Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

BMSC 8210 final exam 2019

In your ***own*** words, answer any of the following **5** questions of your choice, ensuring you answer each part of the selected questions. All questions carry equal weightage. Be direct and succinct in your answers (a maximum of 2 pages for each answer, single spaced). Cited references are not included in the page limit.

Consultation is only allowed with the instructor of the selected question(s) and/or with Dr. Jyoti Jaiswal or Dr. Ed Seto.

Send your completed exams as PDFs with your name, the question number, instructor's name, and date on each page to the **appropriate instructor** (emails are beside each question), **Dr. Jyoti Jaiswal** (jkjaiswal@cnmc.org) and **Dr. Ed Seto** (seto@gwu.edu) by **October 21st at 5 PM**. Points will be deducted for late submissions unless previously approved by me.

Let me know if you have any questions.

Good luck!

Dr. Jyoti Jaiswal
Dr. Ed Seto

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Question 2: Dr. Jyoti Jaiswal (jkjaiswal@cnmc.org)

Q: A cell inhabits an environment deprived of molecule INeedIt (INI), which at high concentrations in the cytosol is toxic. For a specific cellular process this cell must allow entry of INI for 1 minute. Describe the mechanism(s) by which the cell can facilitate the precise control needed to start and stop the entry of INI when:

a) INI is an ion and cell has plenty of energy reserves.
b) INI is a small molecule and the cell is energy deprived.

A)

Two kinds of membrane proteins exist to allow entry of ions into a cell: ion channels and ion pumps. Ion channels are passive conduits that, when open, can allow selected ions to diffuse down electrical and concentration gradients to achieve equilibrium. Ion pumps, on the other hand, work to maintain gradients and consume energy (as ATP) to shuttle ions across a membrane (1).

Ion Channels

Channels will be considered first. Channels are selective towards both ion *type* and ion *concentration*. A channel can be selective towards the *type of* ion(s) it will allow to pass across the cell membrane due to the channel's restrictive size and/or because of its particular amino acid configuration at a region called the selectivity filter. The amino acids at the site of the selectivity filter can allow for ion discrimination in terms of electrochemical properties and hydrophobicity (2). An example of such an ion channel in nature is that of the KcsA K+ channel. This K+ channel uses a selectivity filter made of an amino acid sequence that allows a K+ ion, but not a Na+ ion, to dehydrate, enter, and cross the channel within about 10 nanoseconds (3).

To continue, the precise control of opening and closing ion channels to regulate ion *concentration* is either a function of changing electrochemical gradients in voltage-gated ion channels (4), of cell signaling in ligand-gated ion channels, or of cellular deformation in stretch- and heat-activated channels (5). Voltage-gated ion channels open and close based on signalling from the voltage sensor, a molecular device that detects voltage across a membrane (6). Ligand-gated ion channels open and close depending on the binding of a neurotransmitter to an orthosteric site(s) that triggers a conformational change in the channel. Ligand-gated ion channels can be further governed by the binding of endogenous or exogenous modulators to allosteric sites (7). Finally, stretch- and heat-activated channels can respond to temperature changes, mechanical distortions, stretch receptor signalling, and even sound waves (5).

Ion Pumps

In contrast to ion channels, ion pumps transport ions against a concentration gradient. In the case of the cell in question, this might be necessary because the cell's environment is deprived of INI. A pump consists of ion binding sites that attach the ion

in question on the exterior of the cell membrane and then, using ATP, change the conformation of the pump to transport the ion into the cell. Alternating between opening towards the cytosol and towards the exterior allow pumps to move ions from areas of low concentration to higher concentration using ATP (8).

B)
If INI is a small molecule and the cell is energy deprived then the control of INI import might happen via passive diffusion or facilitated diffusion. Passive diffusion is driven by the concentration and electrical gradient of the solute and does not require the use of energy. Small nonpolar gases and small polar molecules can easily diffuse through the cell membrane in a three-step process: partitioning of the membrane, diffusion across the membrane, and release into the cytosol. However, larger polar metabolites have trouble with passively diffusing across the membrane and larger, uncharged polar molecules cannot diffuse across the membrane at all (9). If the molecule is not suitable for passive diffusion, it may be able to use facilitated diffusion. Much like passive diffusion, this transport method does not require energy and fundamentally relies on moving molecules in the thermodynamically-favorable direction of concentration gradients. However, it differs from passive diffusion in that the small molecules are shuttled across the membrane through protein mediators. Polar and charged molecules (such as carbohydrates, amino acids, and nucleosides) can travel across the membrane via facilitated diffusion without the need for these molecules to interact with the cell's hydrophobic interior (10).

