TWISTS OF FATE

CELL BIOLOGY

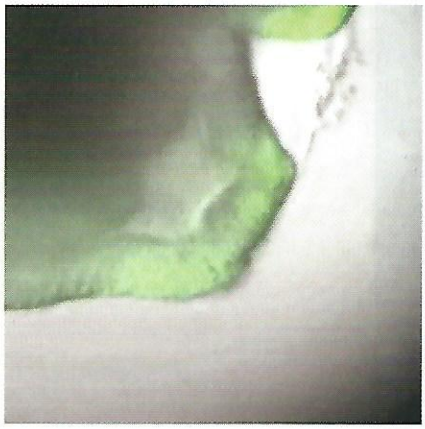
Physical pushes and pulls on a cell, not just genes, determine whether it will become part of a bone, a brain—or a deadly tumor

By Stefano Piccolo

Illustration by John Ueland

Stefano Piccolo is a professor of molecular biology at the University of Padua in Italy. His laboratory studies how cells sense their environment and use this information to build tissues.

THE HUMAN CELLS



in our laboratory looked mild-mannered. They were normal cells, not cancer cells, which are able to proliferate rampantly, invade nearby tissues, and ultimately can kill.

But something disturbingly malignant occurred when we forced these cells to change their shape, stretching them by pulling on their edges. This maneuver, flattening out their rounded mounds, increased the activity of two proteins within the cells, YAP and TAZ. As the proteins peaked, our benign cells began acting cancerous, replicating uncontrollably. It was stunning to see how these changes were triggered not by gene modifications but by a physical force.

Modern biologists tend to explain the lives of cells in terms of genes and proteins: gene A gives rise to a protein that controls gene B, which in turn produces protein X, and so forth, and these molecules determine cell behavior. Ultimately genes do tell cells how to act. Yet it is becoming increasingly clear that some of the most important processes in a cell are sparked by mechanical yanks and pushes that originate in their surroundings, such as nearby cells or fluids.

Over the past several decades scientists who study the way cells interpret such pushes and pulls, the province of a field called mechanobiology, have learned just how powerful these forces can be. For instance, cells with room around them keep dividing, whereas cells clustered together with thousands of others grow much more slowly or stop. Tissue stiffness matters, too: a class of stem cells, which have the potential to morph into a variety of types, will become neurons in surroundings that mimic brain stiffness but will become muscle cells if they encounter muscle-type stiffness. These mechanics guide the self-assembly of stem cells in a lab dish into complex organs, such as parts of eyes or structures normally found in the brain.

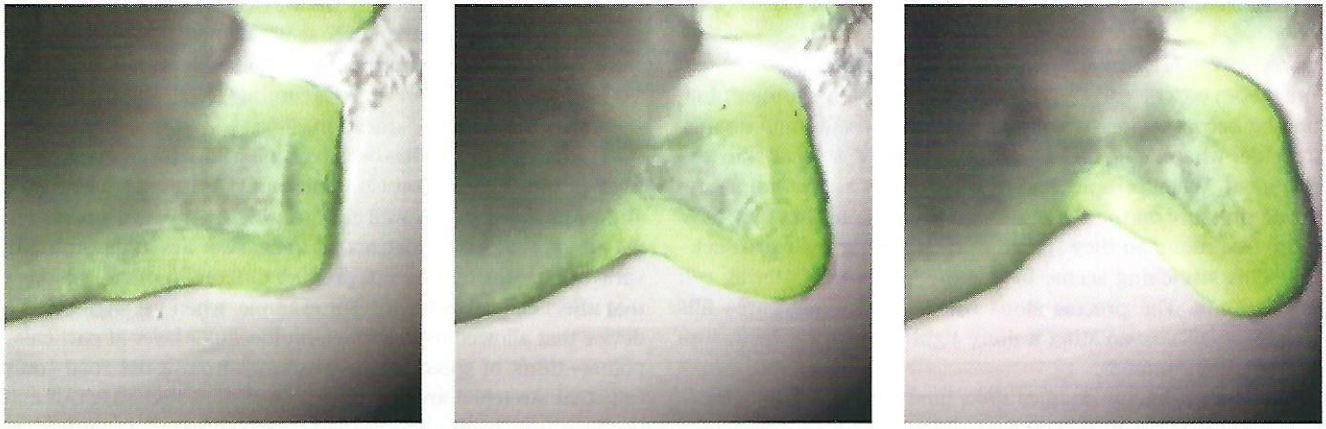
IN BRIEF

Physical forces affect the human body on a microscopic level, acting on each of its cells. Those forces can have impacts that are as profound as the effects of genes, and they are generated by a cell's surroundings.

