Mar 6th, 12:00 AM

10. Genetics

Southwestern Oklahoma State University

Difference in Coding Sequence Between Bats and Humans in DNA Repair Gene Rad50

Erin Reynolds

Abstract

Of the 19 species of mammals that live longer than humans relative to their body size, 18 are bats. Thus, bats are optimal organisms for research concerning age-related diseases and cancer. We investigated differences between the nucleotide and amino acid sequence of the RAD50 gene in bats and humans. Rad50 protein is known to play a role in the repair of double-strand DNA breaks (DSB). When left unrepaired, DSBs lead to loss of genetic information, cell death, mutations, or uncontrolled division. We took skin samples from the wings of three bat species (Myotis velifer, Tadarida brasiliensis, and Eptesicus fuscus). The RNA was separated, converted to cDNA, and the RAD50 gene was isolated using PCR and gel electrophoresis. We obtained 1550 base pairs of DNA sequence representing approximately 1/5 of the coding length. Our results revealed 108 (19.6%) differences in nucleotides among the various bat species and humans with 34 (6.5%) resulting in an amino acid change. Of those variances, 55 (3.5%) were unique to humans, which altered 19 (3.6%) amino acids. The presence of differences in nucleotide and amino acid sequence in the RAD50 gene between bats and humans was confirmed in this study. However, this gene was found to not be under positive selection. This is a preliminary study that provides direction for future research concerning the role of RAD50 in the DSB repair pathway in bats.

Unraveling the Role of Nardilysin in Heart Function and Development

David Seo

Abstract

Nardilysin (NRDC) is a protein localized in the mitochondria. Loss of Drosophila Nrdc (dNrdc) causes developmental defects and neurodegeneration. Human patients carrying loss-of-function variants in NRDC exhibit developmental delay and neurological manifestation. NRDC is highly expressed in neurons, but also hearts. The role of NRDC in hearts, however, has not been defined. The goal of this study is to determine whether heart-specific loss of dNrdc exhibits heart defects in development and adult stages in fruit flies. An RNA interference (RNAi) strategy and heart-specific Gal4 driver (Hand-Gal4) were utilized to reduce dNrdc expression in Drosophila heart at two expression conditions: 25°C (moderate expression) and 30°C (higher expression). To determine dNrdc knockdown effects on development, we scored fly death during development. To assess dNrdc knockdown effects on adult heart function, we scored how many adult flies exhibit wing heart defects: flies with abnormal wing morphology. Our studies show that heart-specific dNrdc knockdown causes developmental defects and a wing-heart defect in fruit flies. Both developmental and wing heart phenotypes were more significant at 30°C compared to 25°C, suggesting that deficiency of dNrdc lead to the phenotypes. This is the first study to demonstrate the effects of dNrdc knockdown in Drosophila on heart function and development, and it shows that dNrdc is required for normal development and adult heart function.

Adipose-specific expression of microRNAs regulate body fat in fruit flies

Jin Seo

Abstract

Obesity is one of the fastest- growing epidemics across the globe and is responsible for various obesity- related diseases such as diabetes and cardiovascular diseases. MicroRNAs (miRNAs) are small non-coding RNAs that have an important role in gene expression. In previous studies, we screened 160 miRNA mutant lines of Drosophila melanogaster and identified 46 miRNAs which regulate body fat. Here, we tested whether the 46 miRNAs regulate body fat in an adipose tissue- specific manner. Using the Gal4/UAS binary gene expression system, which contains adipose tissue specific- Gal4 and Upstream Activating Sequence (UAS)-miRNAs, we overexpressed the miRNAs and measured their body fat contents by quantitation of triglyceride. We have identified adipose-specific expression of multiple miRNAs altered body fat in fruit flies. Considering the conserved genes between fruit flies and humans, our findings could help treat obesity, diabetes, and other obesity-related diseases.

Optimizing 16s and 18s rRNA metabarcoding of microbial communities colonizing freshwater turtle shells

Cameron Kedy & Dr. Matthew Parks

Abstract

Metabarcoding is a DNA sequencing strategy enabling taxonomic dissection of complex microbial communities, with microorganisms identified through informative DNA sequences rather than by morphology. Our project seeks to apply DNA metabarcoding techniques to characterize microbial diversity present on the shells of freshwater turtle species across aquatic environments in Oklahoma. During summer 2019, 30 Trachemys scripta and one Pseudemys concinna turtles were trapped across diverse aquatic sites primarily in central and southeast Oklahoma. Shell scrapings were taken from eight top and bottom scutes of each turtle, and from submerged substrates at each site. DNA was extracted from scrape samples using a two-filter protocol; resulting DNA concentrations ranged from 0 to 361 ng/µL (avg=48.7 ±73.4). We are now applying a two-step PCR protocol to selected DNA extractions. In PCR1, hypervariable regions of 16s and 18s rRNA loci are amplified with fusion primers containing sequence complementary to conserved flanking regions and in-line sample-specific indices. In PCR2, PCR1 product is amplified to further incorporate Illumina sequencing motifs. Modifications to amplification procedures have improved amplification success from ca. 50-75% to 100% on sample subsets for 16s and 18s PCR1 amplifications. Amplifications have now proceeded to a bulk (96-well plate) format, with Illumina sequencing and bioinformatic analysis using the QIIME platform planned for late spring 2020.

Genetic Investigation of the Impact of Single Nucleotide Polymorphism (SNP) on Caffeine Metabolism

Muatasem Ubeidat

Abstract

SNPs are single base-pair mutations in a particular region of DNA. In the human genome, SNPs appear approximately every 300 bases on average. If the human genome is 3.1 billion bases, that means there are approximately 10 million SNPs! Because SNPs can occur anywhere in the genome, they can have dramatic effects on protein expression and function or no effect at all. Caffeine is a widely used drug by 90% of the world population on a daily basis with 150 million regular coffee drinkers in the United States alone. Coffee consumption is beneficial. It makes us energized in the morning and showed linked to a decreased risk of type 2 diabetes, Parkinson's and Alzheimer's diseases, and tea drinking has been linked to a lower risk for some cancers. Too much caffeine can also have negative effects. Some people become jittery after drinking a single cup of coffee, while others can drink several cups of strong coffee Part of that variability and not wake up a bit. Is it genetics? Is it adaptation to caffeine? We know caffeine is primarily metabolized by the liver enzyme cytochrome P450 1A2 (CYP1A2). Our goal is to produce a PCR product for accurate sequencing of the targeted sequence in the small population. An accurate single Nucleotide Polymorphisms (SNPs) for each subject will be achieved. We will be looking for a SNP in an intron of DNA for CYP1A2. This SNP (rs762551) has been linked to how fast CYP1A2 metabolizes caffeine in those of each ethnic group.