The above methods of small molecule transport rely on the existence of a concentration gradient. However, the cell in question may not have such a gradient of INI given that its environment is deprived of this needed molecule. In addition to not having a favorable concentration to facilitate diffusion, the cell is deprived of energy. In such a situation, it is possible that secondary transporters may be able to play a role. Secondary transporters couple the energy of the transmembrane electrochemical potential of one solute to that of another (11). Perhaps the cell has an abundance of another solute and it can use the energetically favorable release of that solute in exchange for INI. In such a case, the mechanism of transport is still *active* in the sense that it requires energy but it does not require ATP; instead it utilizes a *secondary* energy source, the energy generated by the flow of an ion down an electrochemical potential gradient previously created by a pump (8).

If the cell cannot use passive transport or secondary active transport then it may not be able to obtain INI. When cells do not have access to essential extracellular nutrients they engage in the self-catabolic process of macroautophagy. (12)

1. Gadsby DC (2009) Ion channels versus ion pumps: The principal difference, in principle. Nature Reviews Molecular Cell Biology 10(5): 344-352.

2. Ashcroft FM (2000) Ion channels and disease, (Acad. Press, San Diego [u.a.])
3. Morais-Cabral JH, Zhou Y & MacKinnon R (2001) Energetic optimization of ion conduction rate by the K+ selectivity filter. Nature 414(6859): 37-42.
4. Catterall WA (2000) From ionic currents to molecular mechanisms: The structure and function of voltage-gated sodium channels. Neuron 26(1): 13-25.
5. Purves D, Augustine GJ & Fitzpatrick D (2018) Neuroscience, (Sinauer Associates, New York)
6. Francisco Bezanilla (2000) The voltage sensor in voltage-dependent ion channels. Physiological Reviews 80(2): 555-592.
7. Alexander SPH, Mathie A & Peters JA (2011) Guide to receptors and channels (GRAC), 5th edition. British Journal of Pharmacology 164(Suppl 1): 1-2.
8. Clarke RJ & Khalid MAA (2015) Pumps, Channels and Transporters : Methods of Functional Analysis, (John Wiley & Sons, Incorporated, Hoboken)
9. Yang NJ & Hinner MJ (2015) Getting across the cell membrane: An overview for small molecules, peptides, and proteins. Methods in Molecular Biology (Clifton, N.J.) 1266: 29-53.
10. Cooper GM & Hausman RE (2007) The cell, (ASM Press, Washington, DC)
11. Forrest LR, Krämer R & Ziegler C (2011) The structural basis of secondary active transport mechanisms. BBA - Bioenergetics 1807(2): 167-188.
12. Palm W & Thompson CB (2017) Nutrient acquisition strategies of mammalian cells. Nature 546(7657): 234-242.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Question 4: Dr. Linda Kusner (lkusner@email.gwu.edu)

Q: The extracellular matrix promotes mobility, proliferation, differentiation, and survival of the cell. Discuss one of these aspects of the role of extracellular matrix on the cell. Design an experiment that will test the hypothesis that the composition of the extracellular matrix is responsible for the role. Provide literature support for your hypothesis.

Durotaxis and the Role of Extracellular Matrix on The Cell

Durotaxis is the process whereby cells migrate up stiffness gradients in the extracellular matrix (ECM) (1). Altered ECM composition in vivo has been shown to accompany tissue stiffness changes as well as changes in cellular migration (2). Specifically, the mechanical properties of the ECM have been shown to influence fibronectin fibril assembly (3), cytoskeletal flexibility (4), and the strength of integrin-cytoskeleton linkages (5). All of these factors are known to affect cell locomotion in durotaxis(6).

Durotaxis is important in development, homeostasis, and disease. Lung fibrosis, for example, is characterized by local increases in tissue stiffness mediated by an increase in collagen I concentration. In breast cancer, tumor stiffness is also associated with increased levels of collagen I and laminin. In atherosclerosis, thickening of the arterial walls is mediated by changes in the mechanics and composition of the intimal matrix (2).

