

## **The Role of Mechano-chemical Signaling in the Regulation of Cancer Cell Movement**

### **INTRODUCTION**

Cell movement is one of the greatest accomplishments of evolution. It is essential for embryonic development, as single cells and groups of cells must migrate from isolated parts of the embryo in order to form organs in their correct locations. Cell movement is necessary in various physiological functions, including wound healing and the functioning of white blood cells in fighting bacterial infections. While cell movement is necessary in adult organisms, uncontrolled cell migration is the trademark of a cancer cell. Cancer cells have unrestrained division, move quickly, and are not well differentiated. These characteristics explain metastatic tumor cells' ability to invade tissues surrounding the tumor, spread into the body's circulatory system, and establish secondary areas of growth (Lodish et al., 1999).

Cell movement can be broken down into four processes: the protrusion of the front cell edge, which forms a lamella; its attachment to the substratum by forming specialized adhesion structures; the forward flow of the cytosol; and the retraction of the rear of the cell. The molecular machinery that is responsible for cell movement is the actin cytoskeleton. The actin cytoskeleton is a filamentous polymer that fills a large part of the cell cytoplasm, maintains cell shape, and permits directed movement of organelles, chromosomes, and the cell itself. The actin cytoskeleton controls the shape of a cell by assembling or disassembling itself, allowing the cell to perform the above processes and to move along a surface (Lodish et al., 1999).

Cytoskeletal function must be controlled for cell movement to successfully occur. There must be a coordination of protrusion of the lamella with retraction of the rear edge. In addition, the formation of new adhesions at the leading edge of the cell must be synchronized with the dissociation of older adhesions at the rear of the cell. Cell movement is also regulated by environmental signals. During chemotaxis, for example, cell movement is directed toward the source of a certain diffusible chemical. A well-studied example is the chemotactic movement of

white blood cells toward a bacterial infection (Alberts et al.1994). Cells also respond to the biochemical and physical composition of the substratum. An example of this is the growth cones in developing nerve cells, which resemble highly motile, crawling fibroblasts. These growth cones follow topographical features of the substratum and respond to the presence of chemicals, so the developing nerve cells grow along defined paths (Karp, 1999).

Intracellular calcium,  $[Ca^{2+}]_i$ , is responsible for many molecular processes involved in cell movement. For example,  $[Ca^{2+}]_i$  regulates actomyosin-based contractile forces and the formation and disassembly of cell-substratum adhesions. Calcium sensitive actin binding proteins regulate the structure and dynamic behavior of the cytoskeleton (Lee et al., 1999). Moving cells have recently been shown to respond to their physical environment in a process called mechano-chemical signal transduction. This involves the conversion of mechanical force to a biochemical signal, such as a change in  $[Ca^{2+}]_i$ . A change in  $[Ca^{2+}]_i$  can then trigger the cell to respond by altering some aspect of cytoskeletal function. In fish keratocytes, transient  $[Ca^{2+}]_i$  increases occur more frequently in cells that have become “stuck” to the substratum, or when the cells have been subjected to mechanical stretching. This is because the rate of retraction of the rear of the cell becomes significantly slower than the rate of lamellar extension, the cell becomes elongated, and tension increases between the ends of the cell. This tension inhibits lamellar extension and decreases the speed of the cell. When cytoskeletal tension reaches a threshold, stretch-activated calcium channels (SAC’s) are activated and calcium is released from intracellular stores. This transient increase of  $[Ca^{2+}]_i$  activates detachment mechanisms that lead to the retraction of the rear of the cell. Cytoskeletal tension is consequently decreased in the cell, allowing lamellar extension so the cell may move. Thus, in fast moving keratocytes SAC’s act as mechano-chemical signal transducers that allow cells to sense and respond to increased substratum adhesiveness (Lee et al., 1999).

When two membranes make contact, normal ruffling activity of the edges ceases and the cells form junctions. The paralysis quickly spreads to the whole cell and immobilizes it. This is

called contact inhibition of migration (Bray, 1992). Metastatic cells are less affected by contact inhibition than normal cells. The cytoskeleton of metastatic cells is less organized, and their rate of movement faster than that of normal cells. Substratum adhesion, as well, is reduced in metastatic cells (Demetroiou et al., 1995). These characteristics underlie the ability of tumor cells to invade other tissues (Schmidhauser et al., 1990).

My project will examine the role of mechano-chemical signaling in regulating the movement of metastatic cells by observing whether stretch-induced changes in  $[Ca^{2+}]_i$  occur during movement or cell-cell contact. This has not been investigated previously, and is important in that it may shed light on whether the abnormal movement of cancer cells is related to alterations in their ability to respond to their physical environment.

### **SPECIFIC AIMS**

The aim of this project will be to investigate whether mechano-chemical signal transduction is involved in regulating the movement of metastatic cells. To do this, I will see if stretch induced calcium transients occur in moving cancer cells, and whether they are required for movement. I will also see if any changes in intracellular calcium occur following cell-cell collisions, to determine if mechano-chemical signaling is involved in contact inhibition of locomotion and whether this contributes to the loss of contact inhibition in metastatic cells.

