IMAG/MSM PI Consortium Meeting April 11-12, 2006
Questions for MSM PI's

PI Name: Georg Luebeck
PI Project Title: Scales of carcinogenesis: cells, crypts and cancer

1. Please highlight your scientific progress from year 1, where did you hope to be after year 1?

See our year 1 progress report (attached). Our project does not follow a 'linear' time-line, but evolves on different levels of neoplastic progression to cancer. By and large, the model development (concept and implementation) at the tissue and population level have progressed as expected. At the lower end of the modeling spectrum, the cell and crypt level, we are still in discovery and learning mode. However, we have identified new and exciting aspects of cell behavior (kinetics and kinesis) in response to mechanical stresses experienced by the cell, which we wish to incorporate into a node-based adaptive-shape cell model.

2. What challenges did you experience?

Several: One recurring challenge is that the models encoded by the programmers become quickly complex, and even among experienced programmers (in this case JAVA), the code is NOT easily discussed, inspected, or readily modified/adapted by others. The important question here is: how can complex agent-based models be shared in an easy to understand modular framework – that allows for the transparent transport of a model from one platform to another, or from one context to another.

Specific to our project: we lack appropriate (epi)genetic data to answer a number of questions related to the spreading of mutant cells and crypts in the tissue of primary interest: Barrett's esophagus. This is in part due to budget cuts.

3. What unexpected outcomes did you encounter?

In terms of model development & computing: the speedy development of agent-based computer programs is offset by their rapidly increasing complexity. Typically, such programs are special purpose codes that help us answer specific what-if questions. Code efficiency also dictates structure. For stochastic modeling, an event-based queuing system was superior to a discrete-time version with small probabilities for each possible event in a time slice.

In terms of model predictions: wounding in a tissue composed of normal and mutant structural units leads to unexpected spatial growth patterns, including fractionation of the clones into spatially distinct regions.

4. What are the major advances that have occurred in your field this year?

In cancer biology and cancer modeling, the "*tumor stem cell"* is a hot topic – and recent work has identified a subset of such cells in colon cancers . We are planning to utilize models of multistage carcinogenesis to predict the fraction of tumor stem cells in adenomatous polyps in colon based on the observed incidence of colon cancers.

Another highlight is the work by Celeste Nelson and colleagues (Princeton University) on "*Emergent patterns of growth controlled by multicellular form and mechanics*" (PNAS 112(33), 2006). We are currently developing a spatial node-based adaptive-shape cell model following the approach by Tim Newman (Arizona State) to explain the intriguing observations by the Nelson group.

5. How successful were your proposed tools, and did you adopt new tools?

We use primarily mathematical tools in cancer modeling and computational tools to implement the mathematics for the purpose of simulation and parameter estimation. Strictly speaking, no new tools very adopted.

For spatio-temporal simulations of crypt-structured tissues we have developed a random event-driven queuing approach. This approach is more efficient compared to discrete time computing with infinitesimal time slices. The drawback is that the queuing approach is more complex – resulting in lengthier and more complex code.

6. Please share your individual experiences of collaborating with the broader community.

This is our first entry into the field of cellular multiscale modeling – we are definitely newbies, but are catching on. There is a huge gap between traditional genetics-based multistage modeling of cancer, rooted in the simple idea that cancer is the outcome of an accumulation of mutational rate-limiting events and clonal selection, without reference to spatio-temporal properties of cells and tissue, cell-cell and cell-substrate adhesion. We have only begun to identify potential collaborations in this field, related to modeling the behavior of aberrant/mutant cells under various conditions, including noxious challenges. Once we are beyond preliminary explorations, we hope to discuss our approach and findings with interested colleagues. Dr. Glazier and his colleagues were very receptive and helpful in our initial inquiries concerning spatio-temporal modeling of migrating cells in contact with one another and with the substrate.

7. Please highlight your plans for year 2.

a) extent the adaptive-shape cell model to allow for cell proliferation, quiescence and cell death, in response to mechanical stress related to substrate-mediated forces and cell-cell contact. Explain the surprising findings reported in the Nelson paper.

b) study neoplastic progression in vitro using transformed and untransformed mutant cell cultures derives from primary esophageal adenocarcinomas.

c) use the fluctuation analysis developed by this group to estimate the cell kinetics of cells under a variety of conditions (i.e., in log-phase, near confluency, one cell population at a time, two cell populations in competition, with and without wounding, and more). Martin Suchorolski, a Ph.D. student in the Reid lab at the FHCRC, is performing these experiments for us.

