January 2009 Issue | Helene Langevin, MD Associate Professor of Neurology

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Welcome to the January 2009 issue of *Functional Medicine Update*. It is the start of a new year, the start of a new age, and the start of a new period of opportunity. Let's start this new age with a very interesting concept, one that I think makes functional medicine stand out clinically in a way that we probably would have never dreamed possible. I'll start with this: Unspecialized loose connective tissue forms an anatomical network throughout the body. We recognize now that connective tissue functions as a body-wide, mechanosensitive signaling network. Three categories of signals are included, including electrical, cellular, and tissue remodeling, each potentially responsive to mechanical forces over different time scales. I am paraphrasing from a paper authored by Dr. Helene Langevin, Department of Neurology, University of Vermont College of Medicine, titled "Connective Tissue: A Body-Wide Signaling Network?"[1]

You are going to have the pleasure of hearing directly from Dr. Langevin about her work in the area of extracellular matrix physiology and looking at the mechanistic and cellular roles that mechanical and electrical stimulation have on whole-body physiology. Before we get to Dr. Langevin and her pioneering work, I'd like to set the context for what-to me-is emerging to be one of the most remarkable stories that gives substance to the concepts of functional medicine, which we have been describing for over twenty years. It is the story that surrounds an aspect of this connective tissue, the collagenous matrix that holds us together. We have thought of connective tissue as being a structural component of the body, but now we recognize it is a composite not only of structure, but also function because it participates in transduction of signals. Signals from the environment are transduced into the interior of cells, to the very heart of the cell where our book of life resides within the library called our genome. These signals then unlock portions of our library, and certain chapters in our book of life are read to create the stories that become the phenotype of those cells and alter function as a consequence. This mechanism of mechanical transduction of signals to the interior of cells to produce different function is now an extraordinary new component of the evolving understanding of what we have really meant by a systems biology approach to medicine that we call functional medicine.

With that in mind, we are going to focus on extracellular matrix physiology and connective tissue in this month's issue of *Functional Medicine Update*. The place I want to start this discussion is with a condition that we all think we know quite a bit about: osteoporosis. I would like to cast the term "osteoporosis" through the lens of the functional medicine matrix to produce a different sense of what is emerging to be understood about osteoporosis, the relative loss of bone over time with increased risk to fracture.

Osteoporosis as Viewed through the Lens of the Functional Medicine Matrix

Let's start with the presumption that osteoporosis is partly related to things like calcium nutriture, vitamin D status, vitamin K status, and the relationship to estrogens and other hormones. We know these factors play a role in maintenance of bone integrity, but we also recognize that bone loss, leading to decreased bone mineral reserve and reduced integrity of the bone mineral matrix, can occur as a consequence of inflammatory conditions. Let's consider post-menopausal osteoporosis. Could it be more than just the loss of estrogen that then induces some kind of an anabolic change in the bone remodeling unit? Could it be considered, in part, an autoimmune disease? Why do I bring that up? I bring that up because it is now being recognized that there is a role of T-cells and the relationship of their secreted inflammatory mediator, tumor necrosis factor alpha (TNF-alpha), along with other cytokines, in the pathogenesis of bone loss that occurs in systemic inflammatory diseases. The relevance of these T-cell activities in bone loss due to estrogen deficiency has been investigated now by a number of different groups that have shown in recent years that the increased presence of TNF-alpha-producing T-cells is essential for the changes in bone metabolism during estrogen deficiency.[2] The lack of estrogen increases the secretion of interferon gamma by helper T-cells, through which complex class 2-related expression of major histo-compatibility class 2 antigens then enhances the activation and proliferation of the TNF-alpha-producing T-cells. Is osteoporosis an autoimmune disease, or is it a problem related to an isolated difficulty in the bone itself? In other words, does it have a systemic connection, or is it a localized problem that is seen only in bone?

Those are very interesting questions that move us beyond looking at individual organ parts (in this case the skeleton) in isolation from the rest of the body, to looking at the body (the skeleton) in the context of what we call systems biology (from a functional approach). In order to better understand the connection of estrogen and mechanical stress or exercise (weight-bearing exercise, as it is often said, or resistance exercise), or nutrition of calcium, or magnesium, or phosphorus, or vitamin D on bone metabolism, we have to know something about the cell biology of bone metabolism itself, and that's really advancing dramatically in terms of our understanding of how the bone remodeling unit works at the cellular and even molecular level.

Let's dig a little deeper into this question of the cellular biology of bone metabolism. There is a wonderful review that was just published in 2008 in the *Journal of Clinical Pathology* titled "The Cell Biology of Bone Metabolism."[3] Bone (in the macro look) is a specialized connective tissue that is hardened by mineralization with calcium phosphate in the form of hydroxyapatite. We know that it has rigidity and shape and it protects and supports the body, but it's more than that. Contrary to popular belief, bone is a highly dynamic structure undergoing constant remodeling throughout the whole of our life, and, in fact, is an integral part of the neuroendocrineimmune system. Now, you heard what I just said. Let me say it again: bone is an integral part of the neuroendocrineimmune system. That's a different perspective on looking at skeletal health and integrity. We'll come back to talk much more about why I think that position is now justified in light of what we've learned about bone metabolism and the cellular biology of bone.

Regulation of Bone Status: Osteoclastic and Osteoblastic Activity

To make a simple story out of a much more complex topic, bone status is regulated, in part, by the activity of two different cell types: the osteoblast, which produces new bone, and the osteoclast, which is involved with bone resorption. In youth we have osteoblastic activity exceeding that of osteoclastic activity, and we have net skeletal growth. In mid-life, we'd like to think that our bone resorption equals

that of our bone reformation and so we have a long period of skeletal homeostasis under constant remodeling. It's a little bit like the hummingbird flapping its wings. It looks as if the bone is staying in the same state, but actually if we were to look at it with time-lapsed photography, what we would see is remodeling of the bone occurring all the time with new bone replacing old and maintaining that skeletal equilibrium. And then in older age we know osteolysis exceeds that of osteogenesis and we start to get skeletal loss. That can occur, however, even at younger age with specific types of disorders that are associated with osteoclastogenesis, where you actually see the osteoclastic activity exceeding the osteoblastic activity, so we have net skeletal resorption.

What causes this to occur, be it either in the alveolar bone of the jaw (or the mandible), or the trabecular bone of the spine, or the cancellus bone of the wrist? What is it that results in this dynamic equilibrium and the shift between osteoclastic and osteoblastic activity? I think that is really the theme of this new emerging understanding of the cell biology of bone.