It is therefore important to understand cellular responses to stiffness gradients and how these are modulated by the composition of the ECM. It is known that in the process of durotaxis, cells communicate with their environment through focal adhesion complexes which mediate interactions between the cell and the ECM. The ECM acts as a signaling hub that communicates with the cells depending on mechanical cues (7). What remains to be uncovered is the role that different components of the ECM play in this communication and in the process of durotaxis (8).

Hypothesis and Experiment

One way to hone in on the underlying mechanisms involved in the process of durotaxis would be to determine which kinds of matrix proteins allow for the process and to what extent. It has been shown that durotaxis can occur in a matrix of fibronectin but the role of other proteins such as collagen and elastin in durotaxis remain unclear (2). A possible hypothesis, therefore, is that collagen and elastin allow for durotaxis in the same way that fibronectin does.

To test this hypothesis, cultured bovine cells could be placed on a polyacrylamide gel featuring gradients in mechanical compliance and be experimentally coated with fibronectin (a positive control), elastin, collagen, or nothing (a negative control). The cells could be allowed to migrate across the stiffness gradients (i.e. to durotax) and then imaged to determine the effects of different matrix proteins on their motility.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Given the time and budget, it would be fascinating, following the above preliminary results, to perform single-cell RNA-seq on animal-model breast tumor tissue, lung fibrosis tissue, and the healthy analogs for the two previously mentioned cell types. If possible, paired samples would be even better, i.e. obtaining breast tissue cells from a breast tumor and non-diseased tissue *in the same organism*. Following the analysis of RNA-seq data, it may be possible to identify genes which are correlated with tissue stiffening and motility. These genes could be selectively knocked out in mammalian cell cultures while retaining wild-type cell cultures as controls. Then the motility of these knockout cells could be reexamined on the aforementioned polyacrylamide gels to see if the knockouts make a difference in the cells' ability to durotax. The experiment as designed would help develop a clearer picture regarding the genetic and cellular mechanisms that underlie durotaxis and help elucidate a role for the composition of the extracellular matrix in the motility of cells. Therapeutic, bioengineered cells which could navigate via durotaxis to diseased tissue sites could be a future direction for the results of this type of study.

1. Feng J, Levine H, Mao X & Sander LM (2019) Cell motility, contact guidance, and durotaxis. *Soft Matter* 15(24): 4856-4864.
2. Christopher D. Hartman, Brett C. Isenberg, Samantha G. Chua & Joyce Y. Wong (2016) Vascular smooth muscle cell durotaxis depends on extracellular matrix composition. Proceedings of the National Academy of Sciences of the United States of America 113(40): 11190-11195.
3. Halliday NL & Tomasek JJ (1995) Mechanical properties of the extracellular matrix influence fibronectin fibril assembly in vitro. *Experimental Cell Research* 217(1): 109-117.
4. Wang N, Butler JP & Ingber DE (1993) Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260(5111): 1124-1127.
5. Choquet D, Felsenfeld DP & Sheetz MP (1997) Extracellular matrix rigidity causes strengthening of integrin–cytoskeleton linkages. *Cell* 88(1): 39-48.
6. Lo C, Wang Y, Wang H & Dembo M (2000) Cell movement is guided by the rigidity of the substrate. *Biophysical Journal* 79(1): 144-152.
7. Lachowski D, et al (2018) FAK controls the mechanical activation of YAP, a transcriptional regulator required for durotaxis. The FASEB Journal 32(2): 1099-1107.
8. Sapir L & Tzlil S (2017) Talking over the extracellular matrix: How do cells communicate mechanically?. Seminars in Cell and Developmental Biology 71: 99-105.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Question 5: Dr. Russel Cruz (crcruz@email.gwu.edu)

Q: Name a disease that results from pathology in the apoptotic machinery, explain how deficiencies in apoptosis result in the signs and symptoms of this disease, and propose a possible strategy for treatment targeting the cell death pathway defect.