Cells with room around them keep dividing, while cells clustered together with others grow much more slowly or stop. This space-driven behavior may determine the regenerative properties of stem cells.

A protein switch connects these physical and biological works, and flipping it can determine a cell's destiny and whether it is normal or becomes a dangerous tumor.

Until recently, no one knew exactly how cells translated physical pressures into directives to change activities. But my lab's experiments, carried out during the past several years, point to one elusive link. Our work shows that YAP and TAZ constitute a molecular switch that connects forces in the outer regions of a cell to genes in the cell's nucleus that ultimately carry them out. When cells get pulled in certain ways, YAP and TAZ react to the force and activate genes that determine how the cell behaves. Along with exciting research by other scientists around the world, the discovery is providing new insights into the workings of an array of biological processes, from embryonic development to tissue maintenance and wound healing. It also suggests new avenues for attacking cancer and for advancing efforts to grow new organs in the lab.



SELF-CONTROL: Floating in an uncrowded lab dish, human embryonic stem cells self-assemble into a nascent eye during several days (*left to right*).

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FORCES OF NATURE

A MYRIAD OF MECHANICAL FORCES operate in a living body, although most people know of only the most obvious ones, such as heart pumping, muscle flexing and blood flow. Biologists have long appreciated the large-scale effects of these repeated contractions and stretches. For instance, mechanical loading from physical exercise fosters bone mineralization and prevents osteoporosis, and rhythmic expansion of blood vessels protects them from arteriosclerosis.

Yet physical forces also profoundly affect the human body on a microscopic level, acting on each of its estimated 40 trillion cells. The forces arise because of the way cells connect to one another. Every cell has an inner framework, the cytoskeleton, consisting of specialized sets of proteins that serve as cables, struts and tie rods. These proteins buttress and shape the nucleus, various other structures known as organelles and the cell membrane. Outside the membrane, adhesive proteins on the cell surface connect that inner cytoskeleton to the outside world. They anchor themselves to a lattice of external filamentary proteins called the extracellular matrix, which in turn is linked to other cells.

The cell's cytoskeleton and the surrounding extracellular matrix are in a constant tug-of-war. For example, a nearby deformation of the matrix pulls the adhesion sites outward. If unopposed, this action would stretch the cell. The cell, however, responds to such pulls with an equal inward contraction and a restructuring of its cytoskeleton. This back-and-forth stabilizes cell shape. But it is clearly dynamic: it can rapidly reset if the cell encounters a different pattern of mechanical stresses, eventually changing the overall cell shape.

Starting in the late 1970s, scientists began to appreciate that mechanical signals affecting these structures were essential for the control of cell reproduction, also known as cell growth. Donald Ingber of the Wyss Institute for Biologically Inspired Engineering at Harvard University and Fiona Watt of King's College London developed methods to engineer cell shape by attaching the cells to different sticky dots of extracellular matrix proteins that were printed onto glass slides. Remarkably, the cells reproduced only when they were anchored to a large area, which allowed them to stretch and flatten out. If the exact same cells were rooted to a small area, they rounded up, stopped dividing and switched on genetic programs that led them to differentiate (mature into specialized cell types) or to die.

These findings garnered a lot of attention. But something was missing from this picture. To regulate cell reproduction or differentiation, mechanical forces had to affect the core of the cell, its genome, and turn on a number of genes responsible for growth or death. What linked the physical and biological worlds? How was cell mechanics translated into perfectly orchestrated changes of gene activity?

These questions attracted me and my colleagues at the University of Padua in Italy. About five years ago Sirio Dupont, a member of my research team, followed a trail of clues in the best tradition of scientific detectives. He began by searching a computer database for genes activated by mechanical stress. (If you pull on a cell, these are the genes that swing into action.) He then searched for proteins associated with the control of those genes. He found two: YAP and TAZ.

