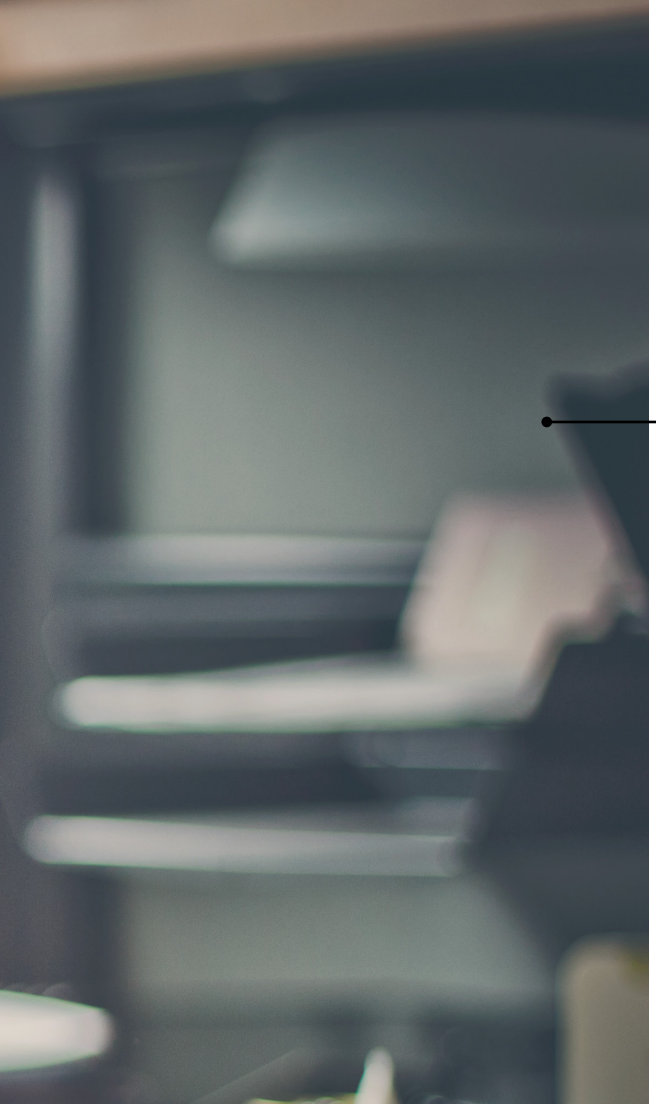
dr.sheila singh

The complexity of brain tumours

The Meducator recently sat down with Dr. Sheila Singh, Associate Professor and scientist at the Stem Cell and Cancer Research Institute at McMaster University, to discuss her ground-breaking research on brain tumour-initiating cells and her hopes for future generations of scientists. Dr. Singh explained to us how she was able to make her discovery and why the science of tumours is such an intricate one. She also shared her insights about the long road ahead for clinician-scientists and described why students should take their time with their careers.

COULD YOU TELL US ABOUT YOUR EDUCATION AND RESEARCH, SPECIFICALLY ABOUT THE BRAIN TUMOUR-INITIATING CELLS?

I started my program here just over five years ago, in August 2007. Prior to that, I completed my undergraduate degree in neurobiology at McGill University and came to McMaster University for medical school. I was then accepted into the neurosurgery residency at the University of Toronto, where during the course of my residency I undertook my Ph.D. within the Surgeon Scientist Program at the Hospital for Sick Children, in the Arthur and Sonia Labatt Brain Tumour Research Centre. I then worked in the lab of Dr. Peter Dirks. In his lab, we made a discovery in 2003 that there were abnormal stem cells that seemed to drive the formation of brain tumours. This was the first published description of the finding, so it opened up a new field of the cancer-stem cell hypothesis. After that discovery our work gained a lot of recognition, and now many people around the world use our model. I wanted to build on that momentum of discovery by opening my own laboratory here and following up on the work done in my Ph.D. When I finished my Ph.D., I did a paediatric neurosurgery fellowship at the Hospital for Sick Children, before finally coming here.



Dr. Sheila Singh, M.D., Ph.D., F.R.C.S.(C) Neurosurgery, is currently the Canada Research Chair in Human Cancer Stem Cell Biology

WHEN DID YOU FIRST GET A HINT OF THIS IMPORTANT HYPOTHESIS THAT CAME OUT OF YOUR RESEARCH?

