Testing the Role of Fibroblast Growth Factor Signaling in Ovarian Cancer Using Zebrafish

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ABSTRACT

Ovarian cancer is the fifth leading cause of cancer deaths in women and the most lethal of the gynecological cancers[1, 2]. A cancerous phenotype may be promoted by inappropriate activation of developmental regulatory pathways. Currently, there are very limited ovarian cancer models. The Draper lab has shown that fibroblast growth factor 24 (Fgf24) is required for proliferation, morphogenesis, and differentiation of the early somatic gonad[3]. Fgf24 signaling downstream leads to MAPK and Etv4 activation, both of which are known to be dysregulated in cancer. In addition, Fgf receptor overexpression correlates with poor ovarian cancer prognosis[4].

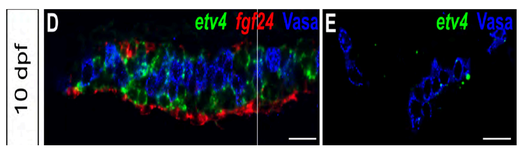
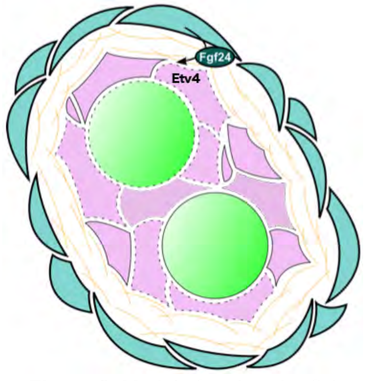


Figure 1. Fgf24 is required for gonad development. Fluorescent *in situ* hybridization of WT (D), and Fgf24-nulll (E) zebrafish gonads at 10 days post fertilization of *etv4* (green), *fgf24* (red), and *vasa* (blue)[3].

Our hypothesis is that overactivation of Fgf signaling in ovarian somatic cells will lead to a cancerous phenotype. To test this, we are generating transgenic zebrafish that inappropriately express Fgf24 in a subset of early somatic gonad cells using a *Tol2* transposon-base system and the *gonadal soma-derived factor* (*gsdf*) promoter, which is expressed in granulosa cells. We are also examining other Fgf expression profiles in juvenile zebrafish to learn more about gonad development.

INTRODUCTION

- Adult ovary and testis develop from a bipotential gonad that consists of primordial germ cells and somatic gonad precursors



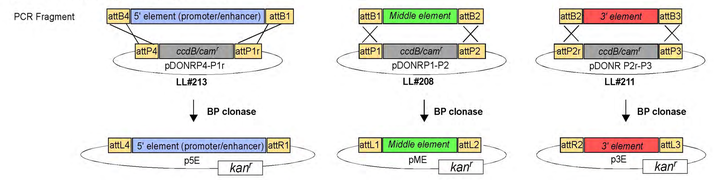
- During normal zebrafish development, *fgf24* is expressed in the epithelial layer that surrounds the early gonad
- In response to Fgf24 signaling, MAPK pathway is activated in mesenchymal cells located in the interior of the gonad, leading to the expression of the transcription factor, Etv4

Figure 2. Model of early zebrafish gonad[3], day 12, including outer epithelial cells (teal), inner mesenchymal stromal cells (purple), and germ cells (green).

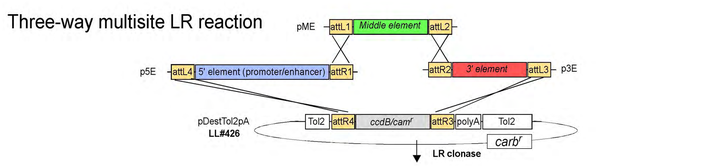
- MAPK activation along with Fgfr and Etv4 overexpression are also correlated with various cancers, including ovarian cancer[3]

METHODS

a) Step 1: Constructing "Entry" plasmids



b) Step 2: Recombining "Entry" plasmids into a "Destination" plasmid



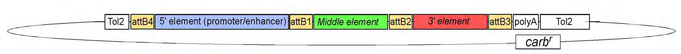


Figure 3. Outline of Gateway recombination based cloning used to construct plasmids. Individual entry clones are made using a PCR product with specific recombination sites and backbones (a). The plasmid is then transformed into a bacteria and selected for on kanamycin. To create the final plasmid, the entry plasmids are isolated and ligated in a multisite reaction into a new backbone (b). Figure adapted from Kwan, *et al.*[5]