We then confirmed through lab experiments that YAP and TAZ indeed form a switch that turns a cell's response to physical forces on and off. We could take command of cell behavior, overriding any changes in cell shape, by experimentally increasing or reduc-

ing the amount of YAP and TAZ produced by cells. For example, if we raised YAP and TAZ levels in small rounded cells that had stopped growing and dividing, we could restore proliferation.

The switch appears to work like this: Generally, YAP and TAZ sit in the cytoplasm of the cell. When the cytoskeleton gets stretched out, they move to the nucleus, park themselves on selected spots of DNA and activate particular growth-inducing genes. If levels of YAP and TAZ increase, more of the proteins can make this move and become active. Conversely, in rounded cells confined to small areas, YAP and TAZ remain in the cytoplasm—deteriorating while they are there—and stay out of the nucleus.

These two proteins are close siblings, although they have different names. Their molecular structures are very similar, and they perform overlapping functions. Consequently, they are usually referred to as one: YAP/TAZ.

KEEPING ORGANS IN SHAPE

THE IMPORTANCE OF THIS YAP/TAZ switch for the body's proper functioning becomes clear when it is studied in tissues and organs. Consider what happens if tissues are wounded, such as skin getting a cut. When cells are lost because of this kind of injury, reduced pressure on the remaining cells tells them that they have more free room. So they spread out, stretching their cytoskeleton. This stretching seems to activate YAP/TAZ, fostering cell proliferation. The process stops when the wounded area fills with new cells, re-creating a more tightly packed, growth-suppressing environment.

Some experiments on mice show how this sequence operates in real organs. Duojia (D. J.) Pan of Johns Hopkins University demonstrated that YAP is instrumental for regenerating the cellular lining of intestines in mice after inflammatory damage (colitis). And Eric Olson of the University of Texas Southwestern Medical Center demonstrated that YAP/TAZ was able to promote partial cardiac muscle regeneration after a heart attack. When researchers genetically engineered mice to produce extra YAP in their skin—work done by both Elaine Fuchs of the Rockefeller University and Fernando Camargo of Boston Children's Hospital—the outer skin layer thickened and stratified in abnormal ways. So it appears that levels of YAP/TAZ have to be "just right" for proper tissue regeneration. Too little translates into a failure to heal, and too much means that cells might pile up in aberrant tissues, which carries a risk of tumor development.

Damage repair is not the only reason why a properly functioning YAP/TAZ switch is crucial to health. Many of our organs need to replenish cells constantly even without wounds or disease. The need arises because organs live for many decades, but the life span of each cell within them is typically much shorter. New cells have to compensate for the death of old ones, or else the organ will wither and die.

The balance in cell numbers is only one aspect of organ maintenance, however. A second aspect is controlling where in the organ those new cells grow. Organs are like tightly packed apartment buildings—they are a collection of various cell types, each lodged within a sophisticated three-dimensional architecture. And this spatial organization is also replenished one cell generation after another. Where is the information about "what goes where" coming from? New findings suggest that, once again, the answer involves YAP/TAZ and the way it responds to the organ's three-dimensional shape.

Organ architecture is complicated. It is a collection of various structures, such as pits, borders, convex or concave curves, and flat layers, all defined by the way cells fit together in their associated extracellular matrix scaffold. Because that scaffold actually lives longer than the cells attached to it, it can work as the spatial memory for new incoming cells, answering that vital "what goes where" question.

The puzzle, though, has been how the scaffold does this. Celeste Nelson, now at Princeton University, and Christopher Chen, now at Boston University, as well as Mariaceleste Aragona, working in my group, provided evidence that the answer lies in the scaffold's varied shape. Such variations produce different mechanical forces that affect cellular behavior. For example, when we engineered a device that allowed us to curve a multicellular layer at particular points—think of speed bumps rising up from a flat road—only cells that stretched around curved areas activated YAP/TAZ and proliferated. This finding has led us to propose that local tissue anatomy controls the behavior of constituent cells by influencing activation of YAP/TAZ. The amount of YAP/TAZ that gets activated and moves to the nucleus peaks in areas where tissues stretch or curve and drops within flat, densely packed cell layers. In this way, tissue architecture can form a template that perpetuates organ shapes through many cell generations, embodying memories for body components that have none of their own.

