Abnormal brain tumour stem cells open therapeutic opportunities

Dr Sheila Singh is an Associate Professor of Surgery and Biochemistry and Chief Paediatric Neurosurgeon at McMaster Children’s Hospital. As a surgeon-scientist, she is leading the way in finding new drug targets for the most aggressive forms of brain tumours in the hope of discovering new therapy avenues to bring back to the clinic. Following her discovery of a population of abnormal stem-like cancer cells in some malignancies, her team are now investigating the genes responsible for driving the tumour’s ability to escape therapy.

Medulloblastoma is the most common malignant brain tumour in children. It originates in the cerebellar region of the brain, which is located in the hindbrain, and plays an important role in movement and coordination. Thanks to advances in multimodal therapies consisting jointly of surgical resection, radiation and chemotherapy, 75% of medulloblastoma patients can now expect a five-year survivorship. However, these regimes are extremely harsh and can be particularly damaging to a child’s developing brain. Therefore, because of this, severe neurocognitive and psychological deficits are often experienced by the patient.

Large-scale studies of paediatric medulloblastoma samples have revealed the presence of four tumour subgroups, two of which are particularly aggressive and associated with the worst outcomes, lowest survival and higher rates of relapse. Groups 1 and 2 are associated with more favourable outcomes and are classified by their continuous activation of certain molecular signals that appear to drive the tumour’s development. Groups 3 and 4, however, lack this characteristic signalling, and are defined only by their metastatic status and propensity to tumour relapse.

Fortunately, a significant breakthrough came when Dr Sheila Singh at McMaster Children’s Hospital identified a small population of cells within Group 3 and 4 tumours that seemed to drive the growth of the tumour. These rare cells were clonal in nature, and had the ability to initiate, proliferate and maintain tumour growth through stem cell-like behaviour. These cells were in fact a type of abnormal stem cell called brain tumour initiating cells (BTICs) – the first isolated cancer stem cells in the central nervous system. The implications of this discovery were huge as, in theory, it would now be possible to understand how brain tumours start, and potentially develop radical new treatments.

Brain tumour initiating cells

Dr Singh and her team set to work on unravelling the exclusive molecular mechanisms which govern BTICs within the most aggressive of the tumour subgroups. Once these have been identified, it should then be possible to develop new therapies that specifically target these cells, rather than the bulk of the tumour.

In contrast to BTICs, all other cells in the tumour have limited ability to proliferate and differentiate into different neurones. BTICs can differentiate into a variety of neurone types, and so therapies could potentially be developed that differentiate BTICs, thereby limiting their capacity for self-renewal and tumorigenicity. BTICs are also more resistant to radiotherapy and chemotherapy which may explain the poor responses observed in current bulk-tumour approaches. Identifying the genes that mark BTICs will provide insight into the biology of the cells actually responsible for driving tumour formation.

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Bright-field image of medulloblastoma spheres propagated in vitro. The expanded human medulloblastoma (MB) cell cultures can be used to screen drugs to identify the ones that can selectively inhibit proliferation and self-renewal of MB cells.

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The hypothesis being that removing these (programmed cell death) and differentiation, and observe the out these genes in vitro. They feel are the most promising. CRISPR-the BTICs that fuels the aggressive nature of responsible for the self-renewal capacity of some molecules that they believe are evade therapy. They have already identified responsible for the tumour’s ability to grow and spread, this will broaden treatment options and improve survival rates. Another key component of the research is to identify better surface markers for the BTICs so that they can be isolated more specifically and easily. In the clinical setting, this would greatly improve the accuracy of patient prognoses.

THE CASE OF TWO CHRISTOPHERS The story began when Dr Singh was in medical school and observed the tragic cases of two young boys with medulloblastomas. Coincidently, both were named Christopher, both were five years of age, and both were treated with the best available therapies. One of the boys flourished, the other died – leaving Dr Singh with a legacy of questions: “Why should two small boys with the same disease fare so differently?” What is different about each individual’s tumour? These questions fostered her interest in research and she continues to search for the answers to these questions to the current day.

CUTTING-EDGE RESEARCH Dr Singh and her team have devised an array of experiments using cutting-edge genome editing tools to identify the genes responsible for the tumour’s ability to evade therapy. They have already identified some molecules that they believe are responsible for the self-renewal capacity of the BTICs that fuels the aggressive nature of medulloblastoma. Her team have identified genes specifically upregulated in relapsed tumours and will now investigate the four they feel are the most promising. CRISPR-Cas9 technology will be used to knock out these genes in vitro and observe the consequences on proliferation, apoptosis (programmed cell death) and differentiation, as well as tumour size and survival benefit. The hypothesis being that removing these putative drivers of relapse will prevent relapse and metastatic dissemination in vivo. First, they will examine the effects of decreasing the expression of some of the genes in cells grown in a petri dish. Then, the ones showing the most promising results will be verified using mouse models. The team have already established a novel human-mouse model using cancerous tissue from the primary tumour of medulloblastoma patients.

Who are your collaborators and what are their roles in the project? We have multiple exciting multi-disciplinary collaborations with Prof Patrick Gunning, a medicinal chemist with whom we iteratively test new classes of STAT3 inhibitors and other novel compounds in our preclinical models of medulloblastoma; Prof Jason Moffat, with whom we explore the clonal composition of medulloblastoma in our dynamic model using cellular DNA barcoding and CRISPR-Cas9 technologies; Prof Michael Taylor, with whom we model and explore the genomic composition of treatment-refractory medulloblastoma; and Professors John Valliant (radiochemist) and Nicholas Bock (an imaging physicist), with whom we are developing new neuro-imaging protocols and molecular probes using MRI and PET to image medulloblastoma stem cells.

What are the strengths of the mouse model you have developed? Our patient-derived xenotransplantation model allows for the intracranial implantation of enriched medulloblastoma stem cells into an immuno-compromised host, and can serve as both a biological model for discovery of new therapeutic targets in brain tumours, and as a platform for preclinical testing of newly designed therapies. Because we are describing human cancer stem cell populations, discoveries in these models are one step closer to translation into cures for patients. Were you surprised by any of the genes of interest that your results generated? Many of the genes that we have found to be functionally important in driving medulloblastoma are key regulators of stem cell self-renewal. It’s not the nature of the genes, but the profound functional effect that they have on tumourigenicity that surprised us.

How could a drug differentiate of BTICs? A drug that disables the essential defining property of stem cells (such as self-renewal) will automatically drive differentiation, as in a sense, a differentiated cell is the opposite of a stem cell. A stem cell is primitive and committed to becoming a stable, mature cell type that no longer self-renews. Why are BTICs so resistant to current therapies? BTICs have multiple mechanisms by which they can escape therapies: they can remain in a “quiescent” state, and therefore avoid therapies like radiation and chemotherapy that target only actively proliferating cells; they have enhanced DNA repair mechanisms and can survive DNA-damaging therapies such as radiation; they have an increased expression of drug transporter channels and can efflux chemotherapy drugs rapidly out of their cell bodies.