Neuronal apoptosis has been shown to play an important role in the pathogenesis of Alzheimer's disease (AD). Aberrant expression of cyclins has been found in post-mortem brains of Alzheimer's patients (1) and caspases are apparently involved in some of AD's upstream pathological events (2, 3). Evidence also indicates that slightly upregulated ceramide levels and/or inefficient glucose utilization in AD patients can stimulate the generation of reactive oxygen species and have negative consequences for neuronal survival (4). Oxidative stress goes hand-in-hand with mitochondrial dysfunction and ultimately apoptosis and neuronal loss.

The process by which apoptotic machinery is affected in AD is illustrated in figure 1:

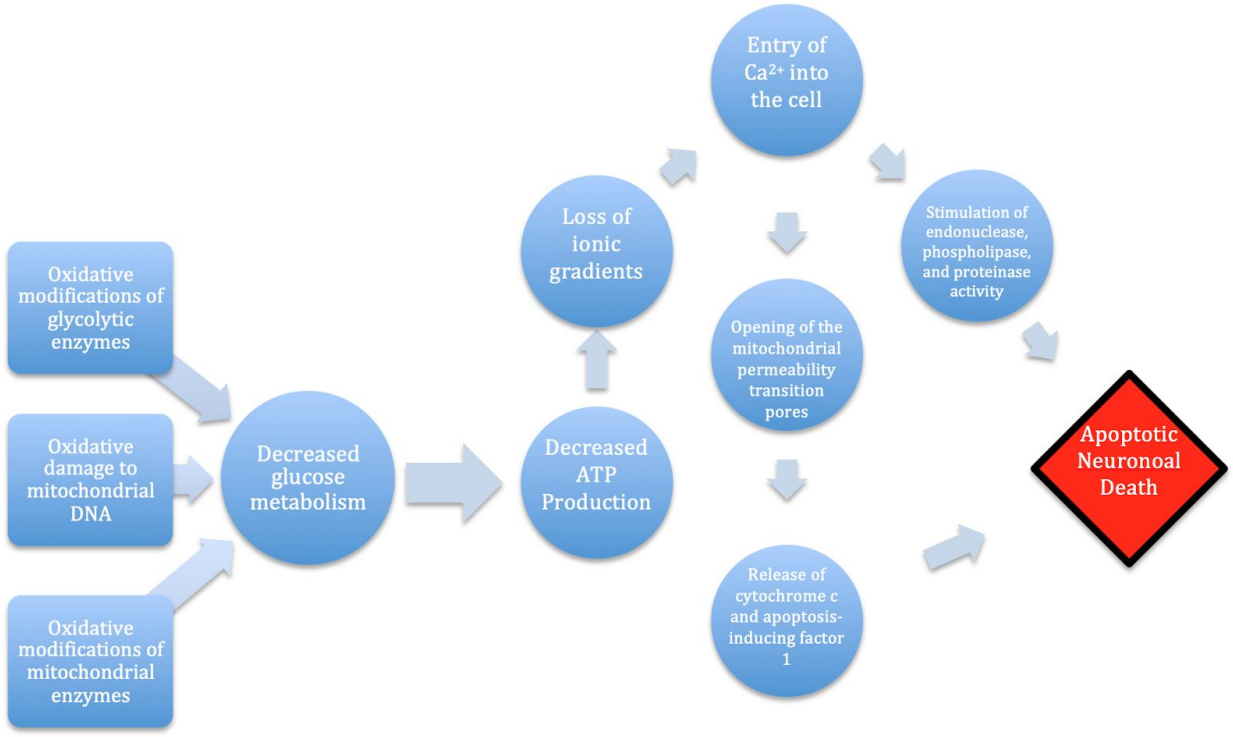

Figure 1: An illustration of how upstream mechanisms cause pathologies in apoptosis in AD (4)

In AD, neuronal loss accumulates over time and results in the seemingly sudden appearance of the clinical and pathological symptoms of AD (5). Such symptoms are those which are common to most forms of dementia: progressive cognitive impairment, memory failure, spatial disorientation, deceptions, and delusions. Coincident with these

symptoms are physiological effects: brain atrophy, plaques in brain matter, and neurofibrillary tangles throughout the cerebral cortex (6).

