Therapeutic breakthrough for malignant gliomas?

Dr Isabelle M Germano is a Professor of Neurosurgery, Neurology and Oncological Sciences and Director of The Comprehensive Brain Tumor Program at Mount Sinai School of Medicine. In the following interview, she discusses her use of stem cell-derived brain cells to treat brain tumours

Firstly, can you provide an overview of your current research efforts into malignant glioma therapy?

My laboratory conducts translational research of high-grade gliomas, the most aggressive type of primary brain tumours. My focus is to identify substances and genes that can kill brain tumours without interfering with healthy brain cells. I also study the mechanisms of cancer cell death and how to potentiate them. Recent findings indicate the importance of tumour stem cells – cells from which the tumours originates. These cells seem resistant, therefore we are aiming to develop therapeutic strategies to kill these brain tumour stem cells.

Why is the management of malignant gliomas such a challenging issue for neuroscience?

One of the main reasons why gliomas are so hard to target is that glial tumour cells intersperse themselves within normal brain tissue, making it harder to remove all tumour cells without damaging or removing normal healthy brain tissue. In addition, studies show that brain tumours derived from tumour stem cells do not respond to current therapies.

Another major challenge is the blood brain barrier (BBB). BBB restricts the passage of chemotherapy into the brain. To overcome this, 'vectors' are used to deliver substances that can selectively kill tumour cells. This approach was first attempted using viruses as vectors and genes as tools to kill tumour cells.

Furthermore, could you discuss the inherent limitations of employing viral vectors for therapeutic delivery to glioma tumour cells?

My team and I were the first investigators in New York State to use an adenovirus as a vector to kill tumour cells in glioblastoma patients. Results showed that patients in this trial had a longer survival rate than the historical controls, and without serious complications. However, subsequent trials and additional pre-clinical work showed that the use of viruses as vectors is limited by many factors, including lack of migration within the tissue, inefficient delivery, toxicity and a risk of changing the DNA of normal brain cells. In the search for new vectors, we turned to embryonic stem cells (ESCs), as they provide several theoretical advantages.

How might the use of embryonic stem cells (ESCs) be utilised to provide a more precise and permanent alternative to viral vectors?

We first introduced the concept that ESC-derived astrocytes can be used as gene therapy vectors. This theory was based on the fact that stem cells can migrate to brain tumours despite the BBB and can be manipulated to express therapeutic molecules. Together, these characteristics and the difficulties encountered in the use of viruses in gene therapy have prompted us to explore ESCs as vehicles for cell-based brain tumours gene therapy .

What impact does the migratory capacity of ESC-derived astrocytes have on tumour treatment, progression and general health?

Our ESC-derived astrocytes are programmed to produce and deliver the gene of choice only when they are turned 'on' by an internal switch. By inserting a promoter into their DNA, we can control gene transcription and activation. To turn on the gene we use an antibiotic called doxycycline. This mechanism allows for the cells to remain dormant in the patient until tumour progression, at which point the patient will start doxycyline intake. Our preliminary data shows that the implanted ESC-derived astrocytes do not cause any cognitive changes in the rodent model. More studies in higher mammals will have to substantiate these preliminary findings.

Could you elaborate on your studies into the apoptotic qualities of ESC-derived astrocytes? Do they cause cell death in healthy cells as well as in gliomas?

We have engineered ESC-astrocytes containing a promising gene called *mda-7/IL-24*, a cytokine that induces apoptosis in a variety of cancer cells while sparing healthy cells. When co-cultured with glioma cells, *mda7/IL24*-expressing ESC-derived astrocytes increased glioma cell death. Additionally, cell sensitivity to radiation went up and cells overcame resistance to temozolomide (TMZ), an oral chemotherapeutic agent currently considered the gold standard of malignant glioma treatments.

How soon could ESC-mediated delivery treatments be available to cancer patients? How do you see your work progressing in the future?

My current research focuses on how to move this successful tool from the bench to the bedside. I believe the main limitation is patient specificity. However, recently published results show that we can produce astrocytes from the patient's own skin, using induced pluripotent cell (iPSC) techniques. This technique allows for patient cells to be reprogrammed to pluripotent stem cells, and subsequently can be differentiated into astrocytes. This would allow for patient specific cells that would not induce an immune response upon introduction into the patient. These iPSC-derived astrocytes can be obtained with the protocol we recently published and be readily available, as a skin biopsy of the patient's arm is all that is required.