If we look at the ultimate activity at the bone remodeling unit at the osteoclast and at the osteoblastic cell, what we'll find is there are many, many different signal transduction agents that are involved in signaling to the book of life-the genome-within those cell types, what they are going to express in terms of proteins that then regulate their function. This intercellular signal transduction process within the bone remodeling unit is regulated by exposure to environmental factors, as well as the genetics of that individual. And that signal transduction process is emerging to be much more complex than we ever would have believed, in that it is influenced by, and it also influences, activities within the nervous, immune, and endocrine systems. Factors that would be considered hormones, or inflammatory mediators, or even mechanical signals can all influence the regulatory signal transduction within these cell types that make up the bone remodeling unit. Those activities of the bone remodeling unit cells secrete (into the plasma) substances that have influence downstream on other tissues and influence the neuroendocrineimmune system in other places of the body, so it is a feedback process. Once again, it is a system of biological function. Bone doesn't work in isolation.

Signal Transduction Agents Influencing the Phenotype of Bone

Let's look a little bit more in detail at these signal transduction agents that regulate the expression of genes that ultimately control the phenotype of bone and may have influence on other functions of the body. One of the principal processes in the signal transduction process related to skeleton and bone formation is the so-called Wnt/beta-catenin system that connects with bone morphogenic protein pathways and modulates key transcription factors within the regulatory units of the genome of these bone cells. The Wnt/beta-catenin signal transduction process becomes a very important part of the process that regulates, then, osteoblastic activity and its interrelationship with osteoclastic activity.

I'm going to talk about this in more detail and I want to make this fairly complex story as easy to understand as possible. I will ask for a little poetic license, here, in that there are very significant levels of detail below what I am going to be speaking about, but I'm going to take kind of a higher level view. What I am really saying is that the nuclear regulatory factors that control the expression of genes within the osteoblastic and osteoclastic cells are influenced by environmental factors that then signal through the Wnt/beta-catenin signaling pathway. Therefore, if we wanted to understand something about ultimately what is seen clinically as a bone demineralizing situation, we'd want to know something about its upstream regulatory effects on these signal transduction processes. We're going to be focusing both on the Wnt/beta-catenin pathway and we're also going to be looking into the osteoclastic cell (the bone

resorption unit) at another interesting signal transduction pathway, the NF kappa B pathway. On the surface of the osteoclastic cell resides receptor sites that then signals into the osteoclastic cell, down through a signal transduction process that ultimately causes either NF kappa B to translocate to the nucleus and upregulate, in the osteoclastic cell, the production of factors that lead to bone resorption, or RANK-RANKL to lead to osteoclastogenesis, where you actually increase the activity and number of the osteoclastic cells at the expense of lowering the activity of the osteoblastic cells.

The Wnt/beta-catenin signaling pathway regulates, to some extent (or to a major extent), the osteoblastogenesis, which means increasing bone reformation, whereas the RANK-RANKL signal transduction pathway activates osteoclastogenesis, which primarily regulates bone resorption. I hope this is becoming at least somewhat understandable now. Environmental factors that activate the RANK-RANKL system are involved with bone loss, whereas those that activate the Wnt/beta-catenin system are more related to bone reformation.

The RANKL system (the Receptor of NF kappa B ligand) is an inflammatory mediated pathway, down through NF kappa B, translocation to the nucleus of the osteoclast and upregulating the expression of genes that then regulate osteoclastogenesis and bone resorption. The Wnt pathway is a pathway that is regulated by many environmental factors that I'm going to go into and discuss, including weight-bearing exercise and mechanical stimulus, including various hormones like vitamin D in its hormonal form (1,25-dihydroxyvitamin D3) and estrogen, and including a variety of other signal transduction agents that may be nutritionally derived, for which we have only recently started to recognize play a role in bone maintenance (in fact, they would not be on the short list of most people who think of various nutritive factors that are associated with bone regulation). These other agents are phytochemicals that might favorably influence the Wnt/beta-catenin signaling pathway in osteoblastogenesis.

It is further important to understand that the osteoblast and osteoclast equilibrium is just that: an equilibrium. You can shift the teeter-totter toward more osteoclasts and higher activity resorption by activating the RANK-RANKL system. You can also shift the teeter-totter the other way, toward more osteoblastic formation and more bone formation by activating the Wnt/beta-catenin system. This is a regulatory intercellular signal transduction pathway that then is influenced and communicates with the outside world and environmental factors, both endogenous substances like hormones and exogenous substances, activities, or functions, including things like mechanical transduction or energy medicine.

With all of that as kind of a summary background, let's now talk about what this skeletal remodeling unit that is composed of the osteoblasts and osteoclasts does, both locally and systemically. Locally it is going to regulate the structure and function of both cancellous and trabecular bone. The formation of this spongy bone is porous and forms a very high tensile-strength structure that is actually very lightweight compared to its strength. This architecture of the bone is regulated not just by calcification with hydroxyapatite, but by the bone mineral matrix itself, as supported by the protein that is the structural protein (the connective tissue protein) that regulates the ultimate structure and function and strength of the bone. This is the local effect of this process on bone strength and function.

Endocrine Regulation, Energy Metabolism, and the Skeleton

Beyond that, however, it has been recognized now that healthy bone or unhealthy bone is secreting substances that go into systemic circulation as a consequence of the bone's response to its environment, and that then influences tissues at a distance. What am I really speaking about? If you recall, last year

in *Functional Medicine Update* I talked about a remarkable paper that appeared in the journal *Cell* in the August 2007 issue, titled "Endocrine Regulation of Energy Metabolism by the Skeleton."[4] This was work that came out of the department of pathology and genetics at Columbia University College of Physicians and Surgeons looking at the role that various factors secreted by bone could have on things like the endocrine pancreas beta cells, the Islets of Langerhaus cells that secrete insulin, or the adipocyte cell that secretes adiponectin. What was found was that a substance called osteocalcin that was secreted by the osteoblastic cell (in an uncarboxylated form), upon stimulation by the Wnt/beta-catenin intercellular signal transduction process, had positive impacts on the adipocytes secreting adiponectin, which is, as you know, anti-inflammatory and insulin-sensitizing. It also had positive effects on the beta cells of the endocrine pancreas in secreting more insulin. In fact, it leads to proliferation of the beta cells. That leads to what? Improved insulin stability, improved glucoregulation, improved appetite regulation, and improved weight.