Given that the entry of Ca^{2+} into the cells is upstream of the two pathways which lead to apoptotic neuronal death (Fig. 1), it stands to reason that inhibition of Ca^{2+} entry into neurons might be an effective therapeutic target to slow and/or reduce the progression of AD. Indeed, recent research findings suggest that hypertensive drugs known as calcium channel blockers, which have been traditionally used to treat cardiac pathologies, may be useful in treating AD (7). More research should be performed on these existing drugs and their potential for drug repositioning, a process in which new uses are developed for existing drugs (8).

1. Busser J, Geldmacher DS & Herrup K (1998) Ectopic cell cycle proteins predict the sites of neuronal cell death in alzheimer's disease brain. Journal of Neuroscience 18(8): 2801.
2. Rohn TT (2010) The role of caspases in alzheimer's disease; potential novel therapeutic opportunities. Apoptosis : An International Journal on Programmed Cell Death 15(11): 1403-1409.
3. Favaloro B, Allocati N, Graziano V, Di Ilio C & Laurenzi V (2012) Role of apoptosis in disease. Aging (Albany NY) 4(5): 330-349.
4. Butterfield DA & Halliwell B (2019) Oxidative stress, dysfunctional glucose metabolism and alzheimer disease. Nature Reviews. Neuroscience 20(3): 148-160.
5. Jazvinšćak Jembrek M, Hof PR & Šimić G (2015) Ceramides in alzheimer's disease: Key mediators of neuronal apoptosis induced by oxidative stress and aβ accumulation. Oxidative Medicine and Cellular Longevity 2015: 346783.
6. The Swedish Council on Technology Assessment in Health Care (2008) Dementia - etiology and epidemiology : Vol. 1, (Statens beredning för medicinsk utvärdering (SBU), Stockholm)
7. Mark A. Lovell, et al (2015) Calcium channel blockers, progression to dementia, and effects on amyloid beta peptide production. Oxidative Medicine and Cellular Longevity 2015: 787805-9.
8. Ashburn TT & Thor KB (2004) Drug repositioning: Identifying and developing new uses for existing drugs. Nature Reviews Drug Discovery 3(8): 673-683.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Question 8 : Dr. Jason Triplett (JTriplett@childrensnational.org)

Q: A critical feature of actin networks is their ability to be organized in different ways, which allows cells to take different shapes in response to extracellular cues. For instance, treatment with epidermal growth factor (EGF) drives fibroblasts to take on a flattened, malleable morphology; whereas treatment vascular endothelial growth factor (VEGF) drives them to a rigid, cylindrical morphology. Describe the actin organizations that could underlie each of these cellular morphologies, including the accessory proteins required to achieve them. Would the same structures be formed if the cells were co-treated with latrunculin? Why or why not?

One key difference between animal and plant cells is the ability of the former to change their shape and move. The machinery that allows for these dynamic changes largely depends on actin, a globular protein that polymerizes into filaments of different organizational structures. (1) These filaments are approximately 7nm in diameter and can be several micrometers in length. Actin filaments within cells are organized into higher-order structures such as bundles and complex actin networks. Actin-binding proteins are critical components of the actin cytoskeleton which are involved in filament and network assembly. (2) Polymerization of actin structures relies on actin assembly-promoting factors including actin nucleators and actin elongation factors. Actin nucleators respond to cellular signals and modulate the timing and location of filament formation. Actin elongation factors control filament growth and regulate the rate of actin subunit addition. Furthermore, myosin motors also contribute to the process by moving filaments past one another or along substrates (3). All told, actin nucleators, elongation factors, myosin motors, and actin monomer-binding proteins work together to create actin morphologies with specialized architectures and functions (4).

Protrusive actin-based structures are especially common at the plasma membrane (5). These structures are composed of actin filaments that are held together by cross-linking proteins (6). Two classes of these higher-order structures have been identified by researchers: those which are sheet-like and those which are finger-like (5). The actin organizations that underlie each will henceforth be considered.

<u>Flattened, malleable morphology</u>
Actin filaments often loosely pack themselves into crisscrossed networks to form flattened, malleable morphologies. These networks are bound together by the longer actin cross-linking proteins such as filamin, spectrin, and dystrophin (6).