Stem cell solution

Researchers at Mount Sinai School of Medicine have successfully created stem cell-derived astrocytes that can deliver treatments to malignant gliomas, offering hope to patients suffering from brain tumours

GLIOMAS ARE TUMOURS derived from glial cells that form in the brain. These tumours are rarely curable and prognosis is generally poor. Glioblastomas are a subclass of gliomas that have the worst prognosis, with a life expectancy of less than one year and the five-year survival rate being less than 5 per cent. Treatment may include a combination of surgery, radiation and chemotherapy.

The brain tumour cells grow in complex arrangements within normal brain tissue and consequently, removal of the tumour hardly ever removes all malignant cells without damaging healthy tissue. For this reason, the glioma is rarely completely removed and tumour recurrence is inevitable. The critical point in creating a successful therapeutic strategy is ensuring it targets tumour cells while sparing healthy ones, passing the blood-brain barrier (BBB) to reach tumour cells. This barrier is created by tight junctions between blood vessel cells in the brain and creates high selectivity of what can leave the vasculature and enter the brain. To bypass this selectivity and allow for drugs to reach the tumour cells in the brain, physicians and scientists turn to so-called vectors, which can act as a vehicle.

GENE THERAPY

The first vector that scientists created to kill tumour cells was a virus, in an approach also referred to as viral-based gene therapy. This technique consists of an insertion or modification of a current gene in an individual's cell that fights cancer.

The first approved gene therapy procedure was performed on a four year old patient at the National Institutes of Health (NIH) in the U.S. in the early 1990s. The patient presented with severe combined immunodeficiency (SCID). Nearly a decade later, Germano and her team were state-wide pioneers in their use of an adenovirus as a vector to kill tumour cells in glioblastoma patients.

> ESC-derived astrocyte-mediated delivery of the gene tumour necrosis factor related apoptosis-inducing ligand (TRAIL) significantly induced apoptosis of malignant gliomas *in vitro* and *in vivo* while sparing normal brain cells.

The trial led by Germano started in 1997 and in 2003 their findings were published in the Journal of Neuro-Oncology. Patients who had enrolled in the trial had a longer survival rate than the historical controls, and did not present any serious complications. However, since then, subsequent trials and pre-clinical work has shown that the use of viruses is restricted by several factors including limiting migration in tissue, and subsequent inefficient delivery as well as toxicity ie. insertional mutagenesis. Based on these results, Germano and her team turned to stem cells as a new vector, which have several theoretical advantages.

EMBRYONIC STEM CELLS AS A NEW VECTOR

Germano's theory of using astrocytes derived from embryonic stem cells (ESCs) was based on the fact that stem cells can easily migrate to brain tumours through the BBB. In addition, these cells can be manipulated into expressing therapeutic molecules that can target tumour cells. These ESC-derived astrocytes can be administered into the brain tissue following surgery or through a needle if no surgery precedes the treatment. Both the post-surgical or needle-based approach would require targeting to the tumour. However, researchers continuously explore whether these astrocytes can also be administered intravenously, since stem cells are attracted by the tumours to a certain extent, and may locate their target independently.

These cells have the capacity to be turned on only when their activity is required. More specifically, Germano engineered their DNA to contain a promoter sequence that precedes the gene that needs to be transcribed. This promoter can be turned on when doxycyline, an antibiotic with

TRANSGENIC IN VIVO DELIVERY OF MDA7/IL24 FOR MALIGNANT GLIOMA THERAPY

OBJECTIVES

This research explores the concept that the migratory characteristics of ESC-derived astrocytes combined with the capability of carrying transgenes tightly regulated by external control, makes ESC-mediated gene delivery a promising new treatment modality for human malignant gliomas.

KEY COLLABORATORS

The following collaborators, presented in alphabetical order, were fundemental for this research. We regret we cannot list all the people involved in the successful execution of these experiments.