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YAP/TAZ's response to cellular surroundings could explain another mystery: how organs know when to stop growing. At the time my lab discovered the duo's role in conveying mechanical signals to the nucleus, YAP/TAZ was already a focus of intense interest because scientists had observed that animals whose cells have higher than normal activity levels of these factors develop giant organs. Because tissue architecture can affect the pair's activity and because mechanical forces change as an organ grows, we suspect that when organs reach their correct size, the resulting balance of forces shuts off YAP/TAZ activity and stops further growth.

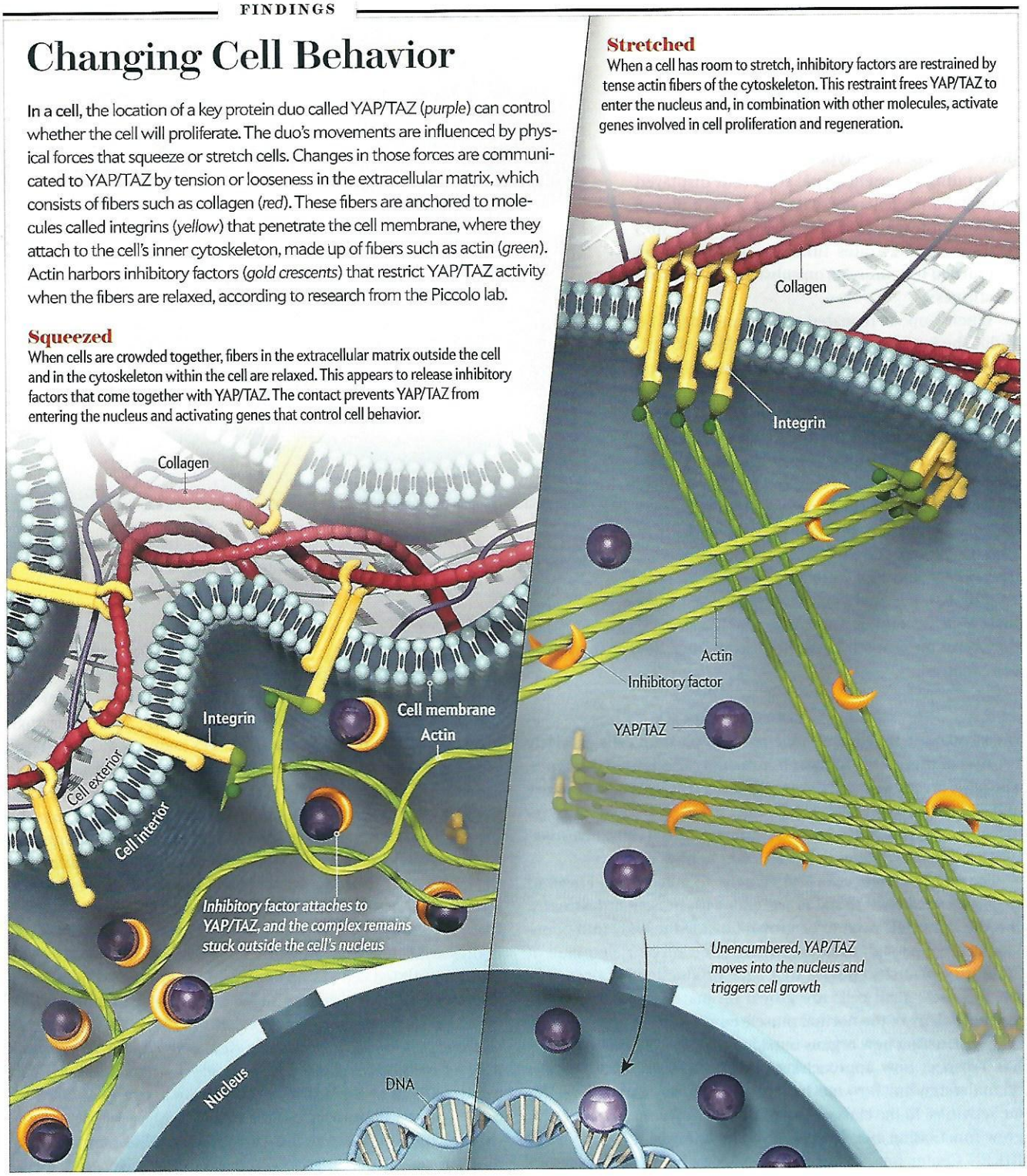
Topography is just one feature in an organ that can influence

