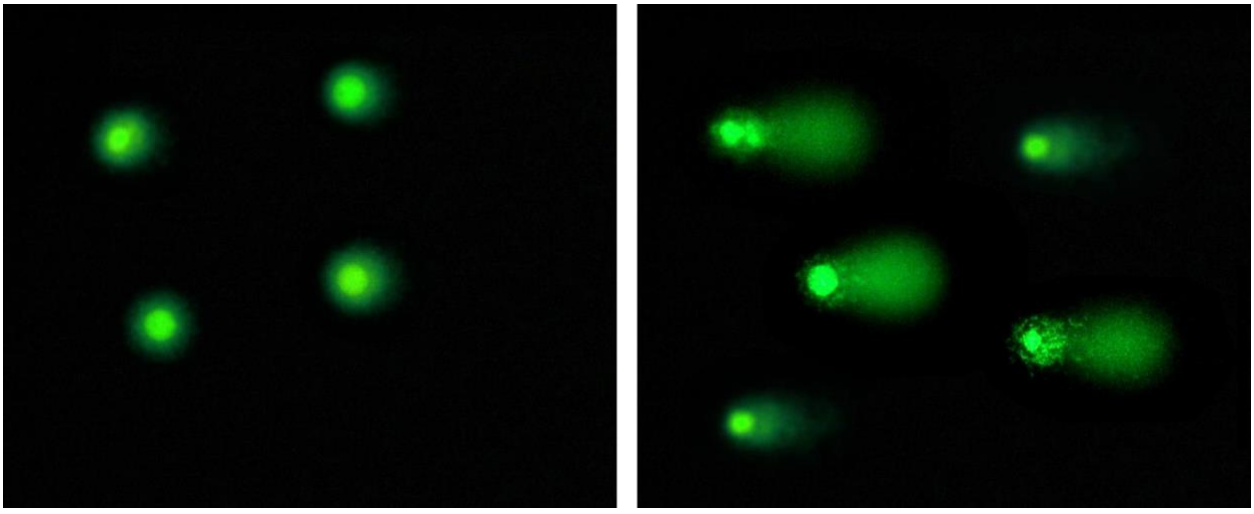
Midterm Exam 2: Studying DNA Damage & Repair via the Comet Assay

Summary

These data are collected from the Comet Assay - also known as single cell gel electrophoresis. Cells are damaged at baseline by exposure to a genotoxic agent, which causes DNA single & double strand breaks. Cell repair mechanisms very quickly start to repair this damage. One challenging feature of the data is that, even if the experimental dose is the same for a batch of cells, it is not possible to get exactly the same dose to each cell. This leads to inevitable heterogeneity among cells in their level of exposure, even at the same experimental dose.

The amount of DNA damage in each individual cell is measured by single cell gel electrophoresis, which produces an image that resembles a comet with the head of the comet on the left of the image and the tail on the right (towards the location of the right anode). If the cell DNA is intact then the comet just looks like a ball & there is little to no tail. As the damage increases the amount of DNA in the tail increases. This is illustrated in the enclosed figure, which shows comet images for representative cells having little to no damage (left panel) and those with higher levels of damage (right panel).

Automated software provides multiple features describing the comet - Comet area (CArea), HDNA (DNA in head of comet), TDNA (DNA in the tail of the comet), ratio (TDNA/HDNA), tail moment (provides a measure of size of tail), Olive tail moment (a different type of tail moment designed by Dr. Olive), and tail length (tailL), which may be subject to outliers/difficulty of measuring individual pixels being in tail or not.



Goals

Your goal is to develop a routine method of analysis for data of this type, noting that data will be collected under the same protocol for many different chemical exposures and for different immortalized cell lines corresponding to humans with different genotypes. Key goals of the current analysis include: (i) develop a statistical model for

relating dose of exposure (H_20_2 in this case) to DNA damage, allowing for variation across the replicates and for impact of repair time; (ii) Is there a statistically significantly increasing trend in DNA damage with dose of H202? (iii) What is the first dose level showing a significant increase relative to no exposure to H202? (iv) How does repair time impact these inferences? (v) Is there significant variation across replicates? (vi) Of the different surrogate variables for DNA damage, which is the single most reliable – if you had to choose a single variable in future analyses which would it be? (vii) Comment on extending your analysis approach to allow data of the same type as above but measured for 90 different cell lines i=1,…,n, with n=90, with the goal being to assess the impact of predictors $x_i = (x_{i1},\ldots,x_{ip})'$ on amount of damage and rate of repair.

Variable Key

dose = dose level of hydrogen peroxide

rtime = repair time (dna is repaired very rapidly after damage but some damage is more difficult to repair than others)

rep = replicate

The other variables are summary statistics of the comet image as described above.

Report Guideline

A written report of 3 pages max should be included in your final submission on Sakai; refer to the *Case Studies Written Report Guidelines*. The report is due Tuesday 04/21 at 8:00 pm.

NOTE

You should work completely independently of other students and make no attempt to search the literature for previous analyses of these or similar datasets. You are subject to the honor code in this respect.