8. What is your primary MSM Working Group?

The primary fit is with WG4 (cell level modeling) even though we have no experience with ML-type model descriptions. Yet, what we do is cell and tissue level modeling. WG5 is secondary.

9. Please comment on your MSM Working Group(s), and what needs to be improved?

I feel the working group (WG4) focuses on an important aspect of model development and sharing, but this is mainly technical, hardly scientific. What scientific threads do the members of this WG share, what biological, statistical, and mathematical aspects are of common interest? Perhaps this is the wrong question to ask in this place, but if science and our understanding of cell biology dictate the nature and form of our models, then the way we present them very much depends on the specific traits we see as important. I perceive a need to better understand the nature and purpose of the models used by other researchers in the group.

There is not better way than scientific discussion - person to person – to find out what others are doing in this field.

10. How do you foresee logical linking of models with others in the MSM?

At this point, I don't. To do so requires better definition and understanding of the multiscale levels each team is concerned with. In some cases model-linking will be obvious and the researchers are already familiar with the work of another group. However, this is not typical. For cell and tissue level modeling - especially in conjunction with modeling disease processes - I fail to see a consortium-wide 'model roadmap', couched in terms of interlinked scientific goals, that would motivate and help us to hook up with other model(er)s.

11. Are you writing grants?

Yes. Constantly.

12. Are you finding new collaborations?

We see potential collaborations with other cell and tissue modelers down the road. See 6.

Progress Report (year 1): Scales of carcinogenesis: cells, crypts and cancer (PI: Luebeck)

This report covers research activities and scientific progress in the first 9 months after this grant was awarded (September 1, 2005). At this point, the simulation and software development projects, in collaboration with Dr. Maley's lab at the Wistar Institute, are well on their way and have resulted in a posting of Java code on Sourceforge (see below). Furthermore, we have made substantial progress in accomplishing our goals set forth in specific aim 4: *From a tissue module to a population module* resulting in a publication on *Age Effects and Temporal Trends In Adenocarcinoma of the Esophagus and Gastric Cardia* (Cancer Causes and Control - in press) authored by our graduate student Mrs. Jihyoun Jeon (also supported by this grant), Dr. Suresh Moolgavkar and the PI, Dr. Luebeck.

A. Specific Aims (modification due to budget cut)

Because of the more than 30% administrative cut in the budget of our recent Multiscale Modeling award, we felt the need for adjusting our scope of work accordingly. Under the tightened budget, there is simply not the money to carry out the proposed SNP experiments in Barrett's esophagus to explore the spatial scales and spatial heterogeneity of neoplastic progression in this tissue. Dr. Jennifer Couch (the NCI program director) and the PI (Dr. Luebeck) agreed that this particular objective be removed from specific aim 3. Instead, we shall explore the possibility of using existing FISH data for model validation. Such data were obtained previously from Peter Rabinovitch's lab (UW) from crypts at different spatial resolutions within Barrett's esophagus.

B. Studies and Results (highlights)

Tissue scale: Event-Based Modeling of Barrett's Esophagus (in collaboration with Dr. Carlo Maley's Lab (subcontract))

Our activities for this project relate to specific aim 3: **From a proliferative unit module to a tissue module.** To facilitate this collaboration and to log and disseminate information between the two research teams, we have set up two Wiki's, one at the Wistar Institute (http://kazad-dum.wistar.upenn.edu), and one at the FHCRC (http://wiki.fhcrc.org/icb). In addition all Java-based source code for the 'crypt-tissue' simulation tool under development has been posted by Mr. Tom Eck (Maley Lab) on Sourceforge (https://sourceforge.net/projects/mlabtismodel/). Briefly, the main purpose of this module is to help us gain insights into what determines/controls wound-inflicted promotion in the size and number of neoplastic clones in crypt-structured epithelia, such as colon, gastric, and Barrett's esophagus. In particular, we are interested in the spatial aspects of these clones and their diversity as a function of the rate of wounding or cell killing. This tool will also allow us to explore (at a mechanistic level) the observed 'by-stander' effect in tissues exposed to chronic radiation levels.

