otg, A Heritable T-cell Malignancy in Zebrafish that Exhibits Apoptosis Resistance

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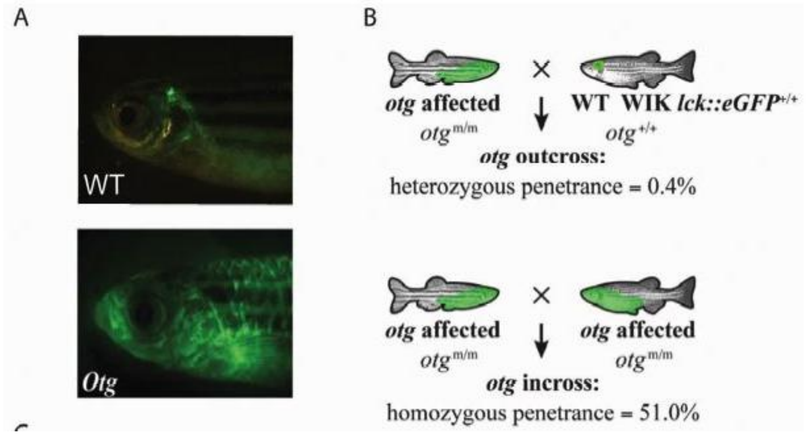
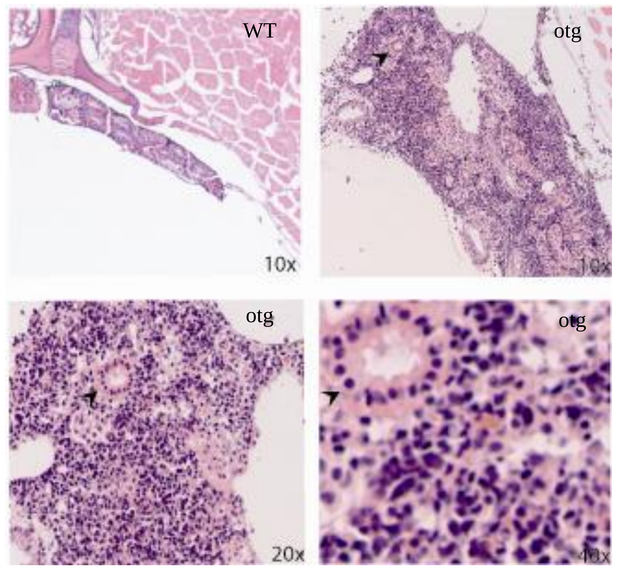
Background

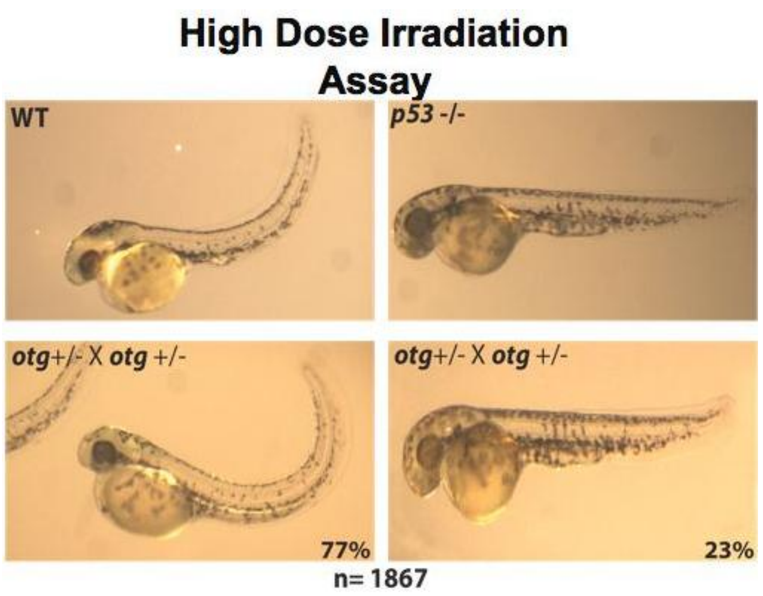
Otg (oscar the grouch), a line of zebrafish identified with a T cell malignancy pseudoposition, has a recessive inheritance pattern with approximately 50% penetrance in presumed homozygotes (m/m). After breeding *otg* with a wild-type lck::eGFP fish to create obligate heterozygotes (+/m), and then in-crossing these siblings, homozygous embryos treated with a high dose of γ-irradiation (100Gy) have been observed to be apoptosis resistant. These o*tg* embryos are irradiated at 30 hours post fertilization (hpf) and are resistant to apoptosis in the neural tube, which is shown by the lack of typical phenotypic curvature in the tail of approximately 25% of the in crossed embryos. Wild-type fish show this curvature in 100% of embryos, while fish with a confirmed mutation in the p53 tumor suppressor gene, who are known to be resistant to apoptosis, show this curvature 0% of the time. The fish with this confirmed mutation are used as a control in this straight-vs.-curled tailed assay because this specific mutation causes apoptosis resistance. Under the direction of graduate student mentor Lynnie Rudner, fish were bred, embryos collected, irradiated, and sorted based on phenotypic expression. The collected embryos have been used to run a genomic panel using PCR in order to compare phenotypic mutant to wild-type fish.



