A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health

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Office of Research Infrastructure Programs

DIVISION OF COMPARATIVE MEDICINE

http://www.exoticfishaquarium.com/zebra-fish-danio-rerio/
A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health

- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.
- Connecting fish genomes to human biology.
Fish provide models for biomedicine

control  miR-140 over-expression

Antarctic icefish

Cleft palate

Osteopenia

Eberhart 2008 Nature Genetics

Fish provide models for biomedicine

- **Wild type**
- **gsdf mutant**

**Reproductive health**

- Follicles mature
- Follicles accumulate
- Polycystic ovary syndrome

Normal ovary
- Developing egg
- Cysts

Polycystic ovary
- Fallopian tube
- Ovary
- Uterus

Fish provide models for environmental health

stickleback

control  perchlorate

few, large thyroid follicles  many, small thyroid follicles

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What makes zebrafish a good biomedical model?

Embryos develop outside the mother.
What makes zebrafish a good biomedical model?

Embryos develop outside the mother.
Zebrafish embryos develop like early human embryos.

28 day human embryo

Zebrafish allows forward mutagenesis
Zebrafish allows forward mutagenesis

Model for Hirschsprung disease
Zebrafish has stereotypic development

Chuck Kimmel
UO Biology

WT
ihha

brachydactyly IHH

NIH National Institute of Dental and Craniofacial Research
Indian hedgehog regulates proliferation of distal margin pre-osteoblasts

brachydactyly IHH
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Fanconi Anemia

• Clinical features
  • Most common inherited bone marrow failure disease
    - red blood cells
    - white blood cells
    - platelets
  • Thumb and radius
  • Rare (1 per 350,000)
  • acute myelogenous leukemia up 8,000X
  • Induced inter-strand DNA crosslinks
  • Hypogonadism
  • Small head & eyes
FA proteins help repair DNA damage.

Does the molecular genetics suggest therapies?

Induced interstrand DNA crosslinks

16 genes, when mutated, give FA

downstream’ components

BRCA-interactors

FA core complex
Does the molecular genetics suggest therapies?

- squamous cell carcinoma of head and neck up 500X

Therapy: bone marrow transplant from a sibling donor

16 genes, when mutated, give FA

‘downstream’ components

FA core complex

BRCA-interactors

BRCA2

16 genes, when mutated, give FA core complex BRCA-interactors downsteam components.

Does the molecular genetics suggest therapies?

- squamous cell carcinoma of head and neck up 500X

Therapy: bone marrow transplant from a sibling donor

Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

We made mutations in four genes:

- RAD17
- ATRIP
- HCLK2

16 genes, when mutated, give FA

‘downstream’ components

BRCA-interactors

FA core complex
Zebrafish FA mutant

- genome instability
- embryonic cell apoptosis

Zebrafish FA mutants have phenotypes like FA patients.

Can a small molecule screen help find alternative therapies?

- germ cell stem cell defect; sterility
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

wild type

control

acridine orange, stains broken DNA

yolk

27hpf

eye
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

- **Control**
  - Wild type embryos
  - fancd1^-/- embryos

- **DEB**
  - DEB increases acridine orange staining of wild type embryos
  - DEB increases acridine orange staining of fancd1^-/- embryos

- **fancd1^-/- mutants** stain about the same as wild types
- fancd1^-/- mutants are more sensitive to DEB
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

Can we find a compound that makes *fanc* mutants look like wild types?
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

163 objects (wild type) vs. 920 objects (fancd1−/−)

731 compounds used in clinical trials

How does warfarin rescue FA zebrafish?
How does warfarin rescue FA zebrafish?

Strong AO staining in fancd1 mutants

Rescues AO staining in fancd1 mutants

Warfarin rescue fails with exogenous vit-k

Knockdown of VORC1 rescues fancd1 mutants

Conclude: warfarin rescues fancd1 mutants by a vitamin-K dependent mechanism.

Which of the several vit-K-dependent proteins is the FA-relevant target?
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- Connecting the zebrafish genome to human biology.

**TGD: teleosts have two copies of 25% of human genes**

- Teleost genome duplication

Vertebrates → Two rounds of genome duplication

1 Hox cluster

Elephant shark

- Rayfin
  - Spotted Gar
  - Fugu

- Zebrafish
  - Stickleback

- Lobefin
  - Coelacanth
  - Chicken
  - Mouse
  - Human

- 7 Hox clusters
- 4 Hox clusters
- 4 Hox clusters

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Gar biology is more similar to teleost biology, but the gar genome is more similar to the human genome.

Gar links human and zebrafish conserved non-coding elements (CNEs)

Amores 2011 Genetics
CNEs:
- Don’t encode proteins
- Sequence conserved for some other function
- Some or most likely regulatory

Gar links human and zebrafish conserved non-coding elements (CNEs)
Mouse as comparator

Chicken
Mouse as comparator

- Chicken
- Zebrafish
- Pufferfish

No teleost CNE
Gar alignments identify CNEs otherwise undetectable.

Gar reveals potential regulatory elements teleosts share with human!

We can test them for function.
We can test them for function

Learn role of CNE by gain of function

Gar reveals hidden shared regulatory elements
Gar reveals hidden shared regulatory elements

mouse Mmu1
(adapted from Klopopcki et al. 2011)
Gar reveals hidden shared regulatory elements
Gar reveals hidden shared regulatory elements

These CNEs aren’t regulating \textit{nhej1}

TGD helps assign function to CNEs

Are these CNEs regulatory?
Are these CNEs regulatory?

Gar and zebrafish can help assign function to CNEs.

Thus, gar and zebrafish can help assign function to human GWAS hits.
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