People started saying, "Just a minute. Are you saying the skeleton has a role to play in energy regulation of the whole body and has an endocrine-like effect by regulating influences on beta cell activity within the pancreas, and adipocytokine production that then influences things like appetite?" The answer is yes. That is what has been seen. In fact, it is now recognized that the signal transduction process that controls the secretion of osteocalcin by the osteoblast is regulated by a gene called esp, and that gene, then, ultimately is controlled by various types of environmental factors. If you have esp gene activities either up- or downregulated, it then has an influence on whether osteocalcin will be produced more or less by the osteoblasts, which then has an effect on either improving or decreasing insulin production and sensitivity, and increasing or decreasing adiponectin production by the adipocyte.

These are really profound, new thoughts about systems biology in medicine, and we want to talk about structure and function. We've moved now to an understanding of cellular physiology that has a whole-body context. We've moved beyond seeing the bone as a structural unit sitting in isolation, to seeing bone as an active contributing member to the overall neuroendocrineimmune system, regulating far-ranging functions such as appetite, insulin sensitivity, glucose, and even anti-inflammatory proteins such as adiponectin. By the way, a very nice review of this whole concept of energy regulation by the skeleton authored by George Wolf from Berkeley appeared in *Nutrition Reviews* in 2008 that I think you might find very helpful if you want to read more about this topic.[5]

It seems like a different view of how the skeleton is part of an overall systems biology relationship is starting to emerge. What we might see as unhealthy bone (bone that means we have increased osteoclastogenesis with increased bone resorption) is reflective not only of increased risk to osteoporotic fracture, but also of maybe systemic problems related to difficulties with regard to insulin sensitivity, to difficulties related to appetite regulation and adipocytokine production, and to difficulties related to inflammatory conditions. It is a push-pull: inflammation increases osteoclastogenesis, and increased osteoclastogenesis then has a feed-forward effect on supporting increased inflammation, so we are into a chronic cycle of inflammatory disorder.

To follow up on this and to show you that extraordinary work is continuing in this area, I want to point out there was a very interesting additional paper published in the August 2008 issue of *Nature*. This came from the Section on Obesity and Hormone Action at the Joslin Diabetes Center at Harvard, and this was titled "New Role of Bone Morphogenic Protein 7 (BMP7) in Brown Adipogenesis and Energy Expenditure."[6]

You'll remember I talked about two signal transduction pathways playing very important roles in osteoblastogenesis. One was that of the Wnt/beta-catenin pathway, and the other was this bone morphogenic protein pathway and its relationship with transforming beta. This whole process of osteoclastogenesis versus osteoblastogenesis is regulated by the way these signals are transduced at the cellular level.

What does this paper in *Nature* tell us? It follows on in the energy-regulation-by-the-skeleton theme. As we all know, adipose tissue (or the adipocyte mass) is central to the regulation of energy balance. It is what fat supposedly is there to do, to kind of store energy for a rainy day in the form of triglycerides. Two functionally different types of fat are present, we know. One is called white adipose tissue, which is the primary site where fats as triglycerides are stored, and also we have the brown adipose tissue, which are more metabolically active forms of fat cells that are specialized in energy expenditure related to thermogenesis and may counteract obesity.

Factors that specify the development and fate of the function of white and brown adipose tissue still remain poorly understood, however, it is now recognized that the family of bone morphogenic proteins support white adipocyte differentiation and that bone morphogenic protein 7 singularly promotes differentiation of brown pre-adipocytes, even in the absence of other hormonal inductions. Bone morphogenic protein 7 coming from bone activates a full program of brown adipogenesis, including induction of early regulators of brown fat, increasing the thermogenic activity and influencing energy economy of the body, so the skeleton can regulate energy expenditure.

Healthy bone is healthy body. A healthy body is a system of biology that produces high function. It's impossible in light of what's emerging in the basic clinical sciences to look at disease as organ-specific in isolation. We have to start looking at things as networked, as interactions, and as systems where we get these interactive components that then set up new cycles of harmonics, and those harmonics become steady states that we call (for the lack of a better understanding) a disease

Let me go back now to where we started this discussion: intercellular signal transduction. For those of you who are not cellular biologists or molecular biologists, let me try to take another run at this, just so we all are on the same page. When I say signal transduction, what I'm speaking to are processes within a cell that take outside information in the form of agents that signal to the cell. These could be mechanical signals, electrical signals, or chemical signals. They are picked up by receptors sites or signal transduction proteins that are generally involved with a family of proteins that we call kinases, which are phosphorylating proteins. The kinases modulate the signals like a relay race: runners passing the baton from one to another from repetitive phosphorylation cascades. A signal gets transduced from outside the cell, then, to the inside of the cell through the cytoplasm, ultimately through the nuclear envelope into the nucleus where the genome resides, and even into the mitochondria (the energy powerhouse of the cell). These transduction processes that are mediated or modulated through these kinase enzyme signaling relay racers (I guess we'd call them) result in altered structure and function of the cell.

Wnt and beta-catenin constitute an important signaling transduction pathway to regulate (as nuclear regulatory factors) the expression within the nucleus on the cassette of genes that regulate osteoblastic cell function, a specific constellation of proteins that then cause bone reformation. The Wnt genes encode a highly conserved class of signaling factors required for the development of musculoskeletal and neural structures. There is increasing evidence that Wnt signaling is critical for bone mass accrual, bone

remodeling, and fracture repair. Wnt proteins bind to cell surface receptors and activate signaling pathways which control the nuclear gene expression, and this Wnt-regulated gene expression controls cell growth and differentiation.

With all of that in mind we might ask, "How can you alter Wnt function and what role does Wnt play in this process?" Wnt is going to stabilize (once it is produced this pathway is activated) a process within the osteoblastic cell that is going to engage osteoblastogenesis (increased osteoblastic activity) and increased bone formation activity, so we're going to actually see a positive influence of Wnt stabilizing the target genes (or activating the target genes) involved with osteoblastogenesis.[7] What this means is if there is no Wnt activity (or there is inhibited Wnt activity) the result would be reduced osteoblastogenesis, where activated Wnt would lead to increased osteoblastogenesis. So you might ask, "Are there people who are born with increased Wnt signaling pathways and what influence does that have on their bone?" The answer is yes, there are genetically unique individuals, and they have very dense bones. What about people on the opposite, who have low Wnt signaling pathways as a consequence of their genetics? Those people also have been identified and are associated with early-onset, very serious bone demineralization issues-things like osteoporosis pseudoglioma. These genetic outliers do exist to confirm the importance of Wnt pathway. The Wnt pathway regulates, at the cellular level, the expression into the phenotype of these particular aspects of osteoblastogenesis and how that then interrelates with osteoclastogenesis.

