Put Your Phone Down: Effects of Cellular Radiation on Drosophila melanogaster

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Experiment Type: Reverse transcriptase (RT) PCR
Feature: Cell phone radiation
Gene: Rad51: Rad51 is involved in the repair of double-strand breaks using homologous recombination

1 Introduction

In this project, we aimed to answer the question: Does cellular radiation affect offspring production and expression of the Rad51 gene in the organism Drosophila melanogaster? It was hypothesized that if Drosophila larvae were exposed to cellular radiation, the total offspring production would decrease, along with a corresponding increase in Rad51 expression. The Rad51 protein is crucial to the propagation of strand invasion and exchange steps in homologous recombination, resulting in the repair of double stranded DNA breaks.

To test our hypothesis, we exposed the experimental group of Drosophila larvae to doses of cellular radiation emitted from an iPhone 6 or 7. Exposure to cellular radiation occurred for 6-minute durations, twice daily, for 3 consecutive days. At the end of the treatment period, RNA extraction from larvae and complimentary Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed on both control and treatment groups to measure the expression of Rad51. Further analyses were performed to look at the fertility of Drosophila larvae that were exposed to cellular radiation.

Due to prior research on cellular radiation exposure and its connection to DNA and sperm damage, it was predicted that expression of the Rad51 gene would show an increase in our treatment groups, along with a corresponding decrease in reproductive ability.

2 Methods

Primer synthesis: Primers for Rad51 were designed using Flybase and Integrated DNA Technologies PrimerQuest. The sequences of the forward and reverse primers targeting Rad51 are as follows: ACTCCGGACTTCAATTACGATAC (Forward) and GCGGCAAGCGAATCAATAA (Reverse). The sequences of the control Gapdh primers are as follows: CGCCAAGAAGGTCATCATCTC (Forward) and CCTCGACCTTAGCCTTGATTTC (Reverse).

Culturing Drosophila: Wild type Drosophila cultures were purchased from Carolina Biological and maintained in Formula 4-24 Instant Drosophila Medium in culture vials as per the manufacturers protocol (Carolina Biological). Briefly, equal volumes of water and dry media were added to the culture tubes along with several grains of bakers yeast (Red Star Yeast). The cultures were incubated at room temperature and were sub-cultured every two weeks. The anesthetizing of the flies was accomplished using CO2 FlyBeds (Azer Scientific) and The Flowbuddy CO2 regulator (Flystuff.com). For the experiment, adult flies were applied to the culture vessels for 24hrs before being removed. The resulting larvae were randomly selected to be in the control or radiation-exposed group. For the radiation exposure, experimental cultures were subjected to six 6-minute doses of cell phone radiation from either an iPhone 6 or 7 over the course of 72hrs. Following exposure, the larvae were randomly selected for subsequent RT-PCR and fertility experiments.

RNA extraction: RNA was extracted from control and experimental Drosophila larvae after 72hrs using Qiagens RNeasy Mini Kit as per the manufacturer's instructions. To prepare for RNA extraction, the larvae were transferred, using forceps, into 35mm petri dishes filled with 1x PBS (ThermoFisherScientific). The larvae were then placed in pre-chilled Eppendorf tubes and incubated in the freezer for 10 minutes. After freezing, a 1000uL micropipette tip was used to grind the larvae for 1 minute. Following the addition of Buffer RLT, the larval tissue was gently passed through an 18 gauge-needled syringe 10 times followed by a 25 gauge-needled syringe 10 times. During RNA extraction, the samples were subjected to on-column DNase treatment using an RNase-free DNase kit (Qiagen).

RT-PCR: cDNA was synthesized using the RevertAid RT kit (ThermoScientific) and following the manufacturer's protocol. PCR was performed using GoTaq Green PCR Master Mix (Promega) using the manufacturer's protocol. PCR amplfication of Gapdh cDNA was used as a positive control for the Drosophila RT-PCRs while the RevertAid Gapdh was used as a positive control for the entire RT-PCR experiment as the reagents for this sample were provided in the RevertAid kit. The No Template Control (NTC) reactions contained all reagents for the RT-PCR reaction, except nuclease-free water was added in place of RNA template.

