TrendsTalk

Mina J. Bissell: Context Matters

Cancers are no longer viewed as collections of genetically altered cells, but as aberrant organs with a plastic stroma, matrix, and vasculature. The coevolution and crosstalk of tumor and stroma is a major determinant of response to therapy, mechanisms of resistance, and metastasis and cancer therapies directed at the tumor microenvironment (TME) are yielding some of the most promising results in years. Among the first to recognize the importance of the cancer environment is Mina Bissell of the Lawrence Berkeley National Laboratory (LBNL) in California. Her unconventional findings, optimism, and persistency produced some of the most fascinating observations on the influence of tissue architecture in the genesis and progression of tumors and became a paradigm shift in how we think about cancer. Dr Bissell shares her thoughts on why we need to see tumors within the broader context of tissue organization and where the field is headed.

You always held a belief that the unit of function in higher organisms was larger than the cell. How did you come to that realization, especially as a former chemist and bacteriologist?

I certainly did not always hold that belief. I discovered these concepts through a mix of curiosity, logic, long hours of experimental work, and, yes, also passion and intuition! The discoveries were made possible also because I had the good fortune of training and working closely with many postdoctoral fellows and students from all over the world at the LBNL, where I got my first appointment after completing my postdoctoral work on RNA tumor viruses at Berkeley, and where I continue to work to this day. Whenever there were no easy explanations for our results, I would devise a model and provide unorthodox explanations. Then we would test them over many, many years.

The process of how I arrived at many of our theories and conclusions may appear convoluted, but it was not really. To me, it was a journey in a rugged and poorly unexplored area, in a road much less traveled, and up an unknown hill with many skeptical onlookers. But also with some thoughtful and supportive colleagues! When I give talks, what became so obvious to me in the late 1970's still shocks quite a few people worldwide more than 35 years later. Still, many only believe what serves their purpose in the face of a large amount of data and irrefutable evidence. It is like Global Warming, where half of our populations have their heads in the sand! One would think that educated scientists should be curious and more willing to look at the evidence. Some still insist that the concept of cell context is all unimportant and that a single oncogene overexpression or mutation is sufficient to render cells cancerous. They advocate that deeper gene sequencing is all that is needed to cure cancers. This is irrational. Whereas we are doing pretty well in some cancers such as breast, even in that case we still see 50% recurrence. In other tumors, our record is simply dismal after 50 years of war on cancer.

During my postdoctoral work, when I switched to the field of cancer viruses and cell biology, I began to wonder about the ways we were growing cells in culture. I wondered what was happening to the cells as we kept changing the temperature and pH every time we would passage or look at them under the microscope and then, usually, measuring one or two markers going up or down at the end. From my past experience as a bacteriologist, I knew well that even a very small change in the pH could make bacteria change their signaling patterns. So, I wondered what it really meant to state that these uniform and elongated cells that we were growing rapidly in a flat dish under these artificial conditions were representative of cells in a normal tissue or organ.

When I started my own laboratory at the LBNL, I decided to get a handle on what was needed to make the environment of cultured cells more physiological. So I started collaborating with Al Bassham, a plant biochemist who co-discovered the path of carbon in photosynthesis with Melvin Calvin. Bassham and I designed a closed Lucite incubator that would allow 30 plates of cells to be maintained at constant temperature and pH without the need to open the system. We called it 'The Steady State Machine'. Then, we adopted the technique that Calvin and Bassham had used for the path of carbon in photosynthesis to animal cells. Using radioactive glucose, we could show that, if we controlled for all the variables, malignant cells always had higher levels of metabolite flow through aerobic glycolysis, just as Warburg had predicted. But, using tritium to label the hydrogen transfer pathways, we showed that, unlike what Warburg had anticipated, the hydrogen transfer pathways were not affected, which means that increased aerobic glycolysis does not need to occur at the expense of loss of function in the tricarboxylic acid (TCA) cycle or other oxidative functions. What mattered most to the pattern of glucose metabolism was the level

of glucose in the medium! In 2014, in an article published in the *Journal of Clinical Investigation*, we elucidated the mechanism by which the level of glucose in the medium has profound consequences for whether mammary cells demonstrate normal or malignant phenotypes in culture.

