

The extracellular matrix and disease: an interview with Zena Werb

Zena Werb's pioneering efforts brought recognition to the idea that the extracellular matrix has a profound influence in determining cell fate. Here, she discusses how a 'rocky' start in geophysics led her to a career that is changing the way we think about cancer.

Everyone is influenced by what goes on around them, and cells are much the same. The signalling molecules and support structures of the extracellular matrix (ECM) direct many aspects of normal cell behaviour, including shape, migration and survival. However, the ECM can also play a role in the development and progression of disease. For example, a cell does not become cancerous on its own – together with intrinsic changes, oncogenesis is encouraged by cues from the surrounding milieu. Furthermore, the aberrant survival of cancer cells is propagated by signals received from the microenvironment, and malignancy depends on the ability of cells to crawl along ECM proteins to invade foreign tissues.

Following decades of research based on isolated cells in culture dishes, scientists are now rethinking how the microenvironment that surrounds cells *in vivo* instructs development, differentiation, inflammation and cancer. Many of Zena Werb's early findings led to this shift in thinking, and she is now creating new technologies that allow scientists to look directly into the cancer microenvironment. By following an interesting and sinuous path, Zena Werb uncovered a great deal about the complex nature of interactions between cells and their support structures.

You intended to be a geophysics major in your earliest days at the University of Toronto. What pre-empted your switch to biochemistry and physiology?

I was interested in geophysics, understanding situations like earthquakes, when

I started college. Ironically, earthquakes became an important part of my life as a resident of San Francisco, but I didn't realise this would be the case then. Back in college, I wanted to go on a summer geology field course to the Rockies. I was one of the top two students in my class, but they wouldn't let me go because I was female – they said that there were not the proper facilities on the site for women. Instead, I found a summer job in the field and actually got paid for the experience I gained, which would not have happened if I'd been allowed into the course. However, I worried that if it was impossible for me to be included in the profession at the level of a summer course, I might always encounter roadblocks that would inhibit my ability to work. The field of geology clearly had strong prejudices.

I decided that biochemistry made more sense. Back in the 60s, the field of biochemistry was still quite male oriented, but I never had the sense that taking a course could be a barrier for me. Even if there was some prejudice, it seemed that the quality of my research and my ability to do it would allow me to advance. Also, there were already a few female faculty in biochemistry.

I was also drawn to the physical aspects of the field. My main study focus was biochemistry, although I minored in chemistry. My second summer job during college was at the Ontario Cancer Institute, where I used optical rotary dispersion and other kinds of physical techniques to determine molecular structures. I found working with structures very fascinating.

Did your interest in physical structures and the techniques that define them stimulate your interest in the ECM?

My interest in the ECM came later. My interest in physical techniques drew me to X-ray crystallography, which is what I set



out to study when I became a graduate student at the Rockefeller. Again, I hit a roadblock. The person in charge of X-ray crystallography at the Rockefeller was Gerry Edelman, who was notoriously difficult to work with. He won the Nobel Prize in 1972 for his work defining the structure of antibodies, but I knew that I did not want to work with someone with his reputation. Instead, I did a rotation in Zanvil Cohn's lab. Within the first few days, I saw macrophages moving in real time under the microscope, and I was hooked! I hadn't thought about the biological part of biomedical sciences until that moment and, all of a sudden, I realised that it was the most fascinating aspect of it. We were actually watching the biology happen. I went on to work on macrophages as a thesis project.

I later went to England for my postdoc. As a graduate student, I had worked mainly on how macrophages are involved in lipid metabolism and how they might contribute to atherosclerosis. Now, I wanted to learn more about proteins. I moved to Strangeways Research Laboratory in Cambridge, England, and examined lysosomes and enzyme function in fibroblasts. The person working next to me was focused on matrix metalloproteinases, and I began to wonder if my cells might make

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these proteinases. When we tested this, it turned out that fibroblasts did generate metalloproteinases that allow them to remodel their surrounding support structures. Suddenly, I had to think about my cells in the context of their extracellular environment. I realised that we were looking at a fabulous biological system, with a careful balance of signals between cells and their environment. This became my area of focus as a postdoc and later evolved into the basis of my research on the ECM.

Once you realised that ECM was important, you incorporated it into your work with cells. However, there is a generation of literature that involved research of cultured cells that did not take the ECM into account. How do you think that what we are learning about the ECM now might influence the interpretation of cell culture studies?

