8-12-2021

Role of Oocyte-Specific cKIT on Development of Ovarian Reserve

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Summer Undergraduate Research Program (SURP)

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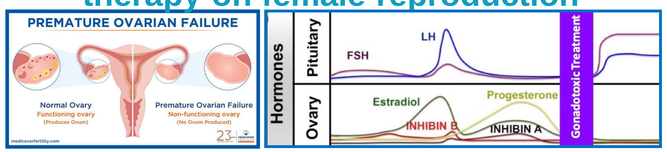
BACKGROUND

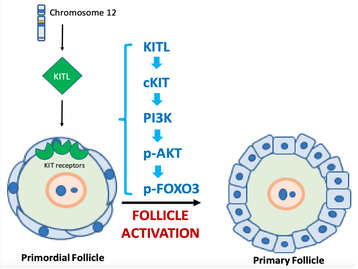
Consequences of cytotoxic cancer therapy on female reproduction



[Premature Ovarian Failure] [Hormonal profile in female]

- Many chemotherapy drugs used to treat common cancers cause **female infertility** and lifelong **hormone imbalances**
- Women who are treated with these drugs have a 40% chance of experiencing ovarian failure and over a **60% chance of experiencing infertility**
- Pre-menopausal women make up **10%** of female cancer cases
- **Premature ovarian insufficiency/failure (POI/POF)** occurs when the ovaries stop functioning normally before age 40
- POF is **irreversible** due to lack of stem cells present in the ovary and is often caused by common chemotherapy drugs

Biological Role of cKIT

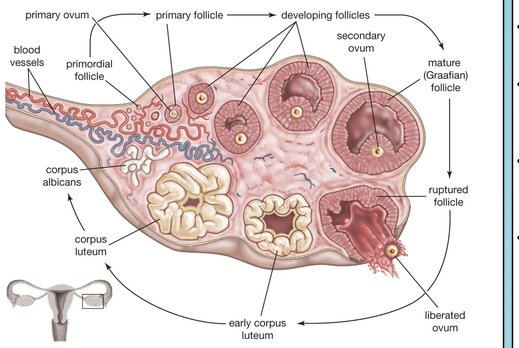


- It has been known that cKIT regulates cell **differentiation.**
- It is expressed in oocyte membrane **from primordial follicles to primary follicles and plays a role in follicle. formation.**
- The ckit signaling starts in fetal mice on embryonic day **18.5** in the fetal ovary.

HYPOTHESIS

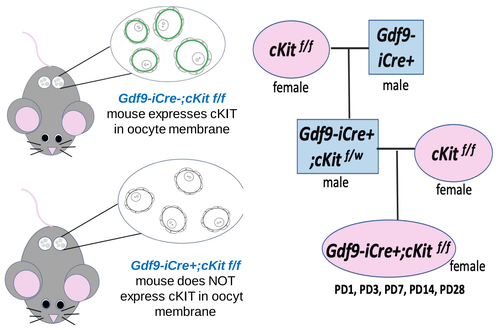
cKIT is critical for the **formation of primordial follicles,** regulating the **transition of oogonia** inside of cyst via cyst breakdown.

Ovarian structure and contents



Strategy for Knocking Out Oocyte-Specific *cKit* Gene

- **Transgenic** mouse line with *Gdf9-iCre* gene inserted
- GDF9-iCRE **cuts out** *cKit* floxed site, preventing expression of cKIT in oocytes
- Mice with **two floxed** *cKit* alleles (f/f) can be **either** positive (+) or negative (-) for *Gdf9-iCre*
- Knowing whether a f/f mouse is (+) or (-) for *Gdf9-iCre* allows us to **directly observe the effects of cKIT** on early stages of oocyte development



RESULTS (continued)

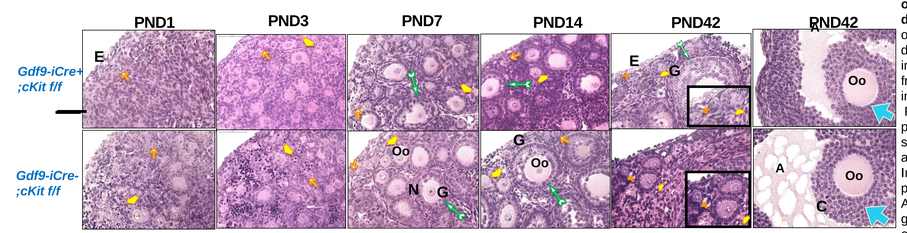


Figure 2. Representative histological images of ovarian tissues stained with H&E during development. Each image shows different class of ovarian follicle. The histological images demonstrate that the ovarian follicles develop irrelevant to the expression of cKIT expression from primordial follicle to antral follicle. Scale bar indicates 50 um. PND, postnatal day.
Primordial follicle:
primary follicle
secondary follicle
antral follicle.
Insets show the presence of primordial and primary follicles with high magnification.
A, antrum; Oo, oocyte; E, ovarian epitherium; G, granulosa cells; C, cumulus cells; N, nucleus of oocyte with nucleoli.