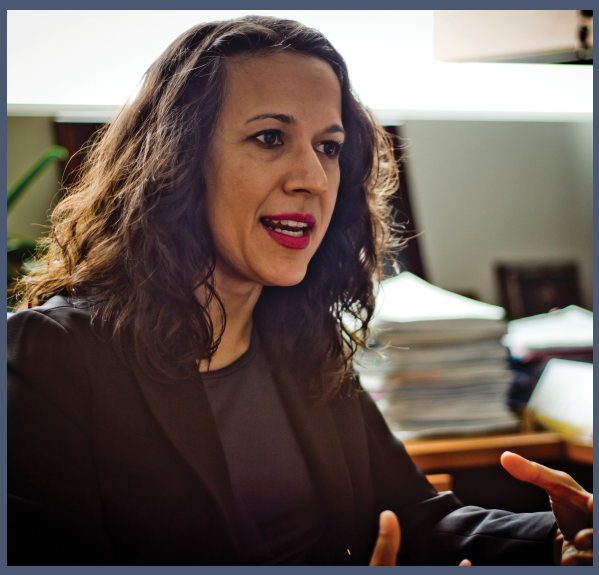
That's an excellent question because we did have a classic epiphany moment. Generally, when people study brain tumours, they use cell lines or study the expression of markers and other proteins of interest. There aren't many other models to study how cancers form, so we went to a famous Canadian scientist, Sam Weiss, who discovered neural stem cells back in 1992. He cultured cells from the subventricular zone of the mouse brain and put them into specific culture conditions. Keep in mind that the majority of cell lines require serum. Dr. Weiss developed these interesting conditions in which he knew that the only type of cell that could survive without serum is a stem cell, due to their unique resilience and ability to grow. In this culture method, you can place cells in serum-free media with specific growth factors, such as epidermal growth factor and fibroblast growth factor, and 99% of the cells in the dish will die because most differentiated cells cannot survive without serum. What you get rising from the ashes is the neural stem cells, which will thrive in those conditions, leaving you with a beautiful selective method of growing only stem cells. Peter Dirks and I used these culture methods. We took some brain tumour tissue from the operating room and grew these cells ourselves, under Sam Weiss's culture conditions. I remember plating the cells on the dish and wondering what would happen after I put them into the incubator. Neural stem cells grow as these beautiful floating colonies called neurospheres. A single stem cell will give rise to a whole colony of cells in one sphere. You then conduct a clonal assay by dissociating these spheres and plating them at single-cell density, then figuring out how many spheres are formed. This gives you a way of estimating clonal frequency of stem cells in your cell population. When you see a sphere, you know you have one stem cell because it has to have arisen from a single stem cell. I remember taking the dishes out of the incubator and looking at them under the microscope, and there in the dish were these spheres. That's when I knew there was a stem cell population in brain tumours. It was really a true moment of scientific epiphany.

From that, the idea emerged that tumours are made of cells that histologically all look the same, but not every cell in a tumour is capable of initiating a tumour, especially in a tumour with a variety of cells in it, like a glioblastoma multiforme (GBM). They all serve different functions within the tumour; some cells may form the support network. However, the model doesn't apply to tumours that are monoclonal. For example, B-cell lymphoma is not a good model for the cancer-stem cell hypothesis because that probably is a tumour that forms when one cell goes wrong and generates every other cell in a clonal manner. Similarly, melanoma may not be a cancer-stem cell type of tumour. But tumours that have that complexity, that are heterogeneous, that have multiple different lineages of cells within the same tumour, probably originate from a stem cell. So this model system gives you a way to study tumour heterogeneity, to order cells and figure out what they are.

IN YOUR TECHNOLOGY, EDUCATION AND DESIGN TALK, YOU MENTIONED YOUR DISCOVERY OF THE INTERCONVERSION BETWEEN $CD133^+$ AND $CD133^-$. COULD YOU ELABORATE ON THAT?