Cellular scale: modeling the impact of cell cycle effects on carcinogenesis.

We have made considerable progress in developing a combined (and in a sense unified) model of the cell cycle and multistage carcinogenesis. We created a model of the cycling of cells on short time scales in mouse intestinal crypts, incorporating homeostatic regulation in the presence of low-dose radiation, while following these processes over long time scales to study the probability of developing cancer. The model explicitly incorporates cell cycle states, simple and complex damage, checkpoint delay, slow and fast repair, differentiation, and apoptosis in the presence of low-dose ionizing radiation.

In modeling the mouse intestinal crypts, we incorporate homeostatic regulation at the individual crypt level to maintain a target of approximately 16 stem cells per crypt. As this target number is approached, the model causes the rate of progression out of the G1 cell cycle state to diminish. Thus cells in G1 stage become blocked, effectively going into G0 state. The model incorporates differentiation out of G1 based on a circadian rhythm, with homeostatic regulation then leading to division of a few stem cells. The dividing cells are much more susceptible to radiation. Thus the model predicts a few (3-4) highly sensitive stem cells that apoptosis with low-dose IR, with the remaining stem cells in G0 highly resistant to radiation. These model results are in general agreement with experimental data. We have one manuscript on this work accepted for publication in 'Advances in Space Research', and a chapter describing our combined cell-cycle and multistage carcinogenesis model that will be published in a book on stochastic carcinogenesis models edited by Prof. Wei-Yuan Tan. Numerical computations for this project are currently based on the Mathematica package, however, we will seek to make this model also available in form of Opensource code.

Population scale: from aberrant cells and crypts to cancer in populations.

This analysis by our graduate student Mrs. Jihyoun Jeon and the PI, Dr. Luebeck, is an attempt to explain the incidence of esophageal adenocarcinoma and its temporal trends by a stochastic multistage model that incorporates multiple scale of the carcinogeneic process. Briefly, a number of hypotheses have been advanced to explain the rapid increase of the incidence of esophageal adenocarcinoma in the US. A major problem in identifying and understanding the nature of this increase is the difficulty in untangling age effects from temporal trends due to cohort and period effects. To address this problem, we have developed multistage models that describe the age-specific incidence of adenocarcinoma of the esophagus and of the gastric cardia with separate adjustments for temporal trends. These models explicitly incorporate important features of the cancers, such as the metaplastic conversion of normal tissue in the esophagus to the metaplastic cryptal Barrett's tissue. These models we fitted separately to the incidence of adenocarcinoma of the esophagus as well as gastric cardia as reported in the Surveillance Epidemiology and End Results Registry (SEER). We conclude that the incidence of both cancers is consistent with a sequence that posits a tissue conversion step in the target organ followed by a multistage process with three rate-limiting events, the first two leading to an initiated cell that can expand clonally into a premalignant lesion, and the third converting an initiated cell into a malignant cell. Interestingly, for both cancers at both sites, the temporal trends are dominated by dramatically increasing period effects. For US males, these period effects correlate strongly with the epidemic increase of male obesity observed in the US over the past 30 years.

C. Significance

The scientific and clinical significance of this work has been previously stated in the grant application.

D. Plans

One important goal, not yet addressed, is how intra-crypt diversity' influences inter-crypt diversity. We have invited Dr. Darryl Shibata to collaborate with us to explore the possibility of using epigenetic tags to 'clock' the Barrett's tissue and to explore the reconstruction of a phylo(epi)genetic tree based on CpG methylation. He has agreed to send us his data. In parallel, we are collaborating with the laboratory of Dr. Charles Laird (UW) in an attempt to improve the sensitivity of the bisulfite PCR assay using batch-stamping and bar-coding. Dr. Paulson (Reid's lab) is currently extracting cells from crypts in Barrett's esophagus for subsequent bisulfite PCR analysis in the Laird lab. High quality methylation data from crypts may potentially be used to assess the degree intra- and inter-crypt diversity during neoplastic progression in Barrett's esophagus.

E. Publications

Jeon J, Luebeck EG, Moolgavkar SH (2006). *From a tissue module to a population module* resulting in a publication on *Age Effects and Temporal Trends In Adenocarcinoma of the Esophagus and Gastric Cardia* (Cancer Causes and Control - in press)