Zebrafish are considered great models for cancer research and for comparison to humans because the vertebrate species does not require much care, fertilizes hundreds of eggs in a matter of a week, and its transparent external structure allows for less challenging screening. This means that the presence of cancer cells or oncogenes is easier to identify, as well as where the cancer forms due to abnormalities during angiogenesis, metastasis, and even cell invasion and intravasation (Stoletov). Fish and flies are both considered to be good model systems to study cancer due to either their short life cycles and oncogenes' abilities to affect neighbor cells or their range of other organisms that the species can be compared to. These two types of organisms will allow for more accurate creation of transgenic models since there is a shorter time between generations in a whole-animal context (Cagan). In "A zebrafish *bmyb* mutation causes genome instability and increased cancer susceptibility," the goal of the experiment was to determine which genes were oncogenes, how it affects mitotic division, and if there are any genes homologous to humans. Along with being a transgenic model, zebrafish are also considered a carcinogenesis model because, during somatic evolution, genetic changes cause the creation of new phenotypes or combinations of them that can cause cancer (Shepard). Fish are especially carcinogenic models because water-borne carcinogen exposure is very common in the wild (Amatruda).

In Figure 1, three morphological images are shown of zebrafish embryos in development, at 16-, 24-, and 36-hpf (hours postfertilization). From one strain of zebrafish, males were exposed to ethylnitrosourea, a mutagen carcinogenic treatment that has been previously known to cause tumors in fish (Cagan). After mutagenesis, the males were crossed with females of the same strain that were not exposed to the carcinogen. The F1 generation produced about 40% of embryos with abnormalities. For each timeframe, shown are both the wild-type and mutant (*crb)* stained with pH3, commonly used as a biomarker for the G2/M phases because it stains

chromatin that is condensed of the nuclear proteins in the DNA (Aarts). By just looking at the images of the embryos, there are slight differences in color and structure between the wild and mutant types: with the *crb* gene in A and C causing a curvature in the spinal region. These changes are not as drastic during 16-hpf since embryological development is still in its earlier stages. The darker regions of at 24-hpf and 36-hpf signify apoptosis, or programmed cell death, providing some proof that the early exposure of the males with a carcinogen that caused the mutation causes irregularities in growth, especially in the S phase. The table in Figure 1 also specifies that the introduction of 5-bromo-2-deoxyuridine (BrdUrd) turns into pH3-positive mitotic cells that can cause cell proliferation to decrease, as well as tumor development. Time is measured on the x-axis from zero to ten hours while the percentage of the BrdU-positive cells are indicated on the y-axis, with the white and black bars representing the wild type and mutant (*crb)* genes respectively. As the number of hours increases, the wild-type cells decrease from around 90% to 20% in about 2-4 hours, and then less than 10% after 10 hours of BrdU incorporation. As for the *crb* cells, the percentage of BrdU-positive cells jumps from 10% to around 65% in about 2 hours and then to almost 90% at the 6-hour mark, but decreases to 50% after 10 hours of incorporation. The relationship between *crb* and WT conveys that BrdU causes the wild-type cells to decrease over time but increase the *crb* expression for only a set number of hours.

The first figure demonstrates how the presence of carcinogens causes abnormal mitoses, but in figure 3, the specific abnormalities are visualized through a cytometric graph. Cytometry allows for the understanding of cell count, shape, and size, as well as DNA content and the presence or absence of certain proteins (Baker). Looking at section B in figure 3, the cytometry graph compares the DNA count (x-axis), where N represents the number of complete sets of chromosomes present, to the cell count (y-axis) of wild-type and *crb* mutants. The two mutants tend to follow the same trend, except for two areas where the cell count of the wild-type mutant

is significantly greater compared to the *crb* mutant at a relative DNA count of 2N. However, at a DNA content of 4N and 8N, cell count is slightly greater for the *crb* mutant. This is likely because the *crb* gene causes abnormalities or disorganization in cellular foci, leading to significantly less or more number of chromosomes, as shown in part C and D where the cells show polyploidy or aneuploidy, an abnormality in the number of sets of chromosomes. This can be proven in section A of the same figure where three dyes were used: blue DAPI to stain the nucleus, along with red alpha-tubulin and green lambda-tubulin that are antibodies that immunostain for specific proteins in the mitotic cell (Schvartz). Normally, cells only have two foci during mitosis that are defined by the centrioles at opposite sides of the cell, as shown in the wild-type (wt). Abnormalities that can be caused by the exposure to carcinogens include monopolar cells with only one polar foci, multipolar cells with more than 2 polar foci, and disorganized cells where it is difficult to determine the structure during mitosis.

With an increase in cell proliferation and the *crb* mutant gene, it means there is a decrease in tumor suppressor genes that slow down the cell division, thus preventing cell development abnormalities. As we saw in the bar graph in Figure 1, *crb* stopped increasing after six hours of BrdU incorporation and that continued with no spontaneous increase in tumor cell development. Due to this issue, Figure 4 represents the stages of the cell after the carcinogenic treatment of N-methyl-N'-nitro-N-nitrosoguanidine or MNNG for a whole 24 hours. In section A, the percentage of adult zebrafish that grew tumors after MNNG treatment is shown on the x-axis while the white bar represents the adults with the *crb* mutant and the black bar as the wild-type individuals. About 0.07% of the wild-type mutants grew tumors while only about 0.04% of the *crb* mutants grew them with a value of 0.014. The next set of structures in the figure show the choroidal vascular plexus of the zebrafish eye of a wild-type mutant(B), the retinal vascular plexus and vascular neoplasm of a *crb* heterozygous mutant (C), spermatogonia and condensed

nuclei in the spermatocytes of a wild-type mutant (D), and a tumor in the testis and undifferentiated spermatogonia in a *crb* mutant individual. As seen in C and E, due to the presence of the MNNG treatment, the *crb* mutants has disorganized and substantial amount of cell proliferation, thus causing the endothelial cells to to create a lot of vascular channels in the eyes as well as the undifferentiated structure in the testis because of the increase diploid cell progeny in the G2/M phases of the cells. The last section, F, in the figure is a chart that represents the signal-to-noise and enrichment scores of the *bmyb* genes based on their rank in the ordered data set. The blue marks under the enrichment score plot is where the p53 phenotype is located. By using the Gene Set Enrichment Analysis (GSEA) method that compares genes of interest, it is found that the blue markers are more prevalent in the beginning of the plot because it is where the *bmyb* signature genes are most concentrated and the *crb* genes are down-regulated, therefore that is where there is a greater retention of the p53 phenotype. Using all the data provided from the figures and testing methods, it was concluded that there is proof that the zebrafish has homologous genes to humans that are seen in *bmyb* expression and its effects on cancer cell proliferation.

In the experiments for the genome instability and cancer susceptibility, multiple experiments were conducted to confirm that the loss of the *bmyb* gene meant that the *crash&burn* (*crb)* phenotype would occur, as well as that decreased levels of Cyclin B meant more *crb* cells during the G2/M phases in mitosis, thus causing delays in proliferation and development. Varying tumor types were identified, with vascular and testicular germ cells have the most. When compared to the human genome, around 40% of the human homologues also contained the bmyb gene, a transcriptional regulator and tumor suppressor gene (Stoletov). Therefore, abnormalities in bmyb would also cause abnormalities in the cell cycle or mitotic progression.

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