCs have similar gene expression to PGCs
- DDX4+ cells are strange:
  - Express PGC marker genes and *DDX4,* but not *DAZL*
  - But transcriptome-wide similarity is closer to oocytes



- DDX4+ cells are not hypomethylated



Conclusions and next steps

- TF expression can make granulosa-like cells and PGCLCs from iPSCs
- Granulosa-like cells can form functional ovarian organoids
- We can also make DDX4+ germ cells
  - But the method still needs some optimization
- Next steps:
  - Screen epigenetic-modifying factors to make mature germ cells
    - Create PGCLC lines from iPSCs with meiosis reporters
  - Optimize ovaroid culture to improve germ cell survival
  - Test TF dosage requirements for inducing granulosa-like cells
  - Investigate granulosa-like cells for oocyte *in vitro* maturation

References and acknowledgements

(1) Kramme, C., Plesa, A. M., Wang, H. H., Wolf, B., Smela, M. P., Guo, X., Kohman, R. E., Chatterjee, P., & Church, G. M. (2021). An integrated pipeline for mammalian genetic screening. *Cell Reports Methods*, 100082. https://doi.org/10.1016/j.crmeth.2021.100082

(2) Yoshino, T., Suzuki, T., Nagamatsu, G., Yabukami, H., Ikegaya, M., Kishima, M., Kita, H., Imamura, T., Nakashima, K., Nishinakamura, R., Tachibana, M., Inoue, M., Shima, Y., Morohashi, K., & Hayashi, K. (2021). Generation of ovarian follicles from mouse pluripotent stem cells. *Science*, *373*(6552), eabe0237. https://doi.org/10.1126/science.abe0237

(3) Pierson Smela, M. D., Kramme, C. C., Fortuna, P. R., Adams, J. L., Su, R., Dong, E., Kobayashi, M., Brixi, G., Kaviraiyuni, V. S., Tysinger, E., Kohman, R. E., Shioda, T., Chatterjee, P., & Church, G. M. (2023). Directed differentiation of human iPSCs to functional ovarian granulosa-like cells via transcription factor overexpression. *ELife*, *12*, e83291. https://doi.org/10.7554/eLife.83291

(4) Giovannini, A., Piechota, S., Marchante, M., Potts, K. S., Rockwell, G., Paulsen, B., Noblett, A. D., Figueroa, A. B., Aschenberger, C., Kelk, D. A., Forti, M., Marcinyshyn, S., Barrachina, F., Wiemer, K., Sanchez, M., Belchin, P., Pierson Smela, M., Fortuna, P. R. J., Chatterjee, P., … Kramme, C. C. (2023). *Improved rescue of immature oocytes obtained from conventional gonadotropin stimulation cycles via human induced pluripotent stem cell-derived ovarian support cell co-culture* [Preprint]. Developmental Biology. https://doi.org/10.1101/2023.03.27.534477

(5) Piechota, S., Marchante, M., Giovannini, A., Paulsen, B., Potts, K. S., Rockwell, G., Aschenberger, C., Noblett, A. D., Figueroa, A. B., Sanchez, M., Barrachina, F., Wiemer, K., Guzman, L., Belchin, P., Smela, M. P., Fortuna, P. R. J., Chatterjee, P., Tran, N. D., McCulloh, D. H., … Kramme, C. C. (2023). *Human induced pluripotent stem cell-derived ovarian support cell co-culture improves oocyte maturation* in vitro *after abbreviated gonadotropin stimulation* [Preprint]. Developmental Biology. https://doi.org/10.1101/2023.03.27.534479

(6) Kramme, C., Smela, M. P., Wolf, B., Fortuna, P. R., Brixi, G., Palepu, K., Dong, E., Adams, J., Bhat, S., Koseki, S., Tysinger, E., Stan, T., Kohman, R. E., Liu, S., Kobayashi, M., Shioda, T., Church, G. M., & Chatterjee, P. (2022). *Efficient Human Germ Cell Specification from Stem Cells via Combinatorial Expression of Transcription Factors* [Preprint]. Developmental Biology. https://doi.org/10.1101/2022.07.11.499564

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