Meanwhile, I began to read the tissue culture literature more widely to understand the definition of a 'normal' – and, for that matter, malignant – cell in a 2D culture. At that time, several events and experiments happily converged. I learned that, in addition to mammary cells that would forget to make milk proteins when grown on tissue culture plastic, all cultured cells lose function rapidly when isolated and grown on a dish, and that under those conditions no cell could maintain a differentiated state qualitatively and/or quantitatively. Coming to this realization led to a comprehensive review published in 1981, in the *International Review of Cytology*, which I believe may still be useful and relevant for scientists doing cell culture. It states that 'Since most, if not all, functions are changed in culture, quantitatively and/or qualitatively, there is little or no "constitutive" regulation in higher organisms; i.e., the differentiated state of normal cells is unstable and the environment regulates gene expression… Our failure to define a cancer cell may stem also from our inability to define the normal state.'

Also, the fortunate arrival of Richard Schwarz, Joanne Emerman, and Glenn Hall at my lab, all thoughtful postdoctoral fellows, taught me important facts about the extracellular matrix (ECM) molecules, culturing mammary cells on floating collagen gels, and context. During the next 6 years, we developed these concepts, and in 1982 we published 'How Does ECM Regulate Gene Expression?' in the *Journal of Theoretical Biology*: I proposed the model of Dynamic Reciprocity, where the ECM would signal via ECM receptors and through the cytoskeleton to the nucleus and then chromatin would signal back. In sum, we had reasoned that tissue and organ specificity is dependent on, and directed by, different ECM molecules and different microenvironments. It was then that I concluded that the unit of function had to be larger than the cell. Years later, Hall and I argued that the organ itself was the ultimate arbitrator of function. Many elements of the model have been proved in numerous laboratories, including ours. I was glad to see that the role of the microenvironment and context in the regulation of gene expression in both normal and malignant cells gave rise to two TME Study Sections at the National Cancer Institute (NCI).

When did you first realize that tumors behave as organs, but are constantly evolving?

This realization came much later, after observing tumor growth *in vivo* and our ability to revert these tumors to a normal tissue by changing cellular architecture. Also, at that time, we were obtaining experimental evidence for the fact that tumors could be reprogrammed. This notion was included in 'Putting Tumors in Context', a piece that Derek Radisky and I wrote in 2001 in *Nature Cancer Reviews*. In the past three decades, I have shown with David Dolberg that even potent oncogenes are not sufficient to form a tumor, only under certain circumstances. They need to collaborate with the immune system and require many other steps and events to make a cell truly malignant. The architecture of the tissues is the key to understanding why cancer is an organ-specific disease.

What have been some of the challenges in studying the TME and how can we overcome them?

There is a huge amount of literature based on work done in tissue culture in the presence of undefined media with serum or even in engineered mice or other animal models, almost all pointing to linear pathways. The conclusions from much of this work, other than discovering the molecules involved, which of course is an important goal by itself, could be misleading in terms of function and regulation.

But ultimately, this is a question larger than the TME field and one that applies to more areas of fundamental biology. In my mind, today, the biggest obstacles in modern biology are the textbooks and the ways we continue to teach and discuss biology in schools and universities and, ironically, even in study sections and top-tier journals! Textbooks are full of yesterday's science and canned dogmatic theories and are often written by authors relying on conventional wisdom rather than addressing new points of views raised in more current literature. We need to pose tough biological questions and to debate different points of view to engage students and to encourage and allow them to think for themselves. Every time I lecture to young people these days, which is quite often, I always say 'Question authority, think for yourselves, don't become arrogant (it kills curiosity and passion) and look at your own data as well as others' data with critical eyes'.

Another obstacle is that, in general, very few accept risk, and as a result scientists with contrary results are not heard and sooner or later give up, since they cannot get funded or rightly published. It becomes a vicious cycle. Debates should be encouraged and questions examined with respect and collegiality. It has taken me 40 years of persistence to help put the field of the microenvironment, ECM signaling, and tissue and organ architecture on the map. Why is a phrase like 'phenotype is dominant over genotype' so controversial? All of the 10–70 trillion cells in our bodies have the same parental inherited genetic information. So the genes in all of the cells within our tissues are the same. This means that something other than the genes is telling these cells of how to function in different tissues! Genes are crucial but not exclusive. Context is what allows the epigenome to change according to the environment the genes find themselves in. These conditions are diverse for different organs. Many scientists focus on discovering one molecule at a time. Very few think about how or why their elbow and their liver do such different things despite

the fact that the sequence of their DNA is essentially the same. It seems to me that we have replaced curiosity and the open mind with quick experiments and rapid publications using fancy techniques that can be easily published in top journals. The desire to go deeper and move science forward along with the joys of doing paradigm-shifting science is taking the back seat. We need keep the young involved and encouraged. We need solutions!