Most of the Cluster Differentiation (CD) markers are exclusively cell-surface receptors. CD133 is a five-transmembrane cell surface receptor. The utility of CD receptors is that they're expressed on the cell surface, so you can literally just use them to mark a cell population. One of the problems with these markers is that when you mark a cell population, you cannot make the assumption that the $CD133^+$ cells you've sorted are all stable. Going back to the principles of physics, we know that everything is constantly in motion; the concept of Schrodinger's Cat is very relevant to our field. No more than whether we know

the cat is dead or alive in the box, we really don't know whether a CD133+ cell will stay positive or if it fluctuates between different states. In all likelihood, we are not studying stable environments; cells are dynamic. So the complexity in science comes from our inability to study proteins or receptors in isolation. I think all scientists are studying one piece of the puzzle. The challenge for the next generation of scientists is going to be putting all these puzzle pieces together.



DR. SHEILA SINGH WAS THE FIRST RESEARCHER TO IDENTIFY THE PRESENCE OF STEM CELLS IN BRAIN TUMOURS IN 2003 WHILE COMPLETING HER PH.D. DURING HER SURGICAL RESIDENCY. DR. SINGH'S RESEARCH HAS SERVED AS THE BASIS FOR THE FORMATION OF THE CANCER-STEM CELL HYPOTHESIS THAT HAS LED THE WAY TO AN ENTIRELY NEW FRAMEWORK FOR UNDERSTANDING SELECT CANCERS AND ULTIMATELY GREATLY IMPACTING THE DEVELOPMENT OF CANCER DRUGS AND THERAPIES.

KEEPING IN MIND THE DYNAMIC NATURE OF CELLS, WHAT ARE THE IMPLICATIONS OF THESE FINDINGS FOR TECHNICAL FEASIBILITY OF POTENTIAL TREATMENTS?

Everything we know about the tumours we study is gathered from an initial pre-treatment biopsy. We take out a tumour and then we subject the patient to a million different treatments, but we're basing all this on one sampling time point of the tumour. There's an evolutionary biologist named Charles Swanton from London, England who applies evolutionary biology theory to the study of cellular populations. He published this breakthrough study in the *New England Journal of Medicine* last year, which showed that if you take ten different samples from a renal tumour you'll see ten totally distinct gene expression profiles. He went back to characterize the tumour and showed that it was a patchwork quilt of different grades within the same tumour. So depending on where the surgeon samples the tumour, that's how the patient will be treated; it's almost random. It shows the problem with cancer treatment. Cancer is a dynamic disease unfolding in a patient but all of our treatments are based on samples taken at the beginning.

What we realize more and more is that we need experimental models that capture how tumours evolve with time. Something we're doing in the lab right now is developing therapy models for our human tumours. We implant human tumours into mice and profile the stem cell population as they evolve through therapy. We treat the mice with chemotherapy and radiation therapy, then conduct a time point analysis at each state to see how the tumours change with treatments, which stem cell populations are responding to the treatments we're giving, which ones are resistant, and which are arising after the therapy. So, one way that developmental biology is applied to cancer is through this method of clonal tracking. We can figure out how the cancer cells are evolving through therapy by labelling a cell population at the beginning, then treating the cancer in the mouse with all the different treatments we use in humans and tracking that cell population over time.

DO THE CANCER CELLS THAT METASTASIZE TO THE BRAIN DIFFER IN ANY WAY FROM PRIMARY BRAIN CANCERS?

Yes and that is what we are currently studying. We are looking for some signature that would indicate migratory homing to the brain, so that we can identify which cells seek brain metastases and understand how and why they are able to form a secondary tumour in the brain. This project is important because, ultimately, these secondary tumours are fatal. We currently have no adequate treatment for such tumours. We do not have answers yet, but we are close to discovering what distinguishes tumour cells that migrate to the brain from tumour cells that originate and develop within the brain. It is important to understand how these cells migrate to the brain and how they evade the normal protective mechanisms like the blood-brain barrier and the immune system. There are many theories in

oncology that try to understand the molecular mechanisms of immune surveillance to explain how cancer cells escape the immune system.

DO YOU THINK THERE WILL BE A CURE FOR CANCER, OR WILL THIS FIELD OF MEDICINE REMAIN TREATMENT-ORIENTED?