What has happened, at long last, is that many people who have been focused on the structural aspects of the ECM are recognising the influence of tissue structure on biology. For example, there was early interest in the structure of the ECM in trades such as leather tanning, which dealt with skin ECM components such as collagen. But it took much longer for people to recognise that the functional unit in the body is not a cell, but a cell and the ECM that surrounds it. The discovery of integrins (cell-surface receptors that bind to ECM components such as collagen and fibronectin) in the 1980s pushed scientists to look at things outside the cell. Until then, people were much more interested in what was happening within cells. Once cell adhesion was recognised as an important determinant of cell fate, scientists began to look at behaviour and function in a more physiologic context – in three dimensions.

Scientists now recognise that making a tissue is really a three-dimensional process. Cells are surrounded by metabolites and signalling molecules, and by a complex matrix that allows cells to polarise. The whole science of polarity has emerged and moved forward with an interest in the ECM.

Although the ECM is important to every cell and probably has some function in the development of most diseases, do you think that there is a disease area that

might be strongly influenced by increased understanding of the ECM?

I think that there are many diseases that people will one day consider to be ECM diseases. There is a strong interest in the ECM with respect to its contribution to cancer – this is one area in which the link is already recognised, even though we don't thoroughly understand it. The contribution of the ECM to arthritis and atherosclerosis also seems fairly clear. However, the ECM is probably also very important for many brain diseases. There are several other examples where changes in the ECM may lead to disease, such as fibrosis and abnormal wound healing. Many diseases – in fact most diseases – are probably ECM diseases.

“There are many diseases that people will one day consider to be ECM diseases”

One disease area that people don't often think of in terms of the ECM is infection. The connection between infection and the ECM is not immediately clear, but, in fact, three-dimensional structure influences how bacteria and host cells respond to infection. The type of matrix that surrounds the host inflammatory cells determines their responses and dictates how well they can search out, find and kill invading bacteria in three-dimensional space. There have been very few studies looking at the influence of the ECM on the response to infection, and we must understand that these complex interactions occur in three-dimensions. When we study infection in two-dimensions, the diffusion of important molecules is very different than what actually occurs in a three-dimensional space. It's not the ratio of bacteria to killer cells that's important – it's the actual concentration of the cells and their secreted chemoattractants that allow them to find each other.

The ECM is a complicated place. Do you think that the technologies are available to take the ECM into account when examining disease physiology?

The ECM is very complicated because it's not homogeneous. Even at the level of a single cell, the matrix can differ over

submicron levels in terms of its structure and its function. The ECM differs on one side of the cell versus the other. So, the interaction of a cell with the ECM may vary across it, with adhesions in one area but not in another. This heterogeneity does not conform well to many powerful technologies, such as proteomics, microarrays or genomics. These technologies do not have the resolution to look at subcellular differences.

The ECM is also subject to many post-synthetic modifications. Many matrix proteins are cleaved in several places and can therefore be structurally quite different, despite their original similarities. Other ECM proteins are differentially spliced and have multiple splice variants. They also get crosslinked to each other and to other proteins. So, the alteration of one molecule can induce changes in a very complex structure. Because the overall structure of the ECM isn't homogeneous, the regulation can be altered quite markedly by subtle changes. These subtle changes can lead to cell transformation, and transformed cells keep making components that normal cells would not make. Therefore, the structure of the resulting ECM can be very different and may have a different influence on cell behaviour.

I hear from my colleagues that you are a respected mentor. What do you do to help students and postdocs become successful?

I give students and postdocs as much freedom as they can handle. I believe that, to become an independent scientist, you have to be given independence while you are developing your skills. As a mentor, I have to allow students to make mistakes. People learn more from making mistakes than they learn by avoiding mistakes. A mentor needs to provide a safe environment that allows students to become independent and to make the kinds of mistakes that can provide them with a valuable learning experience.

Do you customise a student's level of independence relative to what you feel each person can handle?

Independence should come as a student becomes competent enough to deal with it. I value their ideas, but whether I allow them to work completely on their own ideas or whether I vet their ideas before giving them

the go-ahead depends on how ready and confident they are. They have to have the self-confidence to follow through with their ideas, so I judge each person as an individual in that way.

It is also important to give each student examples of how to develop their careers. I try to inform them about everything they need for their careers to move ahead. Most people have lists of how to move along the research career path and get a job. These 'written rules' are relatively easy to follow; it's the unwritten ones that are difficult to identify. It is very important for a mentor to bring these unwritten rules to a student's attention. These include learning to be collegial and a good citizen, how to learn from adverse criticism, how to give credit to others without diminishing your own contributions and how to explain your work to people outside your field in an interesting way.

