q-bio 2016, Cancer Dynamics

 Project Instructor: David Axelrod

*Project Title:* Does colon cancer start “top-down” or “bottom-up”?

*Background:*

Colon cancer starts when a cell in a crypt mutates, proliferates, and forms a crypt filled with mutant cells. Abnormal crypts filled with mutant cells are an adenoma. Crypts are invaginations in the large intestine (colon) that are lined with about 2400 cells, in humans. Stem cells at the bottom may be quiescent, may divide to form stem cells, or divide to form a stem cell and a proliferating cell, or divide to form proliferating cells. Proliferating cells move up to form differentiated cells. The differentiated cells are removed at the top of the crypt (Humphries et al. 2013).

Clinical data have been interpreted to indicate that colon cancer starts when cells at the top of the crypt are mutated (Shih et al. 2001); and other data have been interpreted to indicate that colon cancer starts when cells at the bottom of the crypt are mutated (Preston et al. 2003). A mathematical analysis has concluded that mutations at the top of the crypt, where differentiated cells are located, cannot be discarded (Komarova and Wang 2004).

A computational model of stochastic cell dynamics in human crypts has been developed (Bravo and Axelrod 2013). It has been calibrated with measurements of human biopsy specimens. It reproduces the measured number and variation of each cell type and produces quasi-stationary stochastic dynamics. The behavior of the model has been verified by its ability to reproduce the experimentally observed monoclonal conversion by neutral drift, the robust ability of crypts to recover from perturbation by cytotoxic chemotherapeutic agents, and the formation of adenomas resulting from mutations either at the top or bottom of the crypt. The virtual crypt model is available as an open source program. It can be used to perform simulations to answer questions, by inputting numerical values at the computer interface

*Purpose:*

Students will perform experiments *in silico* (e.g. simulations) in order to determine the effect of mutants initiated at different regions of a virtual crypt: top (differentiated cells), middle (proliferating cells), or bottom (quiescent stem cells).

*References:*

Bravo, R. and D.E. Axelrod Theoretical Biology and Medical Modelling 2013, 10:66-89. <http://www.tbiomed.com/content/10/1/66>

Preston SL, Wong W-M, Chan AO-O, Poulson R, Jeffery R, Goodlad RA, Mandir N, Elia G, Novelli M, Bodmer WF, Tomlinson IP, Wright NA: Bottom-up histogenesis of colorectal adenomas: Origin in the monocryptal adenoma and initial expansion by crypt fission. *Cancer Res* 2003, 63:3819-3825.

Humphries A, Wright NA: Colonic crypt organization and tumorigenesis. *Nature Rev Cancer* 2008, 8:415-424.

Komarova, NL, Wang L: Initiation of colorectal cancer: Where do the two hits hit? *Cell Cycle* *2004*, 3:1558-1565.

Shih I-M, Wang T-L , Traverso G, Romans K, Hamilton SR, Ben-Sasson S, Kinzler KW, Vogelstein B: Top-down morphogenesis of colorectal tumors. *Proc Natl Acad Sci USA* 2001, 98:2640-2645.

*Resources:*

1. Download the article: Bravo, R. and D.E. Axelrod Theoretical Biology and Medical Modelling 2013, 10:66. http://www.tbiomed.com/content/10/1/66

2. Download the Virtual Crypt Model. It is available by clicking “Additional file 1” at the journal website: <http://www.tbiomed.com/content/10/1/66>.

3. Download the open-source multi-platform application Netlogo version 4.1.3, available at: http://ccl.northwestern.edu/netlogo/

4. Any statistics program to record numbers and make bivariate plots.

*Exercise 1.*

1. Open the application NetLogo version 4.1.3. File, Open Virtual Crypt Model. Click on the tab at the top, “Interface”. Details of the model and assumptions can be found in the tab “Information”. The computer code can be found in the tab “Procedures”.

2. At the Interface, click the blue button on the left: “Defaults”.

3. Enter “0.3” in the green input box in the center top, “MutantDivideDiff”

4. Enter “65” in the green input box, center, second from the top “MutateDept”.

 (65 will put a mutant yellow cell at the bottom of the crypt (region of quiescent stem cells), 59 at the lower third (region of proliferating cells), and 20 at top third (region of the differentiated cells). Record the “MutateDepth”.)

5. Click the ‘Setup’ blue button at the top left.

6. Click the “Go blue button near the top left.

7. Wait until time, indicated on the x axis of the plots, is about 100 or more. This will allow the crypt to stabilize from the initial conditions (variation in cell numbers will be quasi-stationary).