Changing Cell Behavior

In a cell, the location of a key protein duo called YAP/TAZ (*purple*) can control whether the cell will proliferate. The duo's movements are influenced by physical forces that squeeze or stretch cells. Changes in those forces are communicated to YAP/TAZ by tension or looseness in the extracellular matrix, which consists of fibers such as collagen (*red*). These fibers are anchored to molecules called integrins (*yellow*) that penetrate the cell membrane, where they attach to the cell's inner cytoskeleton, made up of fibers such as actin (*green*). Actin harbors inhibitory factors (*gold crescents*) that restrict YAP/TAZ activity when the fibers are relaxed, according to research from the Piccolo lab.

Squeezed
When cells are crowded together, fibers in the extracellular matrix outside the cell and in the cytoskeleton within the cell are relaxed. This appears to release inhibitory factors that come together with YAP/TAZ. The contact prevents YAP/TAZ from entering the nucleus and activating genes that control cell behavior.

Stretched
When a cell has room to stretch, inhibitory factors are restrained by tense actin fibers of the cytoskeleton. This restraint frees YAP/TAZ to enter the nucleus and, in combination with other molecules, activate genes involved in cell proliferation and regeneration.



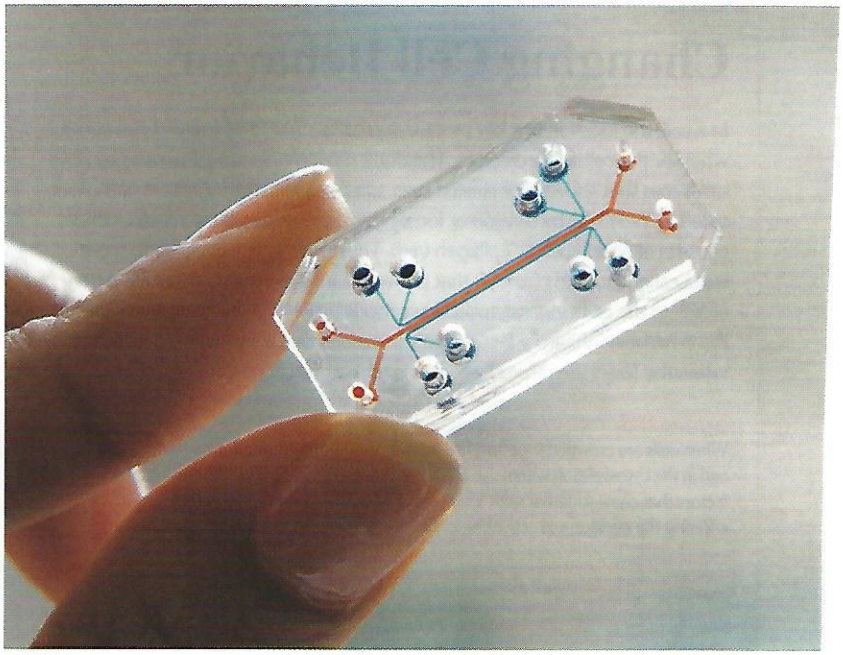
mechanical forces and affect a cell's fate. A second is the different types of ground that a cell can encounter. The extracellular matrix to which cells are secured is indeed not monotonous but has different textures. Some tissues, such as bone, create a stiff, dense matrix, like solid rock. Other tissues, such as brain tissue or fat, develop a much softer version. In other words, each organ's matrix has its own signature.

These signatures appear crucial in organ development and regeneration. Notably, their differing mechanical properties guide the efforts of a very important cell type: mesenchymal stem cells. These cells are found in many adult organs and contribute to repair after an injury. They differentiate into a strikingly diverse array of cell types, including bone, fat, nerve and muscle cells. For years biologists assumed that the cocktail of

Illustration by Emily Cooper

chemical factors that mesenchymal stem cells find at their destination determines their fate. But Adam Engler and Dennis Discher, both then at the University of Pennsylvania, punched a hole in that idea in a 2006 *Cell* paper. They engineered synthetic matrices with a range of rigidities matching those typical of different tissues. Astoundingly, mesenchymal stem cells displayed chameleonlike behavior when they were placed in these different matrices. They morphed into neurons on substrates tuned to brain stiffness and turned into muscles on substrates with muscle stiffness.