Serotonin has Stabilizing and Destabilizing Effects on Signaling Pathways

With all of that in mind, now the question is: what regulates the Wnt pathway? Are there things upstream that we should consider? Here is where the story gets unbelievably interesting. After publication of a paper that appeared in *Cell* in November of 2008, this has to be considered one of those "I can't believe it" kind of responses by the scientific community. It turns out that within this complex signaling pathway, one of the things that stabilizes Wnt or destabilizes Wnt signaling is a hormone that we are very familiar with that has to do with mood; that hormone is called serotonin. Serotonin, we know, is synthesized within the central nervous system; it's a neurotransmitter. The principal place where serotonin is synthesized in the body happens to be in the enterochromaffin cells in the small intestine, in the duodenum. In fact, almost two-thirds of the body's serotonin comes from the gut. We learned this years ago from Dr. Michael Gershon in his classic book *The Second Brain*, which talked about GI hormonal function. Serotonin synthesis in the gut contributes significantly to the level of serotonin in the blood. In fact, the majority of blood serotonin doesn't emerge from the central nervous system; it emerges from the gut.

Gut serotonin does not cross the blood-brain barrier, so it doesn't lead to mood elevation, but it does have impacts on cellular function beyond that of mood. This is where the story gets extraordinarily interesting, because in the November 28th, 2008 issue of *Cell*, an article was published (again out of Columbia University-collaboration amongst researchers in the departments of genetics, neuroscience, psychiatry, and pharmacology)-that was titled "Lrp5 Controls Bone Formation by Inhibiting Serotonin Synthesis in the Duodenum."[8] What is that all about? This is a very, very extraordinary part of the story. Let me, if I can, show you how it connects to our Wnt/beta-catenin regulation of osteoblastogenesis.

Loss and gain of function mutations in lipoprotein receptor 5 (Lrp5), a broadly expressed gene, have been known for some time to affect bone formation. There is a wide body of literature from molecular genetics and animal biology to demonstrate that it causes osteoporosis and the loss of function, or it can result in high bone mass and the gain function. Although Lrp5 has been viewed as a Wnt coreceptor, an osteoblast-

specific disruption of the beta-catenin does not affect bone formation. In this paper, however-the one I'm just describing in *Cell*-the authors demonstrated that Lrp5 inhibits the expression of another interesting gene: Tph1.

Now what does Tph1 do? Tph1 controls the biosynthetic enzyme tryptophan hydroxylase that then results in the formation of serotonin in the enterochromaffin cells in the duodenum. What am I really saying to you? I'm saying that when you have elevated serotonin production at the gut level, you have increased bone loss by activating osteoclastogenesis and reducing osteoblastogenesis because you block the Wnt/beta-catenin signaling process. Serotonin produced by the gut has a very dramatic effect, then, on increasing the potential for bone loss (that's serotonin production in the enterochromaffin cells within the small intestine).

The editorial that follows this article points out that "gut talks to bone."[9] Does this sound at all familiar, relative to the functional medicine and systems biology discussions we've been having over the last many years? In this particular editorial, the author talks about these mutations in the Lrp5-this co-receptor of Wnt protein-that then can result (through the serotonergic signaling pathways) in reducing bone reformation).

What is it that activates serotonin production through this process of the Lrp5? That's where we get to a very interesting connection to clinical medicine. Also in 2008, in *Neurogastroenterology and Motility*, another journal, it was reported that inflammatory signals associated with IL1-beta and lipopolysaccharide-induced serotonin secretion was shown in the enterochromaffin cells derived from patients with Crohn's disease.[10] When we get into chronic inflammatory bowel diseases, or conditions where the immune system of the gut is upregulated, we get increased production of inflammatory mediators that activate this regulatory pathway that is associated, then, with what? With suppression of Wnt and osteoblastogenesis, with increased systemic serotonin production, and increased risk to bone loss. Here we have a condition of gut inflammation connected to bone loss, which is then connected to unhealthy bone, which is connected to lowered osteocalcin, lowered adiponectin, lowered insulin sensitivity, lowered insulin output, and increased obesity and type 2 diabetes.

These are very profound and new types of network thinking. Let's couple this together with a paper I described to you last year titled "A High Fat Meal Induces Low-Grade Endotoxemia: Evidence of a Novel Mechanism of Post-Prandial Inflammation."[11] This paper appeared in the *American Journal of Clinical Nutrition* in 2007. The authors pointed out that if you take apparently healthy people and feed them a high-fat/high-sugar meal, and then you measure bacterial endotoxin in their plasma, you will find it circulating after the meal, indicating gut permeability and an upregulation of their inflammatory pathways at the gut-immune level. You can actually measure the increase in the inflammatory cytokines that occur after giving a person a high-fat/high-sugar meal.

What happens if people constantly activate the inflammatory pathway of their gut, increasing gut enterochromaffin cell production of serotonin, and bathe the bone remodeling unit with increased serotonin that influences the Wnt signaling pathway and shifts the balance of osteoblastogenesis and osteoclastogenesis toward osteoclastic activity and bone loss? Lowered osteocalcin production, increased inflammatory potential, lowered adiponectin, increased regulatory storage, blunting of thermogenic effects on the brown fat, and now we shift that whole-body archetype systemically into a person with

central fat deposition, insulin resistance, metabolic syndrome, high triglycerides, cardiovascular risks, and so forth and so on. The treatment of choice in medicine has generally been to treat each of those outcome variables independently, as if they were isolated, rather than to look at the web of interacting variables. We can say it another way: is there not a co-morbidity between gut inflammatory conditions, autoimmune disease, osteoporosis, cardiovascular disease, and type 2 diabetes? That's a new way of looking at a systemic problem with a functional medicine lens.

With that in mind, let's move to what you've been looking forward to-an extraordinary visit with Dr. Helene Langevin.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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Once again we are at that place in Functional Medicine Update that I know you, like I, look forward to with great anticipation: our clinician/researcher of the month. This month we are fortunate to have a person I think we could call both clinician and researcher. I have looked forward to this interview for probably the better part of three years, as I have been reading her work and becoming more and more fascinated with the way she and her group are approaching this very complicated area; the work is very high level.

I have probably peaked your interest. I'm speaking to Dr. Helene Langevin, who is associate professor of neurology at the University of Vermont College of Medicine and has a very remarkable background. She got her MD at McGill University, did neuroanatomy and neurochemistry at Cambridge, went on to a residency in internal medicine at Johns Hopkins, and then was an Endocrinology and Metabolism Fellow at Johns Hopkins. That's no small background in academic medicine and clinical medicine.