What characteristics are common among graduate students and postdocs who become very successful?

Successful scientists are self-starters. Your career is your own personal responsibility, no matter how much other people may want to help or inhibit your progress. My most successful trainees would never be in a position to say that they didn't do something because no-one ever explained the situation to them. They tend to find out what they need to know to get ahead to advance their own career, and usually know how they will proceed, years in advance.

Successful scientists are always pushing. They are the ones who say that they wish we had a journal club, and so they start one. They always want feedback on their project. They want to join collaborative research projects or progress groups for postdocs, or even start one themselves. Successful trainees look for opportunities to test their work.

Interestingly, the most successful scientists are not always the smartest students. There are many aspects beyond brilliance that influence a career, including how well you get along with other people (so they want to work with you), how thorough you are in getting your answers, the diligence and speed that you bring to your tasks. You may be brilliant, but if you never finish experiments or write about them, then it's like your work doesn't exist. Doing productive science means doing the science,

getting people to work with you in a productive way, getting your science out there so people can see it and being able to bring in the money that's necessary to have continuity in your research. You have to be able to do these things very quickly these days. That wasn't so true when I was moving up in my career, but now within about a year of becoming a faculty member you may no longer actually do any of the research with your own hands.

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If you were going to start your career over today, what would you most like to do?

I would change directions to follow the things that interest me the most. I've never felt too constrained to stay in a specific area. An interesting thing about successful scientists, which may be even more true about women in science, is that they often wander into areas that they can make their own. Often, they find something very interesting that no-one else was interested in and develop an area where they can work at their own pace. Developing areas aren't so competitive that scientists must live 'under the gun' to prevent getting scooped. When the big work does come out, it makes the author a leader in the field. There are several women whose science fits this description. I think this is in part because women were traditionally discouraged from pursuing the hottest science. Women tend to look for the questions that they personally find the most interesting in the context of their postdoc work, rather than following more mainstream ideas.

Do you think women purposefully avoid competitive areas?

I don't think women avoid competition in an obvious way, but they seem to have developed what might be thought of as a mechanism to survive in academic careers. Women scientists look for something that can be theirs rather than fighting for part of something that belongs to the men. I don't think women are even aware that they do it, but I see it happen a surprising amount. It is a basic science survival skill.

One example is Liz Blackburn, who won the Nobel Prize for her work with telomeres in the ciliate *Tetrahymena*. In the beginning, she was working in an area that no-one else was interested in, using a model organism that few other people worked with. This is just one example, but if you look, you will find that almost all successful women were working in something that wasn't interesting until they made it interesting. They had the foresight to know what was interesting. The biggest and most important challenge in science is identifying the right question and developing a meaningful project around it. It's exciting to be able to do this before it is obvious to anyone else.

Like your science, your childhood was also very unique. I understand that you were born in Germany towards the end of WW2?

I was born in a concentration camp, but my childhood memories seem like those of a typical kid. One of my first clear memories is from Italy when I was somewhere between two-and-a-half or three and someone gave me a bag of gumdrops. This was something very special. We were usually fed cornmeal three times a day because the Americans provided cornmeal to Europe after the war. I wouldn't eat anything with cornmeal until I was an adult because I had a negative memory of it. I didn't realise where my negative feelings toward cornmeal came from until later.

I ended up going with my mother to Poland after the war, and it took nearly 6 months to get to Italy, where we were reunited with my father. From Italy we went to Canada. Although it was complicated, I think that my childhood was much like any other childhood. I grew up in an immigrant family that didn't have much money but there was a value placed on education. I learned Newton's laws when I was 4. I always knew that I would go to university and that education would pave the way for my future. It was the hope that my parents had wished for themselves but, as it could not happen for them, it became even more important for their children. I grew up on a farm but I was protected from becoming burdened by too many chores because my studying was more important.

As a result, I'm a very pragmatic person. Like many women, I've experienced discrimination on occasion, but I've always

tried to make the best decision in the situation and then make the best of the result. At every point in life – whether deciding where my science is going to go next or where to take my career – I ask myself how I can make the situation into something successful. This mentality has helped me survive things that would have

made other people fall apart. For example, when I was in England, I had a job lined up in Seattle that fell through at the last minute. I got the fateful letter at the American Express office in Tokyo while I was travelling. What might have made most people fall apart turned out to be the best thing that ever happened to me. I

ended up at UCSF, which is a fantastic place to work.

We greatly appreciate Dr Zena Werb's time and willingness to share her thoughts about medical research. She was interviewed by Kristin H. Kain, and this article was condensed and edited with her permission.