RESULTS

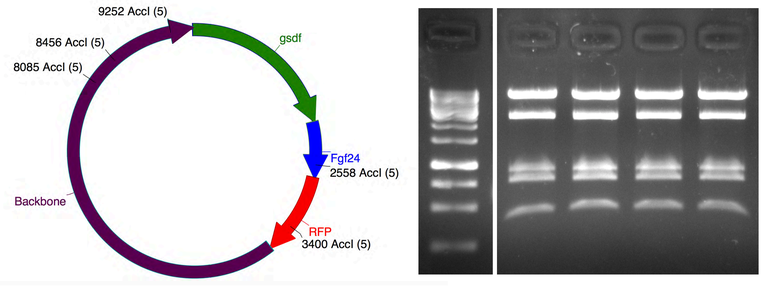


Figure 4. Simplified diagram of the Gateway plasmid construct including the 5' gsdf promoter (green), middle entry Fgf24 element (blue), 3' RFP reporter (red), and backbone (purple). The plasmid construct was confirmed by digestion with AccI restriction enzyme and run on a 1% agarose gel.

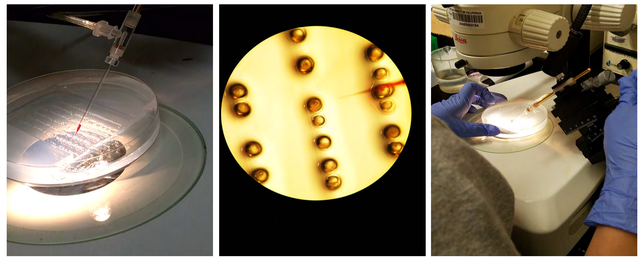
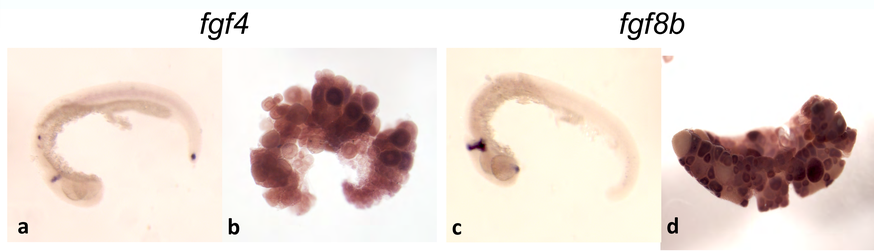


Figure 6. After completion of the Fgf24-overexpression Gateway plasmid, the gene is injected into embryos and inserted into the genome via *Tol2* transposase.



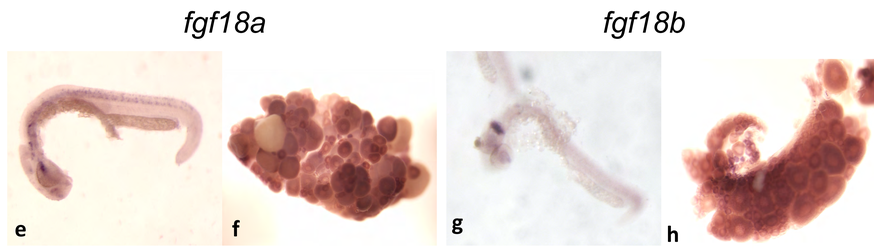


Figure 5. Expression patterns of *fgf*s using *in situ* hybridization. Controls of known *fgf* expression in 24hr zebrafish embryos (a, c, e, g), were compared to 60 day ovaries (b, d, f, h) of *fgf4*, *fg8b*, *fgf18a*, and *fgf18b* using anit-DIG antibody.

CONCLUSIONS

- Fgf24-over expression plasmid has been successfully constructed
- Fgf *in situ* probes created work appropriately and are in line with literature for expression patterns of embryos 24 hours post fertilization controls
- Future directions include investigation of zebrafish gonads 30 to 60 days post fertilization after successful integration of Fgf24-overexpression into embryos to examine gonad morphology and *fgf* expression patterns
- We predict that Fgf24 overexpression will alter gonadal development and result in ovarian tumors

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CITATIONS

1. "Ovarian Cancer Statistics." *CDC*. Division of Cancer Prevention and Control, 21 June 2016.
2. Rosen, D.G., *et al*. Ovarian Cancer: Pathology, Biology, and Disease Models. *Frontiers in Biosci.* **14** (2009): 2089-2102.
3. Leerberg, *et al.* Fibroblast Growth Factor Signaling is Required for Early Somatic Gonad Development in Zebrafish (submitted).
4. Hu, L., *et al.* Fgf19 is correlated with an unfavorable prognosis and promotes progression by activating FgfR4 in advanced-stage serous ovarian cancer. *Oncol Rep.* **34** (2015): 2683-91.
5. Kwan, K.M., *et al.* The Tol2kit: A multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. *Dev Dynamics.* **236** (2007): 3088-99.