Since then, Dr. Langevin has been very actively involved in a research area that I think might lead many "traditionally trained" physicians to think, "Wow, that's something I never thought about." Her area of interest is the extracellular matrix and connective tissue, and how that interfaces with various therapies that are mechanical and mechanical/electric, like acupuncture. These are really very interesting questions that have sat for thousands of years in the Traditional Chinese Medicine literature, but maybe have suffered for lacking some of the Western intervention or mechanistic concepts. This is really where Dr. Langevin has made some extraordinary and very precise measurements with her group.

With great pleasure and privilege, Dr. Langevin, I'd like to introduce you to our listenership at Functional Medicine Update and thank you so much for spending some time with us.

HL: Thanks for having me. It's a great pleasure.

JB:Let's jump right in. With the background I just described, which is formidable, one might ask, "How did you move from that background to the perspective of wanting to look at this whole area of connective tissue, the extracellular matrix, and (I guess you'd call it) the systems biology that connects the nervous system and the immune system to the extracellular matrix-how did that happen?"

Studying the Interface between Needle and Tissue in Acupuncture
HL: Well, it was kind of an interesting path for me. I was practicing medicine (internal medicine and endocrinology), and I had a lot of patients who had chronic pain. I was finding myself quite powerless to do a whole lot about it. A lot of people were asking me about whether they should be having alternative types of treatments, like acupuncture. This was in the 1980s, and there were already quite a surprising number of people who were interested in that. I met some acupuncturists and I became interested in it, and I decided to study acupuncture. I went to acupuncture school part-time, and I was intrigued by the whole completely alternative approach to looking at a patient, looking at the problem. It seemed to me that they were looking at the human being from a completely different point of view. I thought that was very interesting, so I started practicing acupuncture.

One of the things that really intrigued me when I was an acupuncture student was that they would teach you how to manipulate the needles, and then they would say that you had to feel what was happening at the interface between the needle and the tissue. They would say you have to wait until something happens and then you know that you have manipulated the needle enough and you stop. I could really feel something happening under my fingers. The tissue seemed to be tightening. Something was changing--mechanically--the interface between the needle and the tissue. When I asked my teachers-my acupuncture instructors-what was this due to, they would say it was probably muscle contracting. That didn't make sense because there were some acupuncture points on the body where I was inserting needles and there was no muscle there at all. The idea kind of trotted around in my mind that if this phenomenon was truly some kind of tissue contraction that was not due to a muscle, then what could it be? The skin is not supposed to contract, and the connective tissues underneath the skin are not supposed to contract either.

I then moved to Burlington, Vermont and one thing led to another. I sort of finally decided that I needed to really investigate it and I got a research grant to simply try to measure the force that it takes to pull the needle out. That was our first study and we found that there was indeed something going on. After you manipulated a needle, it took more force to pull the needle out. At that point I had no idea what was causing this. We did some studies in animals that showed that it was, in fact, the connective tissue that was winding around the needle and changing the mechanical interaction between the needle and tissue, and then I really got interested in connective tissue because we also kind of looked at the acupuncture points and found that there was a lot of connective tissue there. A lot of these acupuncture points and meridians are located in between muscles in sort of intermuscular fascial planes. It seemed like maybe this acupuncture map that people were drawing thousands of years ago was maybe an indication of where to put the needle if you wanted to your needle to interact with connective tissue. And then I got completely fascinated with connective tissue, and now I am really studying connective tissue-many aspects of it-way beyond acupuncture, at this point, but acupuncture was kind of what got me into that. So that's the story.

JB:It's just an incredible story. You know, I'm always reminded when I have the privilege of speaking to people like you (seekers), that there is some unique characteristic that defines people like yourself-people

who make observations and are willing and courageous enough to follow the process of inquiry to the next level, against what sometimes might be considered barriers that hold other people back. Many people ask these questions, but then they just say, "Oh well, no one seems to be interested in these questions so I'll just move on." Somehow people like you stop and use their resources, their experience, and their wisdom to really dig deep and find things that are unexpected discoveries, and, of course, you've done that.

The first two papers I read of yours' were the paper that was in FASEB journal back in-I think it was-2001 on "Mechanical Signaling through Connective Tissue, the Mechanism for the Therapeutic Effect of Acupuncture," and the other was, I think, a paper in 2002 in the Anatomical Record on the "Relationship of Acupuncture Points and Meridians to Connective Tissue Planes.12,13 When I read those papers, something just dawned on me. You helped guide me to this kind of "a-ha-ism" that maybe the primacy of the cell that we use within biological thought that ultimately directs and focuses its energy into medicine is really not the right fundamental unit. Maybe we ought to be looking at this extracellular matrix as a fundamental unit of organization. I think you have opened a lot of eyes through your work.

HL:I need to sort of specify one thing that's important, I think, for listeners to realize. We think we identified many important things, physiologically: that connective tissue is mechanically responsive, including to acupuncture needle manipulation; and that fibroblasts within the tissue (the extracellular matrix) constantly remodel and they respond to the mechanical stimulus. However, at this point, we still don't know the cause of the mechanical effect of the needle on the connective tissue. What does it have to do with the therapeutic effect? I think it's important to realize that that is still a work in progress. That first paper that you mentioned in FASEB was kind of the original paper where we laid out the hypothesis that, in fact, there could be mechanical signals that could be transmitted by the needle to the tissue via cellular mechanisms. People call this mechanotransduction, which is basically the transformation of a mechanical signal into a biochemical response or cellular response. I'm still very much in the dark about how this could translate into a therapeutic response. I think it is very important for people to realize that.

JB:Thank you. I think that's a really good caveat to put in. Maybe you can tell us a little bit--in summary-about the techniques that you've been using to dig down into this very complex area and try to tease out some of these answers because your experimental methods, I think, are very, very novel and…it's not easy work, it looks like to me.

A Stepwise Approach to Studying Connective Tissue and the Extracellular Matrix
HL: Yes, we have to really go stepwise, and I think right now we have kind of, I would say, a three-pronged approach to this. The first thing is we have identified these really very interesting responses of connective tissue to the needle. That almost has become a cell biology project. As a matter of fact, one of the grants that I have right now is to study the effect of mechanical stimulation on the fibroblasts, and we're just looking at cell biology, almost purely. What are the fibroblasts doing? What are the mechanisms by which they are responding to the mechanical stimulation? What are the responses in terms of cytoskeletal remodeling, gene expression, protein expression, and changes in connective tissue physiology? That is just stuff that is basic research. We're just trying to understand: how does the connective tissue interact and respond to the mechanical forces?