### **METHODS**

I will perform calcium imaging in both normal and related metastatic cell lines, using procedures I have learned in my previous two semesters in Dr. Juliet Lee's cell biology laboratory. I will compare changes in size, frequency and duration of calcium transients that occur during movement of normal and metastatic cells. To check that calcium transients are associated with stretching, I will stain the cells with a calcium indicator and a calcium-independent dye. Using fluorescence microscopy, I will record time-lapse movies of the moving cells. I will use computer image processing techniques to correlate changes in cell shape with intracellular calcium concentration. Gadolinium, an inhibitor of stretch-activated calcium

channels, will also be used to see if calcium transients are due to stretching or not. Other control experiments will be performed to determine the source(s) of the increased  $[Ca^{2+}]_i$ .

A possible difficulty in my project is the multiple cellular signaling pathways that calcium is involved in. These may make it difficult to tell whether changes in  $[Ca^{2+}]_i$  are associated with biochemical signaling or in response to mechano-chemical signaling. This obstacle may be confronted by comparing changes in  $[Ca^{2+}]_i$  that occur during a cell's response to chemotactic stimulus with those resulting from a mechanical stimulus. Cells will be treated with growth factors to induce a chemotactic response while a mechanical stimulus will be applied by tweaking cells with a microneedle and observing the subsequent calcium response.

I have been a member of Juliet Lee's lab for two semesters. In this time, I have studied the movement of fish keratocytes. I have set up cell cultures and learned relevant techniques, including the replating of cells and the fluorescent staining necessary for calcium imaging. I have performed fluorescence video microscopy and analyzed my results. This semester I am studying how cells move on different viscosities of silicone substratum. After my experiences for these two semesters, I am confident in my skills and I am prepared to apply them to the project described above.

## **PLAN OF STUDY**

### University Scholar Plan of Study

### MCB Plan of Study

#### **Spring 2001**

MCB 219, Developmental Biology (3)  
MCB 299, Independent Study (4)  
MCB 214, Exper In DNA Identification (2)  
MCB 213, Concepts in Gene Analysis (4)  
MCB 380, Advanced Cell Biology (3)  
ENGL 212, The Modern Novel (3)

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MCB 299, Independent Study (4)  
MCB 214, Exper in DNA Identification (2)  
MCB 213, Concepts in Gene Analysis (4)  
Elective

For my final three semesters, I will continue to take MCB 299 in Dr. Lee's lab to carry out my University Scholar project. I will take MCB 380, Advanced Cell Biology, which will give me the background necessary to understand the molecular components of cell movement. Juliet Lee's portion of the class goes into detail describing the structures and functions important to cell movement, which will correspond to my project. I will also be able to pursue a second love of

mine, literature, by taking an English Class. I have been putting these classes on hold to take MCB classes, but as a University Scholar, I will be able to take Literature classes and learn not only about works of literature, but about culture, history, and people.

#### **Summer 2001**

I have multiple options regarding my education next summer; I will be applying to universities across the nation who sponsor paid cell biology research opportunities, including programs at University of Michigan and University of North Carolina. As a University Scholar, I will have the opportunity to take summer classes without charge at UConn, so I also will have the option of staying in Storrs for the summer. If I stayed here, I would continue my research with Dr. Lee, and I also would take Biology and English summer classes. This would not be an option without being a University Scholar, because I would not be in a position to give up a summer of paid research and pay to take classes here.

#### **Fall 2001**

MCB 299, Independent Study (4)	MCB 299, Independent Study (4)
MCB 312, Foundations of Structural Biochemistry (3)	MCB 211, Basic Immunology (3)
PNB 274, Enhanced Human Phys & Anat (5)	PNB 250, Animal Physiology (3)
ENGL 286, Women in 20 <sup>th</sup> C. Literature (3)	Elective
MCB 314, Cellular Aspects of Developmental Bio (2)	Elective

As a University Scholar, I will take courses PNB 274 and 275 (I will take PNB 275 in Spring 2002). Though I am a Molecular and Cell Biology major, I am interested in physiology and anatomy as well, and I am interested in how each relates to the other. These courses are more in depth than PNB 250, the course normally recommended non-PNB majors, and involve a lab that PNB 250 does not have. I will take MCB 314, a graduate course that discusses cell movement in development. I will also take MCB 312 to expand my background in biochemistry.

#### **Spring 2002**

MCB 226W, Advanced Biochemistry Lab (3)	MCB 226W, Adv Biochemistry Lab (3)
MCB 246, Virology (3)	MCB 246, Virology (3)
MCB 292W, Senior Research and Thesis (3)	MCB 292W, Senior Research and Thesis (3)
PNB 275, Enhanced Human Phys & Anat (5)	Elective
MCB 325, Str.&Function of Biological Membranes (3)	Elective
ENGL 226, Modern English Literature (3) OR ENGL 264 Study of Ind. Author (3)	

In Spring 2002, I will complete my research and discuss my project and results in my Honors Thesis. By taking MCB 325, I will continue to learn about the structures and functions of the cell. This semester, I will take either ENGL 226 or ENGL 264. If I am interested in the authors

ENGL 264 is studying this semester, I will register for it. Otherwise, I will register for ENGL 226.

## References

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Demetriou, Michael, et al. (1995) Reduced Contact-Inhibition and Substratum Adhesion in Epithelial Cells Expressing GlcNAc-Transferase V. *The Journal of Cell Biology* **130**: 383-392.

Karp, Gerald. Cell and Molecular Biology. New York: Wiley and Sons, 1999.

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