An example of a flattened, malleable actin formation is that of the lamellipodia. These are ribbon-like, flat, thin cellular protrusions resembling pseudopods that are formed at

the cell periphery which are rich in actin filaments. They exhibit a dynamic, malleable morphology, undergo cycles of protrusion and retraction, and play a role in guiding cell movement via their sensory role (7-9). Formation of these structures involves Rac, a member of the Rho family of GTPases. More specifically, lamellipodia formation is likely modulated by the actin reorganization functions of the Rac-WAVEs-Arp2/3 signaling pathway. Rac recruits WAVE proteins to the plasma membrane which help induce curvature via actin nucleation of the Arp2/3 complex. Actin branches are formed from this process and their growth generates a dendritic network (5) which has the force to extend the plasma membrane into lamellipodia (8).

Ruffles and phagocytic cups are two additional examples of cellular structures that are made possible by flattened actin networks. Evidence seems to suggest that their organization is similar to that of lamellipodia (5).

Latrunculin A is a toxin which binds actin monomers and sequesters them from polymerization (10). Treatment of cells with Latrunculin A results in the rapid disassembly of actin filaments. In a demonstration of this phenomenon, treating metazoan cells (which use lamellipodia for locomotion) with latrunculin A has been shown to compromise the motility of the cells (11). It stands to reason that other flat, malleable actin networks would have their formation similarly inhibited with the application of Latrunculin A because they would be unable to polymerize their constituent filaments.

Rigid, cylindrical morphology

Rigid, cylindrical morphologies are made possible by the bundling of actin filaments into parallel arrays. These networks are bound together by the shorter actin cross-linking proteins such as fimbrin and α-actinin (6).

One example of a cylindrical actin organization is that of the cable. Cellular actin cables can be used to create protrusive and contractile forces for the establishment of cell polarity and to provide intracellular tracks for polarized particle movement (12). These processes are powered by the polymerization of actin filaments. Formin proteins at the cell cortex generate bundles which are held together by cross-linking proteins and form the cables (13).

Studies have indicated that Latrunculin-A disrupts cellular actin cable structures within several minutes of application, suggesting that continuous polymerization and depolymerization of F-actin is necessary for the formation of actin cables (14).

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

1. Blanchoin L, Boujemaa-Paterski R, Sykes C & Plastino J (2014) Actin dynamics, architecture, and mechanics in cell motility. *Physiological Reviews* 94(1): 235-263.
2. Cooper GM & Hausman RE (2007) The cell, (ASM Press, Washington, DC)
3. Yu JH, Crevenna AH, Bettenbühl M, Freisinger T & Wedlich-Söldner R (2011) Cortical actin dynamics driven by formins and myosin V. Journal of Cell Science 124(Pt 9): 1533-1541.
4. Hyeong-Cheol Yang & Liza A. Pon (2002) Actin cable dynamics in budding yeast. Proceedings of the National Academy of Sciences of the United States of America 99(2): 751-756.
5. Chhabra ES & Higgs HN (2007) The many faces of actin: Matching assembly factors with cellular structures. Nature Cell Biology 9(10): 1110-1121.
6. Lodish H (2013) Molecular cell biology, (Freeman [u.a.], New York, NY)
7. Belvitch, Patrick;dudek & Steven (2018) Membranes in Pulmonary Vascular Disease, (Academic Press, US),
8. Rikitake Y & Takai Y (2011) Directional cell migration regulation by small G proteins, nectin-like molecule-5, and afadin. International Review of Cell and Molecular Biology 287: 97-143.
9. Conn PM (2012) Imaging and spectroscopic analysis of living cells, (Elsevier, Acad. Press, Amsterdam [u.a.])
10. Fujiwara I, Zweifel ME, Courtemanche N & Pollard TD (2018) Latrunculin A accelerates actin filament depolymerization in addition to sequestering actin monomers. Current Biology 28(19): 318-3192.e2.
11. Keller HU (2000) Redundancy of lamellipodia in locomoting walker carcinosarcoma cells. Cell Motility and the Cytoskeleton 46(4): 247-256.
12. Chesarone MA & Goode BL (2008) Actin nucleation and elongation factors: Mechanisms and interplay. Current Opinion in Cell Biology 21(1): 28-37.
13. Drake T, Yusuf E & Vavylonis D (2012) in Advances in Systems Biology, (Springer New York, New York, NY), pp 325-335.
14. Kathryn R. Ayscough, et al (1997) High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. The Journal of Cell Biology 137(2): 399-416.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Question 9 : Dr. Jason Triplett (JTriplett@childrensnational.org)

Q: In an unbiased screen, the lab has identified a novel compound (X) that accelerates the migration rate of fibroblasts, providing a potential way to speed the wound healing process. Preliminary experiments suggest the compound binds tightly to RhoA. Explain how manipulation of RhoA activity might lead to faster cell migration. Another compound (Y) identified in this screen binds with strong affinity to Cdc42 and keeps it in the activated state. How do you predict utilizing compounds X and Y in combination affect migration? How would you test this prediction?