When Dupont repeated these experiments in Padua, he found that the degree of YAP/TAZ activation in mesenchymal stem cells changed along with the rigidity of different matrices. On very stiff substrates, for instance, YAP/TAZ was more active in cells and guided those cells on a journey toward becoming bone. Yet on the softest environment, high overall levels and activity of YAP/TAZ dropped, and these stem cells turned into fat cells. By experimentally playing with YAP/TAZ levels and activity, we could actually trick these cells: adding a modified version of YAP/TAZ to soft mesenchymal stem cells—the ones that were becoming fat—made them act as if they were on a much harder substrate and turned them into bone instead.



BUILDING A LUNG: When this chip created forces that mimic breathing, blood vessel and lung cells on it formed complex lung structures.

CELL SWITCH THERAPY

THE BIOMEDICAL WORLD has come to focus on stem cells precisely because of this ability to morph into many specialized cell types. The hope is that with the proper guidance, the cells could restore and replenish damaged tissue and even be grown into replacement organs. But to take advantage of stem cells, investigators need to understand how they react to physical forces.

For example, stem cells that give rise to muscle could be used to bolster weakened tissue in patients with muscular dystrophy. But the stem cells need to be grown outside the body into populations large enough to have a therapeutic effect. Helen Blau of Stanford University showed that such production happens only when muscle stem cells are grown on materials matching the exact elasticity of the normal muscle environment.

Constructing new organs outside the body—a science-fiction-like prospect now approaching scientific reality—also depends on understanding how mechanical signals end up altering cellular activities. In the classic sci-fi movie *Blade Runner,* researchers grew functioning eyes in vats. Now the late Yoshiki Sasai of the RIKEN Center for Developmental Biology in Kobe, Japan, and his colleagues have established that it is possible to make embryonic eyes in a petri dish, starting from a ball of initially identical embryonic stem cells floating in a soft extracellular matrix. When the ball reaches the proper size, the cell layer starts to autonomously fold, twist and sink, mechanically self-assembling into eyelike structures as if it were living origami. This phenomenon occurs only when the scientists detach cells from the mechanical constraint imposed by flat walls in plastic dishes and let them follow an inner developmental script driven by a series of mechanical operations: folding, stretching, bending, and softening here and becoming more rigid there.

So-called organs on a chip recently reported by Ingber and his colleagues also obey these physical signals. Rather than growing cells on plastic dishes, Ingber's team grew them in tiny containers that exert pressure on the cells through minuscule amounts of fluids. These devices can change that pressure with finely honed accuracy. In this way, the cells experience the mechanical strains typical of real tissues. For example, lung cells were exposed to cycles of pressure and release that mimicked physiological breathing movements, and intestinal cells were stretched and compressed by motions like those of the digestive tract. Re-creating the normal rhythms and pressures of our bodies awakened some unexpected behaviors in otherwise dull, undifferentiated cell clumps. Some of them underwent a spontaneous change into differentiated organlike structures.

If tissues use mechanical regulation of YAP/TAZ to increase or decrease the number of their stem cells, the protein switch may let us produce more of these cells on demand. Stem cells occur only in secluded locations within tissues—at borders, bulges or the bottom of hollow tubes—that is, in special mechanical niches. These confined areas may be able to instill "stemness" in cells, the ability to regenerate themselves and, at the same time, to generate a progeny of multiple cell types. In several of these locations, stem cells display high levels of YAP/TAZ in their nucleus, which increases their capacity to reproduce, and the locations appear to influence these protein levels. By engineering niches that mimic those in the body, investigators may be able to expand rare stem cell populations in the lab. In a not too distant future, we might be able to manipulate stem cells within living tissues by delivering drugs that stimulate YAP/TAZ activity in the cells. Or drugs could turn it off,

COURTESY OF WYSS INSTITUTE FOR BIOLOGICALLY INSPIRED ENGINEERING, HARVARD UNIVERSITY

SCIENTIFIC AMERICAN ONLINE Watch Donald Ingber explain why physical forces on a cell can be as important as genes at ScientificAmerican.com/oct2014/cell-forces

which should stop expansion and induce the cells to morph into a particular cell type needed by a given tissue.