What is the future of studying the TME and the outstanding questions that remain?

Cancer is an organ-specific disease. We need to understand the normal organ before we can understand that organ's cancer. We need to learn more about the ECM and the basement membrane that surround each tissue and understand why and how the basement membrane of, say, the liver is different from the breast. The current excitement referred to as 'precision medicine' cannot be truly successful and precise if the microenvironment of the tumor and the immune status of the patient are not well studied. We have made some strides in survival in several cancers including breast cancer and melanoma, but often these tumors return rapidly, even when we thought that the patient was cured. What are we missing?

The role of protumor and antitumor immune cells is now being discovered and the potential of using this information for therapy is enormous. We also need relevant models of cell dormancy and metastasis, both in mice and in humans. Although we now have a few promising models of dormancy that could and should be put to good use, much remains to be understood on why cells become dormant and why they wake up. We need to find ways to keep the dormant tumor cells in tissues in that state until we find a way of killing them selectively. The recent and unexpected findings on the role of exosomes in tumors also promise another huge paradigm shift in how we view cancer. What are these membrane-bound vesicles doing, exactly? Can they be harnessed for therapy? Exciting work in a couple of laboratories seems very promising. But of course much remains to be learned.

Given what we know about the role of the TME in both preventing and promoting cancer progression and resistance, what will likely be the best way to approach the treatment of cancer?

There is no way we can eradicate cancer unless we cure aging. However, we can try to eliminate or control the tumor and either cure the patient or turn cancer into a chronic disease. Many years ago, in a *Science* article, my laboratory showed that TGFβ1 has opposing functions in malignant and normal cells. Many were incredulous or even angry because they wanted to treat tumors with drugs for TGFβ1, since this is a growth inhibitor for normal cells. But they mostly have all come around. Curiously, we were not studying TGFβ *per se*, but we wanted to know which wounding agent was responsible for helping tumors get formed and we stumbled on TGFβ. So it is essential to understand for each tissue and organ how normal and malignant cells may interact with the same ligands in their surrounding microenvironment.

We also need to build accurate 3D models of each organ by isolating and reassembling the different cell types or by using viable organoids in TME assays. We need to strive for models that allow us to kill or push the tumor cells to quiescence and then use primary tumor organoids and test how can we create conditions where these tumor cells can be selectively killed without toxicity to normal cells. We have observed that an inhibitory antibody against integrin-β1 has no toxicity in mice but kills tumors cells. What is exciting is the fact that we could reproduce this in relevant microenvironment 3D assays.

In short, if we consider both the tumor and the microenvironment, which includes not only matrix, vessels and immune cells, but now also exosomes, we may allow ourselves to be more hopeful about the future of cancer research!

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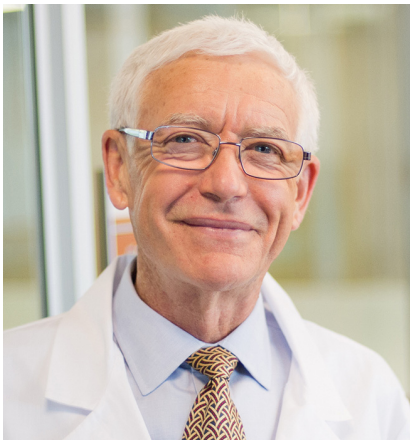
TrendsTalk

Peter Jones: Leaving a Mark on the Cancer Genome



Genome regulation can occur through direct changes in the nucleotide sequence or through epigenetic modifications including chemical modification of histones and DNA, nucleosome remodeling, and noncoding RNAs. The resulting changes from epigenetic modifications play a crucial role in the regulation of DNA-based processes including transcription, DNA repair, and replication. Many epigenetic regulators have been found to be mutated in cancers, sparking interest in understanding how epigenetic modifications regulate tumorigenesis. Furthermore, several cancer drugs targeting epigenetic enzymes have been recently approved for use in the clinic. Pioneering the field of epigenetics, particularly its role in cancer and the development of novel therapies, is Peter Jones, Research Director and Chief Scientific Officer of the Van