I do not think there will be one cure for cancer. What we realize now is that there is not one GBM; every case of GBM is a different beast. A brain tumour that evolves in the brain of a 45 year-old male will be distinct from a similar tumour that evolves in that of an 85 year-old woman because of differences in the microenvironment. This is why patient-targeted and individualized therapy is becoming such a popular topic. I think the only way that we will ever be able to cure cancer will be on a case-by-case basis. You will have to be able to profile the patient's tumour by extracting the cells and conducting quick multi-level analyses to figure out what gene expression is present in each cell population of the tumour. Only then will you be able to determine which drug to give that patient. Another patient may present with the exact same tumour but we may find that a different cell population is causing trouble. Then we will target that cell population with another drug. I think that treating each patient with an individualized cancer therapy is the only way we will ever be able to manage cancer; it will not be a matter of curing it, but more so managing it. Part of successful individualized therapy will be based on detecting these populations at an earlier stage. The dream would be to detect these populations before they turn cancerous and prevent it from transforming ahead of time. But that is down the road as well. I am worried about the amount of money that will be necessary to profile at this level, because such procedures are still very expensive. This will not be sufficiently funded by the Ontario Health Insurance Plan unless the technology can catch up to the point that it can be incorporated into everyday diagnosis. The government should be investing their money into making these technologies high-throughput and affordable.

ARE THERE ANY SCIENTIFIC CONTROVERSIES IN YOUR FIELD?

Huge! One controversy exists regarding which cancers the cancer-stem cell hypothesis is relevant to and which cancers it does not apply to at all, because it is certainly not going to explain the bulk of cancers. We have controversies in our field about which markers actually identify brain tumour-initiating cells, and which sub-populations can be identified. We even have controversies about culture methods. There are so many controversies, and there always will be. You just have to figure out what works in your hands for your program and which methods result in models that are the most similar to the patient's disease course. If the findings in your dish recapitulate what the disease process is in humans, then you know it is a good model. We navigate those decisions everyday in science, trying to decide "Is this right?" or "Is this modelling what is happening in the patient?" If it is not, we do not bother with it. That is another reason for why it is beneficial to be a physician as well. I actually know whether a model is appropriate or not, which is very useful.

WHAT HAVE BEEN THE CHALLENGES OF BALANCING BOTH RESEARCH AND CLINICAL PRACTICE?

Balancing basic research and clinical practice is a constant challenge. Often you feel as if you are walking on a high wire. The only advice I have, which I give my trainees in the Surgeon Scientist program at McMaster University, is that the more continuity and feedback you have between the clinical practice and the research practice, the better. In the past, people would often study things that had nothing to do with the focus of their clinical practice, but the more similarities there are between the two, the better your work will be. When trying to work in two different fields, you have to be even more vigilant and recognize when something is outside your expertise. For example, I focus on brain tumours in my laboratory, and my work has focused and honed my brain tumour clinical practice, giving me more power to become an expert in one thing. If a patient presents with an aneurysm, which is outside my expertise, I will not hesitate to ask for help from my vascular surgeon colleague or even refer the case; it is very important for patients that you are staying within the realm of your expertise. The best advice I have is that if you are trying to work in two different fields in your practise and your research, you must maintain a very tight focus on both, so that you never endanger anyone on the clinical side nor will you claim to be able to do something outside your capacity on the research side. If you do stay tightly focused on one area, you will be able to approach a problem like brain tumours using all of the tools in your tool box. I have clinical ways and laboratory ways to study brain tumours, but it is all one research focus.

WHAT ADVICE WOULD YOU GIVE TO UNDERGRADUATE STUDENTS WHO ARE POTENTIALLY HESITANT ABOUT THIS LONG-TERM JOURNEY?

I would say that the only way to be able to serve your patients the best that you possibly can is to become an absolute expert in the area. And the only way to become an absolute expert in anything is to devote long hours and to work hard. My residency program was one of the toughest residency programs. When I came here and started as a staff paediatric neurosurgeon, Dr. Hollenberg, my senior partner, threw my pager at me and said, "I'm going to the lake for three weeks – bye!", leaving me on my own. The only way you can become equipped to handle those first weeks is through your training. I fell back on

everything from my training to succeed: every long night, experience, and difficult struggle to stay awake. That is what made me competent in the end. Sometimes I think we do not emphasize enough that those struggles are what make you the best physician. My advice to those students would be to not be afraid of struggle and a large workload. Do not worry if it will get you anywhere, or if it is really worth it. Do not think about that. If you love something and want to study it, then commit to it and become the best at it. The hard work will not pay off in the short run, but it pays off in the long run.