At the other end of the spectrum, we are looking at chronic low back pain, because the idea is, "Well, okay, if the needle is doing something to the tissue that is going to be translated into a therapeutic

response, what is it that that would be fixing? What is the problem that this mechanical stimulus would help correct?" The important thing right now (the reality) is we don't really know very much about what causes chronic musculoskeletal pain. We are still very much in the dark about the pathophysiology of the problem. A lot of people have musculoskeletal pain and people have looked into the nervous system in terms of hypersensitization, psychological responses to pain, but the tissues themselves…we don't really know what is it in the tissues that could be contributing to the pain, and in particular, connective tissue has been looked at very little.

We have a study that is ongoing right now where we are looking at a group of subjects with chronic low back pain with ultrasound. We are looking at the structure of the connective tissue to see if it's abnormal. People who have chronic low back pain…they have pain and say they have an initial injury, they will change how they move, or they will move less, or they will change their movement patterns because it hurts to move. Because connective tissue is so exquisitely responsive to mechanical stimulation-it remodels, in good ways and bad ways-if we stop moving or if we change the way we move, the connective tissue changes. For example, if you have an arm in a cast or a shoulder injury and you stop moving your shoulder you can get a build up of connective tissue such that you lose your range of motion. We are hypothesizing that the same thing occurs in the back-that in low back pain, the connective tissue essentially fibroses, or becomes thicker, and that impairs movement and further contributes to the chronicity of the pain.

We are also looking at how tissue, if it is abnormal, responds to the acupuncture needle mechanical stimulation. Are the responses different in people with low back pain versus people without low back pain? To link these two projects, we have an animal model where we actually induce connective tissue abnormalities under control conditions using a combination of inflammation and movement restriction, and then we see how we can impact that with mechanical stimulation.

JB: I was reading the very interesting paper that you co-authored with Karen Sherman on "The Pathophysiological Model for Chronic Low Back Pain: Integrating Connective Tissue and Nervous System Mechanisms."14 This appeared in Medical Hypotheses last year, and I was kind of struck-and I'm sure you've been asked this question before-that not only does it seem like this argument (or story) connects to acupuncture, but to other manipulative (or physical) forms of medicine like deep-tissue therapy, Rolfing, and other things that may send signals through the fascia that implicate remodeling.

Studying Mechanisms that Apply to All Manual Therapies
HL: Yes, absolutely. I think that's very important. The mechanisms we're studying are not restricted to acupuncture or to manual therapies. I think they might be common to a lot of manual therapies-types of treatment even beyond that, even movement therapies, like yoga, for example. Anything that stretches the tissue may have an effect on the connective tissue. The acupuncture needle is an interesting way to mechanically stimulate the tissue because it is focused. The needle, first of all, can penetrate quite deep. When an acupuncturist manipulates a needle, they can impart, actually, some very high forces, but in a very, very focused manner to the connective tissue because the collagen fibers are actually winding around the needle and then the needle becomes mechanically coupled to the tissue. With everything you do-when the acupuncture needle is manipulated up and down or sideways-it transmits a mechanical signal that can affect the deep connective tissue layers, very, very precisely.

On the other hand, manual therapies, where you are applying your hands, or fingers to the tissue, applying

a combination of pressure and stretch, there are some differences, obviously, as to how these mechanical forces are applied to the body, but they still have something in common. And then, of course, movement-based therapies, like yoga, or Feldenkrais or Alexander technique, or techniques that show people how to move differently also apply mechanical forces to the tissue, although this time it is the person, themselves, doing it. We think some of the mechanisms we are studying in terms of connective tissue responses could apply to all of these.

JB:You know, we want to talk about a model of integrative science. You authored a paper (as the lead author, I think) along with a variety of your colleagues, and it included not only your work at the department of neurology, but the department of mechanical engineering and the department of pharmacology, all at the University of Vermont's College of Medicine. This paper was published in the American Journal of Physiology and Cellular Physiology in 2005 and was titled "Dynamic Fibroblast Cytoskeletal Response to Subcutaneous Tissue Stretch Ex Vivo and In Vivo."15 This is a very interesting model for a different kind of research than we have seen in the past.

HL:Yes. We think it is important for several reasons. First of all, from the point of view of cell biology, I would say the overwhelming majority of the work on mechanotransduction in cell biology is being done in cultured cells. These are cells that are fibroblasts or other types of cells that are grown on plastic dishes or collagen matrices-artificial environments. There are a lot of very interesting signaling pathways that are known to be activated by mechanical stimulation in cells, gene expression, all sorts of stuff that we know that cells do when they are mechanically stimulated.

We can look at fibroblasts in their own tissue environment in an ex vivo model, which is where we take a piece of connective tissue out of the body (we use a mouse model), but it's the whole tissue with the fibroblasts in it. They are excised from the animal and they are kept in an organ bath, sometimes for as much as a couple of days, sometimes for just a few minutes or a few hours, but they are still in their environment, and we look at how the fibroblasts respond to mechanical stimulation. We also look at them when the mechanical stimulation is applied in a live animal, then we sacrifice the animal and look at the cellular response (the mechanical stimulus is applied when the animal is still alive). We had a lot of surprises, there, when we compared how our fibroblasts were responding, and how they even looked. The morphological appearance of the fibroblasts looked quite different from the fibroblasts in the dish.

We think that when you are studying the effect of mechanical stimulation, it is really important that the mechanical environment of the fibroblast or the cell is maintained because the fibroblasts growing in the dish are not necessarily going to respond the same way as if they were in the real tissue. We think, from a cell biology point of view, these are important models. And, of course, from a translational research point of view, we think they are important because they will allow us to understand the effect of the dose of the mechanical stimulation.
Obviously, it is really important how much and how long, for example, tissue is stretched. Physical therapists know this in their practices. If you have somebody who has, for example, a contracture, or connective tissue adhesions following an injury, a scar or something like that, and you stretch the tissue just enough, that can cause the connective tissue to remodel and the range of motion to improve, but if you stretch too much, you can actually injure the tissue more, and cause inflammation, and the person has more pain and that can worsen the problem. We don't really understand the mechanisms by which all this happens, and we don't know the dose-these are all things that people do by experience, empirically. Each practitioner knows what enough is and what is too much. But there is quite a lot of controversy. Sports

medicine, for example, is very interesting now. Everybody used to stretch before sports events-athletes and trainers would recommend a lot of stretching-and just recently there is starting to be a whole bunch of publications saying that stretching actually sometimes can impair sports performance and does not prevent injury. There are even more recent studies that have begun to really look at how much people should stretch because stretching too much can be detrimental. I really think this points to the importance of understanding the correct dose of mechanical stimulation that the tissue requires to be healthy, and also what could be a therapeutic dose. If somebody is in a situation where their tissue is not healthy, what is the right dose to promote a healthy response and healing of an injury? Or in chronic pain, for example, what could be helpful to the patient?