Cell migration is a process in which a cell must extend protrusions, form adhesions on its leading edge, translocate its cell body, release cell adhesions, and retract at the rear of the cell (1). GTPases of the Rho family are involved in these multistep processes; specifically, studies indicate that they are involved in the regulation and coordination of the remodeling of the cytoskeleton which is required for the aforementioned events (2). RhoA in particular is involved in the regulation of actomyosin contractility of the cell body migration (3). Rac is another member of the Rho subfamily and it has been shown that RhoA and Rac are involved in an antagonistic relationship that regulates the speed of cell body migration. In experiments, it has been demonstrated that RhoA phosphorylation induces the release of RAC1 from RhoGDI, “trapping” RhoGDI on RhoA and stimulating the migration and adhesion of vascular smooth muscle cells, increasing would repair (4). Evidently, the sequestering of RhoA and the activation of RAC1 make this speed-up possible. Perhaps compound X plays a similar role in the sequestering of RhoA.

Cdc42 regulates the microtubule-dependent establishment of cell polarity. Together with proteins mPar6 and PKCζ, Cdc42 helps reorient the microtubule organizing center and the Golgi to face the wound edge and help encourage cell elongation. Cdc42 is likely involved in the initial formation of cellular protrusions, whereas Rac is essential for the maintenance of the protrusions during migration.

Because of their theoretically synergistic roles, it is plausible that the phosphorylation of RhoA and activation of Cdc42 via the X and Y compounds, respectively, will accelerate migration rate of fibroblasts and encourage wound healing.

As it stands, the hypothesis thus constructed is that RhoA, Rac1, and Cdc42 activation and/or inactivation are modulated by compounds X and Y. Specifically, the hypothesis is that X and Y behave in an additive and synergistic way to encourage wound healing. To test this hypothesis, an experiment could be conducted in the following manner:

Isolate rat aortic vascular smooth muscle cells by enzymatic dissociation and transfect them to express RhoA, Rac1, and Cdc42 in a wild type, always-activated, or always-inactivated manner. Use these in each of the possible permutations to establish controlled experiments, positive controls, negative controls, and to observe any potential additive effects.

Wound closure could be measured by streaking a pipette tip across a monolayer of each cell culture and image over a period of three hours to assess the wound closure and migration abilities of each cell type.

Tyson Dawson
tyden46@gwmail.gwu.edu
Monday October 21st 2019

Measuring adhesion could be accomplished by incubating the cells on glass slides, washing, and counting the cells that remain adhered.

Then, repeat the wound closure and adhesion experiments with the wildtype cells with the addition of either compound X, Y, or a combination of both and compare the effects with those of the transfected cell cultures. The hypothesis will be validated if cell cultures that have inactivated RhoA, activated Rac1, and activated Cdc42 are associated with migration, adhesion, and wound healing and if the wildtype cells treated with both X and Y display similar migration, adhesion, and wound healing results.

1. Webb DJ, Parsons JT & Horwitz AF (2002) Adhesion assembly, disassembly and turnover in migrating cells – over and over and over again. *Nature Cell Biology* 4(4): E97-E100.
2. Besson A, Gurian-West M, Schmidt A, Hall A & Roberts JM (2004) p27Kip1 modulates cell migration through the regulation of RhoA activation. *Genes & Development* 18(8): 862-876.
3. Pertz O, Hodgson L, Klemke RL & Hahn KM (2006) Spatiotemporal dynamics of RhoA activity in migrating cells. Nature 440(7087): 1069-1072.
4. Hodge RG & Ridley AJ (2016) Regulating rho GTPases and their regulators. Nature Reviews. Molecular Cell Biology 17(8): 496-510.
5. Etienne-Manneville S & Hall A (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. Cell 106(4): 489-498.