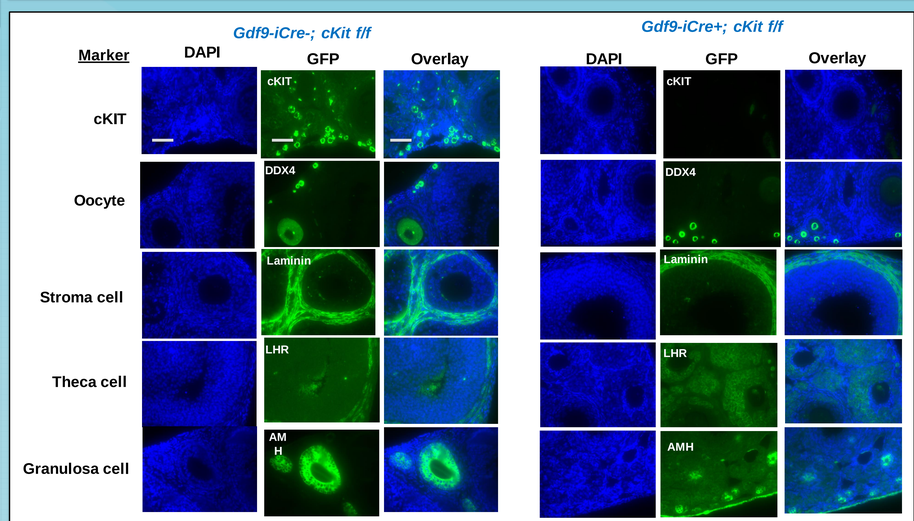


Figure 3. Immunofluorescence (IF) assay of the expression of cell-specific markers in the ovarian section from each genotype.
DAPI indicates nucleus of each cell and GFP presents the expression of molecule. Each staining was performed using PND14 ovary.
The IF assay data demonstrate that there is no difference of the expression of each marker in the ovaries having different genotype. Scale bar indicates 75 um.

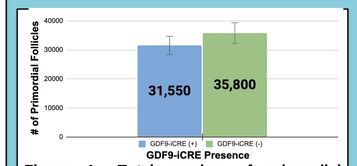
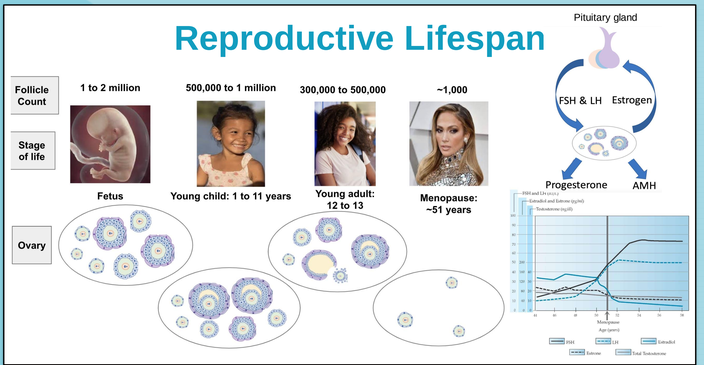
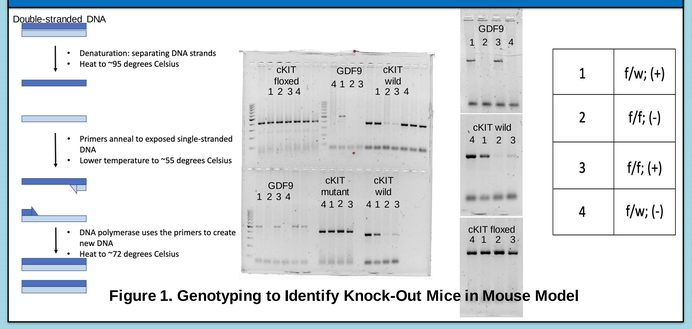


Figure 4. Total number of primordial follicles from the ovaries of PND7 *Gdf9-iCre-; cKit f/f* and *Gdf9-iCre+; cKit f/f* mice. The total number of follicles was counted using every 10th slide of 5 um ovarian sections. The counting data demonstrate that there is no significant difference between the ovaries having different genotypes.

Reproductive Lifespan



RESULTS



1	f/w; (+)
2	f/f; (-)
3	f/f; (+)
4	f/w; (-)

Figure 1. Genotyping to Identify Knock-Out Mice in Mouse Model

CONCLUSION

1. cKIT is **not necessary** for follicle formation
2. Lack of cKIT expression has **no effect** on the expression of other proteins
3. Primordial follicle population is **unaffected** by cKIT

FUTURE DIRECTIONS

1. Gleevec, a common drug used to cure leukemia, binds to cKIT. Based on the results presented in this poster, Gleevec would not harm patients' oocyte development. Further study is needed to evaluate the effects of Gleevec on patients' fertility.
2. Although cKIT knock-out mice display normal oocyte development, the fertility of these mice is unknown and would be worth evaluating.
3. The role of cKIT in the oocyte of primordial follicles is needed to be investigated.

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ACKNOWLEDGEMENTS

Special thanks to Dr. So-Youn Kim, Dr. Seok-Yeong Yu, Yi Luan, and Pauline C. Xu for their invaluable support and assistance with this project.