UROP Proposal
Dr. Leona Samson, Ph.D., Center of Environmental Health Sciences
Mentors: Chandni Valiathan and Ericka Noonan, PhD Candidates in Biological Engineering
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Determining Cell Fate After DNA Damage

Background and Significance
Our DNA is constantly exposed to damaging agents such as UV radiation from the sun and chemicals in the air, our water, and our food. The damage caused by these agents give rise to mutations which may lead to cancer and other diseases. In order to protect our genome from these mutations our cells activate the DNA damage response (DDR), which allows repair of the damaged DNA or initiates cell death if a cell's DNA is too damaged.

Often tumor cells have defects in DNA damage response pathways making them more sensitive to DNA damaging agents compared to normal cells. Chemotherapy applies DNA damaging agents to the tumor under the assumption that the cancer cells have lost DDR and will undergo cell death, but the healthy cells have not and will be able to repair the DNA and regain function. When the cell death pathway is defective, tumor cells become resistant to this type of treatment. Insights into the pathways that control cell death decisions are needed to improve current anti-cancer therapies.

The goal of this project is to take a systems approach to determine how a cell decides to die or survive after DNA damage. Two levels of regulation of the DNA damage response will be observed, protein signaling response and transcriptional response. DNA damage protein signaling events will be monitored by RNAi knockdown, western blots, and by kinase activity assays and will illustrate immediate cell response to damage. Protein signaling regulates gene expression in the cell. The transcriptional response will be observed by a transcription factor activity measurement and gene expression microarrays and will illustrate the role of transcriptional regulation in cell decision after DNA damage. Experimental measures will show a wider response to the damage as it affects processes in the cell required for proper cell death or survival decision. The data from these two levels of regulation and the resulting phenotypes like cell death and cell cycle arrest, will be integrated to form a computational model which predicts cell fate after DNA damage.

My two goals are to collect data from wild type TK6 cells mutagenized with MNNG and to create new cultures of TK6 (human lymphoblastoid) cells with knocked down proteins using mammalian cell RNAi. In the future, these cultures will be used in DNA damage experiments in order to better understand the role of the knocked down protein in the DNA damage response.

Research Design and Methods
Harvesting RNAi Knockdown Cultures. The transfection line of 293T cells will be grown and then plated at 2.5 x 10^6 cells/60mm plate. The following day, shRNA (1µg) specific to the protein to be knocked down will be mixed with psPAX2 virus(1µg), pMD2(0.5µg), water(up to 45 (1µl), and CaCl2 (5µl). HBSS (50µl) should be added dropwise while vortexing between each drop. Incubate the culture for 3 minutes and then pipette 100µl/wel on a 6 well plate and incubate the plates at 37°C for 12-18 hours then change the media. Harvest the virus-containing supernatant and infect TK6 cells by adding 200µl of virus to 200µl of TK6 cells at about 5*10^5 cells/ml.

Incubate for 24 hours at 37°C and then add 1ml of fresh media. After 48 hours, add 4ug/ml puromycin. After 2 days, dilute into 1 cell/well in 96-well plates.

Western Blots. TK6 (human lymphoblastoid) cells will be treated directly with the mutagen MNNG at concentrations of 0.01 μg/ml and 0.1 μg/ml. Lysates will be collected at various time points. A Western Blot using Bis-Tris gel will be run with 30 μg protein taken from the various time points. After incubation in primary and secondary antibody, the membrane will be imaged using Odyssey by Li-Cor. The amount of protein phosphorylation in each sample will be quantified using Odyssey to give the signaling measurement.

Kinase Assay. Antibody specific to the kinase of interest will be washed over well plates coated with either protein A or protein G. Cell lysate will then be placed in the well and the kinase of interest will bind while the rest of the lysate can be washed away. Substrate and $^{32}P(\varphi)ATP$ will be added and then a filter will be placed over the wells and the solution vacuumed so the substrate is left. The filter pieces over wells will be separated and then measured using scintillation counter to detect whether the kinase phosphorylated the substrate with the ^{32}P donated by the ATP.

Transcription Factor activity measurements. In this ELISA-based 96-well plate colorimetric assay, TK6 cells will be treated with a mutagen then cells from subsequent time points will be made into lysate. The lysates will then be incubated in wells coated with a DNA sequence specific to a transcription factor of interest. Excess lysate will be washed off and primary antibody against the transcription factor of interest will be added followed by secondary antibody with Horseradish Peroxide. The density of the color will be used as a measure of transcription factor activity.

Gene Expression Microarray. In order to discern the temporal changes in gene expression after treatment with DNA damaging agents, cells will be collected at five time points following DNA damage by a mutagen. The mRNA from a treated as well as an untreated sample will be purified and washed over a full human genome microarray to determine which transcripts are up-regulated and are down-regulated during the cell decision process. Computational algorithms will be used to reveal gene sets that have different temporal expression patterns in cells that decide to die versus those that decide to survive.

Conclusion

DNA damage can occur from a variety of sources we are exposed to every day. This project explores the various effects of this damage which will aide the progression of science to be able to reverse the damage. My part in this project will teach me many valuable skills in the laboratory and I will have the ability to watch real experimental science as it occurs. I will see the honing of experimental method to get accurate results and the data collection and completion of a project. Through this experience I hope to gain a better understanding of experimental biology, in order to aide me in my future endeavors both during and after MIT.

References

Noonan, Ericka, *Cell Decision Processing in Response to DNA Damage O^6Methylguanine.* Thesis Proposal, 2005.

Valiathan, Chandni, *Temporal Regulation of Gene Expression Signatures for Cell Death or Survival after DNA Damage*. Thesis Proposal, 2007.