JB: That's a beautiful segue, actually, into kind of a translational question. Being a person with a multiple personality-type of background (all the way from your board certification in endocrinology and metabolism and internal medicine, to your being a licensed acupuncturist), you've got your world view on many different planes. What do you think the operator dependence is, and/or the practitioner-dependent component of being successful in implementing these? From what you've said, it sounds to me like there is a lot of art in the skill of understanding how to be part of this process with a patient, and the touch and the feel and the skill that comes from years of experience, or whatever those characteristics are, must be very important in outcome.

Creating Tools to Record Acupuncture Techniques

HL: Yes, absolutely. That's one of the things that fascinate me, actually. What is the difference…For example, what makes a good acupuncturist and a not-so-good acupuncturist? Acupuncturists typically and traditionally are trained by apprenticeship for generations and generations. In China and in Asian countries, acupuncturists typically learn from their families-from their parents, father, mother-and the art and types of techniques would get transmitted along the generations. There are so many different ways to practice acupuncture. If you look at, for example, Japanese acupuncture versus Korean and Chinese acupuncture, people will use different depths of needle penetration, different amounts of simulation. Some people turn the needle one way, some people do up and down combinations. We don't really know what works better for what condition. We're just beginning to scratch the surface, really, and trying to understand what works and what doesn't.

One of the things I'm interested in, actually, is developing some tools to record the needle technique that an acupuncturist employs during treatment so that at least we can document what people do and try to understand, quantitatively, what is more effective in terms of acupuncture techniques. The same, really, is true with manual therapies. I think there are a lot of efforts right now in the chiropractic community and massage to develop techniques to actually measure the forces that are applied during chiropractic manipulation, for example, to try to understand, first of all, what happens during a treatment. We do research to be able to standardize the practices in order to do controlled clinical trials. I think it is very important that we do that and that we pay a lot of attention to what is being done to understand what works better.

JB: When we start looking at the mosaic of your extraordinary work--now many, many papers and many, many different studies--there is something that kind of stands out to me. I'm sure you are probably way ahead of me in seeing this. The landscape tends to suggest that when we look at the body-and, in fact, I think you even spoke to this very eloquently in a paper you wrote not too long ago, I think it was back in 2006 on connective tissue, a body-wide signaling network-that somehow there is this signaling capacity,

from a mechanical stimulus, through the fascia, that has a whole-body effect that more suggests, then, systems biology or network signaling type of phenomena, which is a very different context for physiological response than we have previously been thinking about, which has been point of action and point of response. This is more of a general network change in physiological state function. Can you give us some insight on how you see this information that is emerging relating to this whole network and systems biology concept?

HL: Yes, that's a great question-something I am very interested in-but, again, that is something that is still at the hypothesis level right now, but I think a very interesting hypothesis level. Connective tissue is a network; there is no doubt about that. It's an anatomical network because it goes absolutely everywhere in the body. I think there is sort of analogy-this kind of image-that you could draw a line from any point of your body (a continuous line) to any other point of your body via connective tissue because it actually surrounds every muscle, every nerve, every blood vessel, every organ, so it is an anatomical network. And, in addition, it's a cellular network because the fibroblasts are connected to each other. They make contact with each other, and that's another thing we found by looking at our whole tissue using different microscopy techniques--we are able to actually see the processes of the fibroblasts touching each other. Of course, the question is, "Well, if they are making so many contacts and they reform the cellular network, are they talking to each other?" So far, we have not been able to identify (with certainty, anyway) any signal that gets transmitted over a significant distance-I'm talking about, you know, more than just one cell to the cell immediately adjacent to it. It is a very tempting hypothesis that there might be some kind of a body-wide signaling network and that's the hypothesis I put forward in that paper. So far we have not conclusively tested it one way or another-I can't say there is or there isn't at this point in time. But we're still looking, and I think that one of the things that acupuncture theory kind of suggests or proposes is that the network of meridians in the body, in effect, connect the various parts of the body functionally. Because we found this relationship (interesting relationship, anatomical relationship) between the connective tissue network and the acupuncture meridian network, I think it raises the interesting possibility that what the acupuncturists refer to as "qi," which is what they call a life force. People struggle as to how to describe qi as some kind of active principle, but you could think that it is perhaps some form of signaling, or information exchange, or something like that, through the acupuncture meridian network. That was really the hypothesis I proposed-that perhaps this signaling network does exist. We're still trying to find out what this qi could be, but right now we don't know.

JB: That segues into something I'm sure you've had discussions about. One of the people who worked in the medical school at University of Maryland, in the integrative medical department, was kind of a methodologist/biostatistician and authored a book that appeared last year called Snake Oil Science in which he was reviewing the clinical trials that had been on CAM therapies. As I recall, in that book, he took exception to acupuncture as having any demonstrated proof of outcome when you start looking at it from RCTs. I have some thoughts about those studies and the way he evaluated them, but I'd be interested in your thoughts about translating some of your mechanistic work into the outcome trials in humans. What is your thought about those who criticize and say there is no demonstrated proof of concept?

Randomized Controlled Trials are Problematic for Studying Acupuncture

HL: Yes, this is a very important subject that acupuncture researchers are, right now, obviously very concerned with. Acupuncture research has reached a point where it is kind of a paradoxical situation. There are really three big types of acupuncture studies. One is people do trials where they compare the effect of acupuncture to no treatment or to standard care, and they find there is an effect of acupuncture

(that acupuncture really helps people). But then in clinical trials that compare acupuncture to a sham procedure, very little difference is found between the acupuncture and the sham acupuncture. And then, on the other hand, when we look at physiological studies, where we look at the effect of acupuncture on the brain using neuroimaging, or what I'm doing in connective tissue, or what other people are doing looking at, for example, inflammatory response in peripheral tissues, we find that acupuncture does have very clear physiological effects.