Stem cell therapies do have a dark side: these cells may be ineffective or even dangerous if, instead of differentiating into a desired kind of tissue, they keep growing. Cancer stem cells, the most dangerous type of cancer cell, behave this way. That risk is one reason why many of us who work in mechanobiology believe that any future attempt to inject patients with stem cells for therapy needs to ensure that those cells will end up in the proper physical surroundings. The wrong surrounding forces could nudge these cells down an undesirable path, toward an inappropriate cell type or a cancerous growth.

PULLING THE STRINGS OF CANCER

INDEED, WHILE RESEARCHERS working on stem cells and the field of regenerative medicine are looking to boost the expansion of these cells and regrow damaged tissue, cancer researchers are struggling to do just the opposite: to restrict growth. Here, too, the physical forces tugging at cells may play a decisive role. For 40 years the war on cancer has largely been ruled by the view that genetic mutations drive tumor growth. Although some therapies that block the activity of such mutants have been effective, it is uncertain whether this approach will translate into a wide range of new treatments. Simply put, there are too many mutations, even in a single tumor, to chase and block all of them.

While researchers working on stem cells are looking to regrow damaged tissue, cancer researchers are struggling to do just the opposite: to restrict growth. Here, too, the physical forces tugging at cells may play a decisive role.

Cancer, however, is as much a disease of a disturbed microenvironment as it is a result of disturbed genes. Alterations of cell shape and of the cell's surroundings actually precede the onset of tumors and may even initiate disease. For example, work at Valerie Weaver's lab at the University of California, San Francisco, has shown that increasing the rigidity of the surrounding extracellular matrix prompted nonmalignant cells to switch to a tumorlike program of aggressive growth.

In our experiments, we demonstrated that forced shape changes translated into activation of YAP/TAZ and into more malignant behavior. Michelangelo Cordenonsi, a member of my lab, found that when he artificially raised TAZ in benign cells, these became indistinguishable from cancer stem cells. Indeed, YAP/TAZ is active in breast cancer stem cells, where they increase malignancy. Tumor cells do not invent anything new. Instead they co-opt a key mechanism by which tissues control the number and differentiation of their stem cells.

Because of this work, researchers in my group are pursuing an unorthodox idea about cancer. We think the initial acquisition of malignant properties may not necessarily involve accumulation of genetic lesions. Rather cancer may result from a rift in the body's normal microscopic architecture. Now it appears particularly apt that tumors have long been called "wounds that never heal" for their tendency to endlessly produce cells as if they were needed to repair a gash.

Restoring the environment, then, may be a balm as much as disturbing it is a bane. When Weaver took cancer cells and blunted their unusual pulling capacity by cutting their attachment strings to the extracellular matrix, their growth signals slowed, as did their proliferation. They turned into normal-looking tissue.

My colleagues and I are hopeful, then, that YAP/TAZ may prove to be an Achilles' heel of cancer. Hyperactivation of the pair is common in a vast number of tumor types, and dampening that zeal might help normalize tumor cell behavior or prevent metastasis. This strategy is already being pursued by several research groups.

Yet we and other scientists are mindful that cancer is a complex disease. Indeed, different cancers may have different paths that connect outer forces to genes. Many therapeutic approaches that appeared promising at first in the lab have made little difference to cancer patients. For any future YAP/TAZ inhibitor, the challenge will be to spare normal stem cells while targeting cancer cells, specifically. If direct inhibitors cannot be found, drugs able to relax the cytoskeleton or the extracellular matrix in tumors might do the job indirectly.

The ancient Greek philosopher Aristotle considered shape as the soul of all living entities. Cell biologists are beginning to see the profound role of shape in a more modern sense. Shape exerts a powerful influence on life: on one hand, it affects how cells build and repair organs, and on the other hand, it can become malevolent, undermining health. As we refine our understanding of the power of shape, we may be able to bend it to help people. SA

MORE TO EXPLORE

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An Indirect Way to Tame Cancer. Rakesh K. Jain; February 2014.

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