Gel electrophoresis: The RT-PCR reactions were electrophoresed on a 1% agaorse gel (BioRad) in 1xTBE (VWR). The ImageJ gel analysis program was used to determine the relative intensities of the PCR products for semi-quantitative analysis.

Fertility Assay: Larvae from four control and four cell phone radiation-exposed treatment

groups were allowed to develop until adulthood and the number of offspring from their subsequent mating were counted.

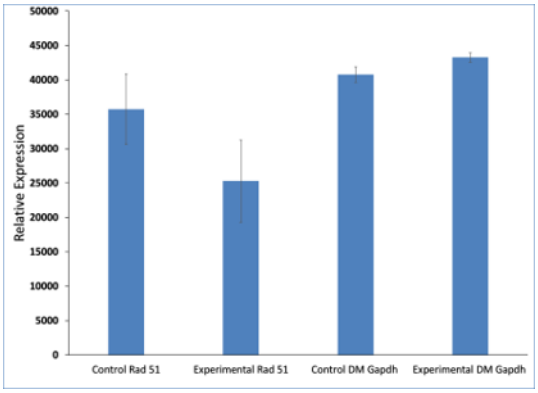
3 Results

The semi-quantitative RT-PCR results indicate that cellular radiation exposure does not result in a change in Rad51 expression, as seen in Figure 1 ($p = 0.23$). Furthermore, cellular radiation exposure did not affect the number of offspring produced by radiation-exposed adult flies, as depicted in Figure 2 ($p = 0.10$). Collectively, these results contradict our initial hypothesis that cell phone radiation exposure would result in an increase in Rad51 expression and a decrease in offspring production.

Future studies should focus on optimizing the exposure time of the larvae to cell phone radiation and increasing the number of experimental replicates. Additionally, a more quantitative measure of gene expression should be used to more definitively determine the effect cell phone radiation has on Rad51 expression.

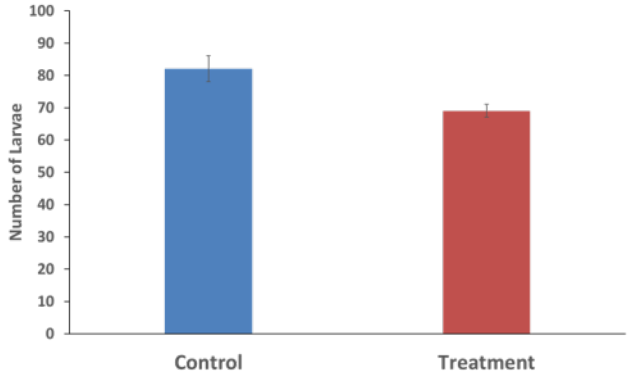
4 Figures

4.1 Relative Expression of Rad51 in Control and Cell Phone Radiation-exposed Drosophila Cultures



The relative expression of Rad51 compared to the control gene Gapdh was measured using semi-quantitative RT-PCR across four control and four cell phone radiation-exposed Drosophila cultures. The error bars represent the standard error of the means for each condition. A two-tailed t-test assuming unequal variance was performed to determine the significance of changes in gene expression between the control and experimental cultures, with p=0.23 for Neur and p=0.12 for Gapdh.

4.2 Average Larvae Counts from Control and Cell Phone Radiation-exposed Mating Pairs



The average number of Drosophila offspring was measured across two control and two cell phone radiation-exposed cultures. The ability of cell phone radiation-exposed adults to successfully mate was not significantly altered compared to control cultures (p=0.10). Error bars represent the standard error of the means and the measure of statistical significance (p value) was determined using a two-tailed t-test assuming unequal variance.

5 Acknowledgements

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