Epigenetic study of methylation changes in rat astrocytes caused by cellular phone exposure

Kevin Leung, James Gomes

Interdisciplinary School of Health Science, Faculty of Health Science, University of Ottawa

Background

There are many ways that gene expression is controlled in eukaryotes, but methylation of DNA is a common epigenetic signaling tool used to control gene expression. DNA methylation occurs at the cytosine bases of eukaryotic DNA. [1]

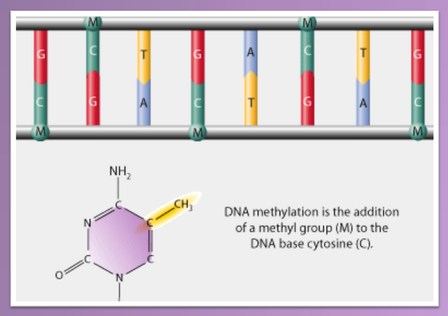

Figure 1: Shows the difference between methylated and unmethylated cytosine. [4]

The methylation of these sequences can lead to changes in the expression of genes, such tumor suppressor genes in cancer cells.[1]

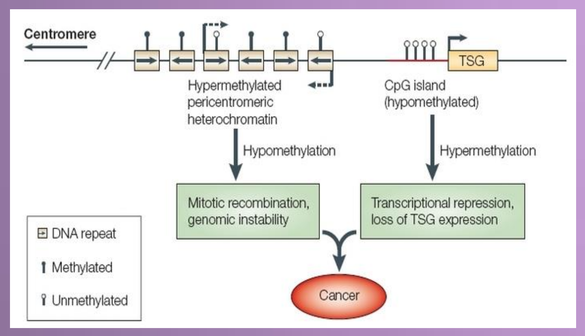

Figure 2: Shows the early effects of methylation on tumorigenesis and the development of cancer.[1]

In this study, the environmental factor is the device that is used daily by individuals in today's society, cellular phones. Cell phones emit radiofrequency energy, a form of non-ionizing electromagnetic radiation, which can be absorbed by tissues closest to where the phone is held.[2] The amount of radiofrequency energy a cell phone user is exposed to depends on the technology of the phone.[2]

Research objective

The objective is to identify and analyze genes that are modified as a result of exposure to radiation from cellular phonesand are associated with the disease development.

Methodology

Cell culture:

Rat astrocyte cells are maintained in DMEM growth media supplemented with 20% heat-inactivated FBS (v/v), 2 mM glutamine and 5 mL of antibiotic solution (streptomycin and penicillin) and incubated at 37°C and 5% CO2. Growth media is changed every other day. Cell culture is grown until 80-85% confluency and then plated according to the protocol.

Time-dependent exposure:

Two controls were used as reference for the exposures. Control 1 consisted of 2 plates being placed in an incubator at 37°C and 5% CO2 for 6 hours. Control 2 consisted of 2 plates being placed in the water bath warmed at 37°C for 6 hours.

Once the number of cell culture plates are confluent and cell count is sufficient, two plates are used to perform the time-dependent exposure. The two plates are placed into a water bath warmed at 37°C. A cellular phone is placed on top of the plate and put on call mode for 6 hours. The cell phone was on call mode with a frequency of 1700/2100 MHz. After 24 hrs, the cells were harvested.

DNA extraction:

Harvested cells were thawed and the DNA was extracted. The concentration of DNA and the 260/280 ratio was measured and recorded

Results

Table 1: Shows the DNA concentrations of the control and exposure sample, the amount of protein contamination and volume of sample.

Sample	DNA Concentration (ng/µl)	260/280 ratio	Volume (µl)
Control 1 (a)	1370.5	1.46	15.0
Control 1 (b)	1207.4	1.57	15.0
Control 2 (a)	1358.3	1.65	15.0
Control 2 (b)	1184.1	1.63	15.0
Exposure 6h (a)	1266.5	1.55	15.0
Exposure 6h (b)	1342.6	1.53	15.0

Note: Control 1 are plates placed in the incubator at 37°C and 5% CO2; Control 2 are plates placed in the water bath at 37°C.

Future work

With the DNA concentrations recorded, the DNA samples will be methylated, bisulfite converted and bisulfitome amplified. With the amplified DNA, the PCR protocol will be performed. Finally, DNA samples will be analyzed and referenced to the house-keeping gene to identify any differences.

Acknowledgement

I would like to thank Dr. Gomes, Dr. O'Reilly and the Endotox lab for their help and guidance. Furthermore, I would also like to thank the University of Ottawa for this opportunity and the support for this research project.

References:

[1] Phillips, T. (2008) The role of methylation in gene expression. Nature Education 1(1):116
[2] National Cancer Institute (2013) Cell Phones and Cancer Risk. Bethesda, MD
[3] Ng, David (2006) Human cloning: science or fiction. Vancouver, BC.

Contact Info

Name: Kevin Leung
Email: kleun106@uottawa.ca
Phone number: (613) 562-5800, ext. 4930

uOttawa