What we don't know is this: what is the relationship between the physiological effects and the therapeutic effects? That's one thing we don't know much about. And the other thing we don't understand is: what's the role of a needle in the therapeutic effect? If you can get the same effect with some kind of sham needle, then does that mean that the needles are not doing anything? I think part of the answer to this sort of paradox is that part of what the sham control trials are doing is they are really asking a very, very specific question. They are saying, does it matter if we put the needle in one place versus another place? Or, does it matter if we manipulate the needle or not manipulate the needle? It is very important to understand that these particular trials are really asking a fake question and maybe some of these trials are showing us that, as far as the therapeutic effect is concerned, maybe some of these factors (for example, where you put the needle) maybe don't matter that much. If the control-the sham control-is putting the needle in a non-acupuncture point, for example, and you find just as good an effect, well maybe it doesn't matter. Maybe the needle position is not that important.

I think we have a long way to go to understand this, but the field of acupuncture is working very hard right now to try to solve these questions. You know, I think that within a few more years, hopefully, we'll have some answers. The relationship between the mechanisms that we are uncovering, in terms of the physiological effects of acupuncture and the therapeutic effects, also, I think we have a lot of work to do there to try to better understand that.

JB: I'm going to follow-up with a philosophical question. There is no obvious right answer to this question, but I'd just like your opinion. Let's just take, for a moment, as a thought, your proposed argument (or hypothesis) that appeared on connective tissue as a body-wide signaling network and the role that acupuncture and other mechanical therapeutics might have on that network, and then ask the question: could it be that the methodology of the RCT is really suspect because, in part, we have this whole Heisenberg uncertainty principle about what is changing, what are the variables? You know, the RCT wants to look at univariant-type of processes, and it doesn't sound like this falls nicely into univariant analysis at all. I'm just wondering, are we painted into the corner with the way that the RCT is done to get a certain negative answer?

HL: That's possible, of course, but I think there are probably some other really (perhaps) more basic explanations for why the RCT is not giving us statistically significant differences between the sham control groups and the real acupuncture groups. For example, it could be there are components of the acupuncture treatment, such as the attention and the time the acupuncturist spent with the patient, the teaching (the reframing of the problem, the traditional Chinese diagnosis), the education that takes place during a real acupuncture treatment that really kind of supplements the effect of the needle. It's very possible those effects are overpowering in magnitude compared with the effects of the needles themselves. It is really the combination of all of this that causes the therapeutic effect, and if you are trying to isolate the effect of the needle by itself, you are looking at an effect that may be important, but is small compared with the nonspecific effects of the treatment.

I think we need to really look at all the components of acupuncture treatments besides the effects of the needles. There may be a synergistic effect, obviously, between all of these things. We would need to really start looking at what happens during an acupuncture treatment, as a whole. The sophistication of the methodology that needs to be developed in order to investigate complex interventions, such as acupuncture, is quite formidable compared with doing a placebo-controlled trial of just a pharmacological agent. I think we are leading the way here-the alternative research, the CAM complementary and alternative medicine (CAM) research community is really at the forefront, I think, of research methodology for developing placebo controls. I think we are doing that out of necessity because these modalities are so complex, and I think we're going to really improve our understanding of how to do clinical trials, in general, via these problems that we're facing.

JB: I want to really compliment you. The answer you just gave to my very diffuse and somewhat nonspecific question was extraordinarily eloquent. You answered that question better than I could have. Thank you very much; that was very nicely said.

Let me close with one last thought. We've been talking about systems, which is a very extraordinary and complex concept. It is sometimes hard to get our heads around this, but I'd like to move it down the ladder now into smaller and smaller units of functional control (regulatory control), to the cellular level. One of the things that struck me in your 2001 FASEB paper was a diagram that showed how matrix deformation could transmit signals through cells by way of membrane-related conformational changes that then would influence intercellular signal transduction through things like kinase signaling through ERK and other kinase pathways that ultimately would modulate things like inflammatory potential, or cell replication, or gene expression. Do you think there is any evidence accumulating now to support the model that there are potential mechanical changes at the cell cytoskeletal structure that then can be translated through intercellular signal transduction in the cell to gene expression patterns that alter its physiology?

HL: Absolutely, yes. There is a very solid body of evidence in favor of this in cultured cells. There is no doubt this takes place and we are starting to understand the pathways involved in mechanotransduction using cultured cell models. So far, what we have done in our ex vivo models is we have confirmed that there is no question this occurs in tissue in response to the mechanical stimulus, whether it's stretch or using the acupuncture needle. The cells are receiving the mechanical signal. They are changing their cytoskeletal morphology in an active, dynamic manner that can be inhibited using specific cytoskeletal inhibitors or cytoskeletal signaling molecules such as Rho kinase and RAC that involve actin and microtubules. Our results are supported by a large body of literature in cultured cells that suggest that applying a force to a very specific part of the cell surface where there are protein complexes that contain integrins (these are molecules that are thought to form a mechanical bridge between the cytoskeleton of the cell and the extracellular matrix). An integrin acts a little bit like a sensor in that it allows the cytoskeleton of the cell to respond to the mechanical force in a direct way, and then that can trigger cascades of signaling events. Obviously we have not tested all of these in our system, but we have tested some of them and, yes, our results are very consistent with what has been described in cultured cells, with some differences, however, as I mentioned earlier. The morphology, for example, of the cells in whole tissue is different, in some ways, to that of the cells in the dish. I think it's important to compare our results to those in cultured cells and see the similarities and the differences.

JB: I knew before we started that this was going one of the most provocative, interesting, and mind-expanding discussions that I've had the privilege of having and it has certainly lived up to that. Your work

is just amazing. I think it's really at the cutting edge of developing a new science as it relates to systems biology and how it pertains to what we used to think of as unproveable hypotheses. I want to really commend you. It's courageous to be involved in this field and I think you are bringing the best of science with the best of tradition together. Thank you very much. We're going to follow your extraordinary work closely because I think it is opening the door to the next generation of evolution in medicine.

HL: Thank you. It was a pleasure speaking with you. We'll see what happens next.

JB: We certainly will. We'll stay tuned. Best to you, Dr. Langevin.

Wasn't that a treat? What an amazing journey we just were taken on with Dr. Langevin in this area of a systems biology look at the interaction between mechanical forces and cellular signaling. That was extraordinary. It reminds, in the 26 years. I think you can probably see that what she was addressing is a new methodology for evaluating, at a basic level of scientific inquiry, things that for millennia were just considered kind of experientially correct but we didn't know the reproducibility, or the variation, or how technologies could be employed with greater senses of positive outcome. Now those new methodologies-the systems thinking-are connected together with historical records of experience in such a way as to, I think, optimize the translation of this into future clinical. What an extraordinary experience we had. I look forward to talking to you in the February issue.

Thank you.

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