8. Click the “MutateOneCell” blue button in the middle near the top.

9. A yellow mutant cell will appear in the image of the Virtual Crypt on the left. If the yellow mutant cells disappears (dies or is removed) then record the “UnboundedSizeTime” as “0”, then click the “MutateOneCell” button again until the yellow progeny cells are maintained. Watch the crypt fill with yellow mutant cells and the run stop.

10. Record the “ “UnboundedSizeTime” with the MutateDepth”. This Time is the interval from the time the “MutateOneCell” button was clicked to the time that the crypt became unbounded in size and overflowed. A crypt with unbounded size filling with mutant cells is an adenoma, an early stage of colon cancer.

11. Repeat Steps 4 through 10 for 10 runs using another value for “MutateDepth”, such as 59. Keep in mind step 9, and record each zero.

12. Repeat Steps 4 through 10 for 10 runs using another value for “MutateDepth”, such as 20. Keep in mind step 9, and record each zero.

*Data Analyses:*

1. Combine data with other students.

2. Plot the distribution of “UnboundedSizetime” for each “MutateDepth”, 20, 59 and 60.

3. Plot Efficiency vs. “MutateDepth”. Efficiency is the number of runs at a depth that became unbounded divided by the total number of runs at a depth. The total number of runs at a depth is the number of runs that became unbounded plus the number of runs in which all yellow mutant cells disappeared (recorded as “0”).

*Answer questions:*

1. Can adenomas form from mutant cells initiated in the regions of quiescent stem cells, proliferating cells, or differentiated cells?

2. What is the dependence on time to form an adenoma on the region of the initiating mutant cell?

3. What is the efficiency of forming an adenoma from mutant cells initiated in different regions of the crypt?

4. Are the results consistent with adenomas formed “top-down” or “bottom-up”?

5. Rephrase the question “Does colon cancer start “top-down” or “bottom-up”? And answer the new question.

*Exercise 2.*

The manual exercise above can be automated by using “Behavior Space” available under the pulldown menu “Tools”, by writing a simple script into a form. This facilitates “parameter sweeping”.

1. At Interface, click “Defaults” button.

2. Tools, Behavior Space, New.

3. Experiment name:

 MutateDepth Date

4. Variables (Default values will be as set at the Interface.) Change the following):

 [“MutateDepth” 20 59 65]

 [“MutantDivideDiff” 0.3]

5. Repetitions: 100

6. Measure runs using these reporters:

 count turtles

 UnboundedSizeTime

7. Measure runs at every step: uncheck

8. Setup commands:

 setup

9. Go commands:

 go

 if ticks = 200 [MutateOneCell]

10. Stop condition:

 ticks > 200 and count cells with [Mutant = true]= 0

 or UnboundedSizeTime != 0

11. Final commands: leave blank

Time limit:

 0

12. OK

13. Run

14. Spreadsheet output: (do not select)

15. Table output (click. Will produce .csv formated file)

16. Simultaneous runs in parallel: 4 (if available)

17. OK

18. Save as: (chose an informative name such as “MutateDepth Date-table.csv)

19. Where: (choose)

20. Save:

 A window appears “Running ….” with the top line indicating the number of the current run, the total number of runs, and the step of the current run. The second line indicates the total elapsed time. You can watch the virtual crypt run, but the simulation will be faster if the slider on the top is moved to the right, and “Update view” and “Update plots and monitors” are unclicked. When all runs are completed, this window will disappear, and the results will appear in the .csv file. Note that the results may need to be sorted by “MutateDepth”, because runs may be completed out of order.

*Notes:*

1. 30 runs takes about 10 min. using 4 parallel processors.

2. The “Behavior Space” script will be saved only if the program (Interface) is saved

 when closed.

3. Parameter sweeping of values of a variable, such as “MutateDepth”, can be

 accomplished by specifying start, increment, and end values.

 Note the extra bracket.

 [“MutateDepth” [20 5 65]]

4.If computer goes to sleep, NetLogo will not abort, but runs will continue when

 computer wakes up.

5. While NetLogo is running, other programs such as Word can be used

 simultaneously.

6.Example results of a few manual runs:

 MutantDivideDiff = 0.3

 MutateDepth = 59

 Overflow: 120, 124, 0, 157

 MutateDepth 65

 Overflow: 131, 0, 142, 124

 MutateDepth 20

 Overflow: 248, 0,0, 304, 0, 0, 0, 0, 0, 0 , 0, 0, 0, 0, 0, 0, 0, 0, 0, 276

*David Axelrod July 6, 2016*

 *Incorporating suggestions of Sudeepti Vedula, 092515*