Ron Benveniste, MD, Assistant Professor of Neurosurgery, University of Miami

Luni Emdad, MD, Research Assistant Professor Neurosurgery, Mount Sinai School of Medicine, New York

Paul Fisher, PhD, Professor and Chair, Department of Human and Molecular Genetics Director, VCU Institute of Molecular Medicine, Virginia Commonwealth University

Gordon Keller, PhD, Director of the McEwen Centre for Regenerative Medicine at the University Health Network in Toronto and Canada Research Chair in Embryonic Stem Cells

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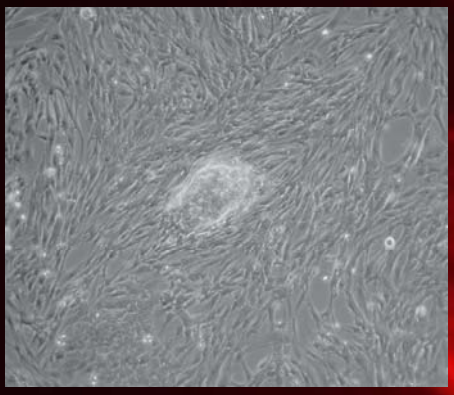
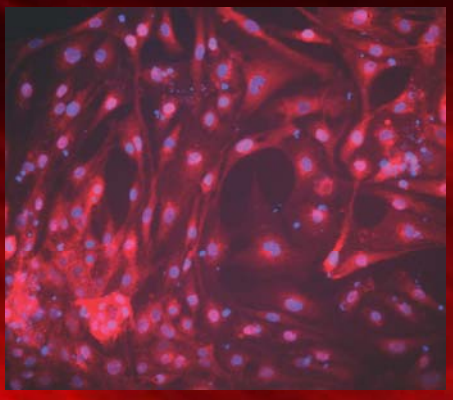
DR GERMANO has a very active basic science laboratory focused on translational research for brain tumors. For the past 10 years, her multidisciplinary research focused on developing preclinical data for new strategies to treat malignant gliomas. Her recent research proposal submitted to the National Institute of Health (NIH) focused on the use of embryonic stem cells to deliver genes to fight malignant gliomas.



EMBRYONIC STEM CELL (ESC) SURROUNDED BY DIFFERENTIATED ESC-DERIVED ASTROCYTES



ESC-DERIVED ASTROCYTES EXPRESS THE INSERTED GENE TO KILL MALIGNANT BRAIN TUMOUR CELLS AFTER ADMINISTRATION OF ANTIBIOTICS TO TURN THE GENE ON

very few side effects, is prescribed. Therefore, unless doxycyline is administered, the astrocytes will lay dormant in the patient until they are needed.

TRAIL AND MDA7/IL-24

Germano's experiments have shown that ESC-derived astrocyte-mediated delivery of the gene tumour necrosis factor related apoptosis-inducing ligand (TRAIL) significantly induced apoptosis of malignant gliomas in vitro and in vivo while sparing normal brain cells. However, since the effects of TRAIL were not uniform across all glioma cells, ESC-astrocytes were engineered containing another promising gene, the melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24), a cytokine that induces apoptosis in cancer cells while sparing healthy ones. These astrocytes containing the cytokine caused increased glioma cell death while sparing healthy brain cells, and additionally these cells increased tumour cell sensitivity to radiation.

CREATING IPSCS

Despite the promising initial results demonstrated by the ESC-derived astrocytes, the findings were not without limitations. One such difficulty was in the complicated search for a specific match, making it mandatory for patients to receive immuno-supressive agents in parallel to avoid rejection. To overcome this challenge, Germano's team focused their recent efforts on creating patient specific cells. They have published results showing how they successfully produced astrocytes from the patient's very own skin, using what is known as induced pluripotent cell (iPSC) technique. Following a minimally invasive skin biopsy, skin cells are re-programmed into stem cells and then astrocytes. Currently, they are working at modifying the patient's DNA to express a conditionally expressed gene tightly controlled by doxycycline as was the case in the ESC cells.

COLLABORATION IS KEY

Germano knows her research is heavily dependent on teamwork. "No man is an island and my research requires significant collaboration," she comments. "It is impossible for a single person to come up with the ideas, techniques, and clinical applications that high-tech translational research requires." It is with collaboration and commitment to research that Germano is able to help her patients, not only through surgery, but by providing them with tailored and improved adjuvant therapeutics.