DO YOU THINK YOU WOULD BE ABLE TO HAVE THAT CONNECTION TO THE RESEARCH IF YOU WERE NOT PRACTICING?

No, I do not think I would be so deeply invested in it. That is another big benefit of being a clinician-scientist – it is hard not to be invested in what you do. You walk across that bridge to the hospital where you treat patients with brain tumours, then come here to sit in your office and work on your research. I am very lucky to have that direct connection. A lot of people study a topic in a lab simply because they are part of that lab and the supervisor chose to. But these people do not really know why they are studying it or what future benefit it will have. In my lab, I think my students do feel very lucky because they know exactly whom they are working to benefit. They are working for all of those patients in the hospital and they have a direct connection to the practise.

WITH THE HELP OF BIOINFORMATION AND COLLABORATION BETWEEN EXPERTS THAT WERE NOT PREVIOUSLY AS INVOLVED, WHAT DO YOU THINK THE NEXT BIG BREAKTHROUGH IS?

Well, just ten years ago we were all looking at the human genome and profiling individual genomes; that was a big breakthrough. I think we are really advancing to the level where we need to take all of that information and translate it into targeted therapies for patients. That is going to be the next big breakthrough. If there were a team of scientists that could collaboratively work on every cancer patient, maybe we could improve survival. Right now, the solutions are not financially feasible because we need the technology sector to help us work on making the tools accessible and cheap. It is about making the new wave of scientific discoveries accessible and trying to figure out how to generalize that technology to the population. In fact, it will take economists as well to work on this kind of problem – to understand how to deliver that degree of technological diagnostic care to the general population. It is all about translating technologies to patients. It will require a lot more people than just scientists in a laboratory. It will require bioinformaticians, health care economists, people in the technology sector, and more. The question is how to get these people to work together to deal with the disease? Just twenty to thirty years ago, science was such a different field. It was all about, "I am a scientist, I study sarcomas and my model is mice and my laboratory is in this building and I work by myself." That mindset does not exist anymore. Science is evolving into a team game. I hope I am going to be able to stay youthful enough in my philosophy to change with the times, and stay up to date with the new technology and new ideas.

YOUR CAREER HAS SPANNED A VERY SHORT PERIOD OF TIME, AND YOU STILL HAVE A GREAT DEAL OF TIME REMAINING IN YOUR CAREER. WHAT DO YOU HOPE YOUR LEGACY TO BE WHEN ALL IS SAID AND DONE?

I hope to be an active participant in this revolution of how therapies are administered to cancer patients, helping to shape how brain tumours are treated and managed in the future. I think that at the end of my career, I would like to have been able to contribute to advancing brain tumour therapy to a point where people survive things that they never would have been able to survive and are able to have a better quality of life. These are some of my patients who have not made it *[she points to pictures of young children behind her monitor]*, so at the end my career, I would like to know that patients with those same tumours are now surviving. That is what drives me and my laboratory program. We are working not towards a cure but to better outcomes and survival, and ways of managing things that we are not able to manage right now. It is bound to happen, so I just want to play a role and participate.

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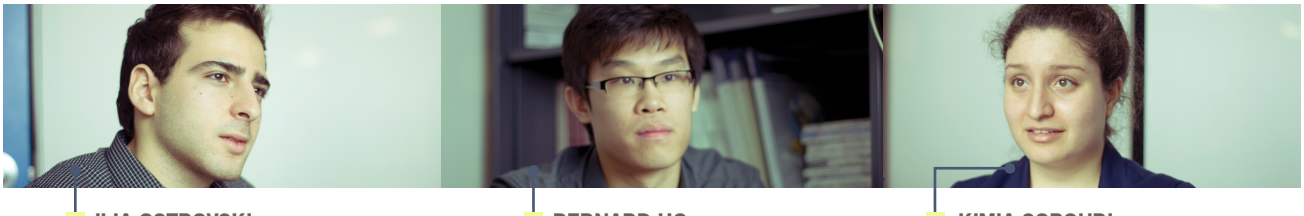

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