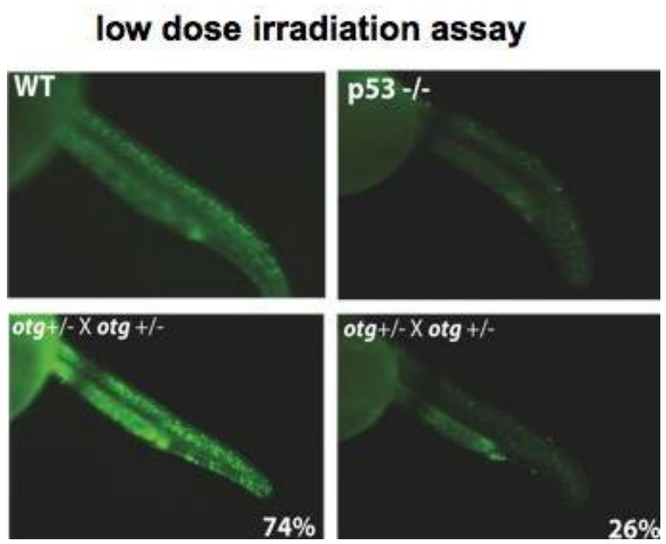
(A) *otg* is a heritable T-cell cancer phenotype from an ENU mutagenesis screen. (B) The recessivity of the *otg* mutation was verified by out-breeding (only 0.3% of heterozygotes acquired disease), and in-breeding (51% of homozygotes were abnormal)



Histological sections of the kidneys, which contain the T-cells of zebrafish, of wild-type and otg mutants demonstrates the difference between normal and malignant cells



Irradiation with 100 Gy at 30 hpf shows apoptosis resistance in the neural tube cells of 23% of embryos from a mating of fish heterozygous for the *otg* gene. The apoptosis resistance is exhibited in the lack of curvature of the tail after irradiation.



After irradiation with 12 Gy at 18 hpf, embryos are stained with acridine orange at 24 hpf. Embryos with apoptosis resistance extrude dye and consequently have less staining.

Continuing Research Design

Aim: Use marker techniques and mapping of the F2 generation to identify the site of the mutation.

Rationale: The goal of this project is to identify which gene has become mutated in this recessive phenotype. Identification of this mutation will improve our understanding of T-cell ALL and direct use of this genetic line of fish.

A genomic panel using predetermined zebrafish markers was ran on collected embryos from irradiation experiments, the banding pattern of mutant verses wild-type fish was examined, differences between the two would signify linkage to the location of the mutation. Even with this genomic panel the location of the mutation has not yet been determined, though many polymorphic regions of the chromosomes have been ruled out. However, since none of the polymorphic regions showed to be linked to the mutation, it is now necessary to more closely examine areas of the genome with are largely monomorphic and as yet are uninformative.

Designs and Methods: Examinations in regions that are largely monomorphic will be done using new markers and using the F2 generation of the congenic CG-1 out-crossed line. These examinations will be underway as soon as embryos from the new line are collected. These examinations will be done using offspring of heterozygous (+/m) in-crosses and then running a PCR and gel electrophoresis on the DNA of these fish comparing phenotypic wild type fish to phenotypic mutants.