Development of stable lines of transgenic zebrafish (*Danio rerio*) to investigate genomic instability

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The goal of our project is to investigate low-dose radiation induced-genomic instability. We describe the development of a whole-vertebrate model bearing a reporter transgene that is designed to indicate the presence of genome instability by somatic homologous recombination (HR) events. The transgenic zebrafish bear two defective copies of the enhanced green fluorescent protein (EGFP) arranged in a tandem repeat. This tandem repeat can be activated by HR or by the single-strand annealing repair pathway, leading to a functional EGFP. To trace the incorporation of the transgene into the animal models, we introduced the reporter cassette into the construct cmlc2:EGFP, which expresses a green fluorescent marker specifically in the heart. Expression of the marker outside of the heart is indicative of genomic rearrangement. This construct also has a unique I-Sce1 cleavage site between the two defective EGFP genes and is flanked by Tol2 transposon sequences. The Tol2 system increases transgenesis efficiency in zebrafish. To establish founder lines, we microinjected the reporter DNA (50 ng μl^{-1}) together with mRNA encoding Tol2 transposase into one-cell stage embryos. After 48 hours, we screened recipient embryos for the presence of EGFP fluorescence in the heart. Embryos that were positive for heart EGFP fluorescence were maintained at 28±1°C until adulthood and then were backcrossed to wild type zebrafish to determine germ line transmission. At this time, we have eight founder families with independent transgene insertions. We are in the process of testing these eight families for frequencies of spontaneous, radiation-induced and I-SceI-induced recombination events. Preliminary results show fluorescence is induced in specific tissues after gamma irradiation and after microinjection of I-Sce1 mRNA in the whole fish. Futures plans include an investigation whether a history of irradiation in early life results in ongoing genomic instability in adulthood as reflected by the increase in recombinational activation of GFP in somatic tissues.

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