Tikanga in the Laboratory: Engaging Safe Practice

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Abstract: The main focus is to investigate how human brain mitochondria are involved in the development of Huntington's disease. The mitochondria are tiny structures involved in energy, cell death and survival. They are thought to be involved in the disease process leading to the loss of brain cells and eventually to death. The emphasis of this paper is on the scientific methods that we use and the processes we have put in place for developing culturally appropriate methods.

In our laboratory we use several methods that are considered to be "cutting edge", including growing cells from post-mortem and post-operative brain tissue, and the use of post-mortem brain tissue for molecular techniques. In short we work with human tissue. As a consequence, we feel it is important to examine the cultural, ethical and spiritual implications of working with human tissue. Part of Melanie's PhD journey has been about exploring the concept and use of tikanga in the modern world, talking to her iwi about this research, and consulting with Māori to develop appropriate tikanga for use in the laboratory.

Keywords: Huntington's disease, mitochondria, tikanga

Introduction

There are two parts to this paper. The first section covers some of the science and methodologies used to examine Huntington's disease at the molecular level. The second part addresses the cultural implications of our work and discusses a number of tikanga practices we have established to ensure cultural safety in the laboratory.

Huntington's disease

The main focus for Melanie's doctoral research is to investigate the involvement of mitochondria in Huntington's disease. The mitochondria are tiny structures involved in energy, cell death and survival. Huntington's disease (HD) is an autosomal dominant neurodegenerative disease. This means that only one copy of the defective gene is required to cause neurodegeneration. The gene involved is called IT15 (Interesting Transcript 15) and is positioned on chromosome 4 (Huntington's Disease Collaborative Research Group, 1993). When the HD mutation is present, a specific pattern of neurodegeneration is produced due to neurons (brain cells which carry information) in the striatum and cerebral cortex dying prematurely (Vonsattel, Myers, Stevens, Ferrante, Bird & Richardson, 1985). The death of these cells leads to a number of specific changes in behaviour, including changes to movement, mood and cognition. The motor disorder is often the most noticeable sign of HD and involves dance-like choreiform movements (Barbeau, Duvoisin, Gerstenbrand, Lakke, Marsden, & Stern, 1981). However, later in the disease course rigidity may also occur (Young, Shoulson, Penney, Starosta-Rubinstein, Gomez & Travers, 1986). It is not unknown for motor symptoms to be altogether absent (Tippett, Waldvogel, Thomas, Hogg & van Roon-Mom, Synek, Graybiel & Faull, 2007). The mood symptoms cover a broad spectrum of psychiatric disturbances, including aggression, depression, suicidal tendencies, psychoses, and anxiety. It is this component which can lead to severe personality change. Moreover, mood symptoms often precede the movement disorder (Kremer, 2002). Cognitive deficits involving attention, memory and learning may also be affected in HD.

Mitochondrial involvement in Huntington's disease

The mitochondria have previously been implicated in Huntington's disease. For example HD metabolic abnormalities have been found in HD patients (Kuwert, Lange, Langen, Herzog, Aulich & Feinendegen, 1990; Leenders, Frackowiak, Quinn, & Marsden, 1986). In addition since all known cell death signals involve the mitochondria; their involvement in neurodegeneration is highly plausible. Some of the most compelling evidence of mitochondrial involvement in HD has been identified in recent years. First, an interaction between huntington protein and the outer mitochondrial membrane has been shown. When the interaction involved mutant huntington, the membrane permeability transition which initiates cell suicide, was accelerated (Choo, Johnson, MacDonald, Detloff, & Lesort, 2005). That interaction has been implicated in the vulnerability of striatal cells, which are lost in HD (Seong, Ivanova, Lee, Choo, Fossale & Anderson, 2005). One of the limitations of this work is that it has been done with transgenic mouse models or non brain-derived cell culture models using lymphoblasts (from blood samples) or fibroblasts (from skin samples) of people with HD. The relevance of this work is limited, since the human studies have been on non brain-derived tissue.

Cutting-edge methods

In our laboratory those limitations are overcome because we use human brain tissue to study Huntington's disease. We use several cutting-edge methods, including growing cells from post-mortem and post-operative brain tissue, and the use of post-mortem brain tissue for molecular techniques. For primary human glial cell culture, post-operative or short post-mortem delay brain tissue (<6 hours) is cut into small pieces and enzymatically digested. The cell suspension is then plated out in optimal growth conditions and the resulting culture is used for experiments, see figures 1A and 1B. The advantage of using these primary human glial cells is that they are brain-derived, non-cancerous and non-transformed (as are often used in cell culture). Of great significance with Huntington's disease primary culture, is that the cells contain the actual genetic mutation and background of the donor. Human brain tissue is also used for immunohistochemistry, a technique used to detect the expression and location of a protein of interest. A primary antibody which is specific to the protein is incubated with the tissue, followed by a secondary antibody which has fluorescent properties which are able to be detected, see figures 1C and 1D.

As shown in Figure 1B (human brain astrocytes from an epilepsy case) these methods are quite amazing. Since, mitochondria are involved in a number of other neurological disorders (e.g. Alzheimer's, Parkinson's and stroke); our work is also likely to have wider implications for diseases other than HD.

However, there are cultural, ethical and spiritual implications of working with human brain tissue. So the question must be asked. Why use human tissue? One of the biggest advantages is that it is the actual tissue which is affected by the disease, rather than a model system which can never give the full story. Moreover, the whakapapa of the tissue is very powerful. Before there were any genetic tests for Huntington's disease, the only way to diagnose HD for certain was to look at the specific pattern of cell loss in the post-mortem brain. Whānau with the disease, often approached our research group and asked if it would be possible to undertake post-mortem diagnostic studies. Sometimes we found that the deceased did not have Huntington's disease; it was like giving the family a million dollars. So the origins of our human brain studies had its basis in whānaungatanga and manaakitanga. That is, people wanting to be able to make informed decisions about the well being of their whānau. From these small beginnings, something bigger grew. It is important to reiterate that we never ask

people to give their roro (brain) to us – the initial approach and final offer comes from the whānau. We talk to the community (Huntington's disease, Parkinson's disease, Alzheimer disease foundations and support groups) and to families about our research. The whānau see our research as giving them hope.

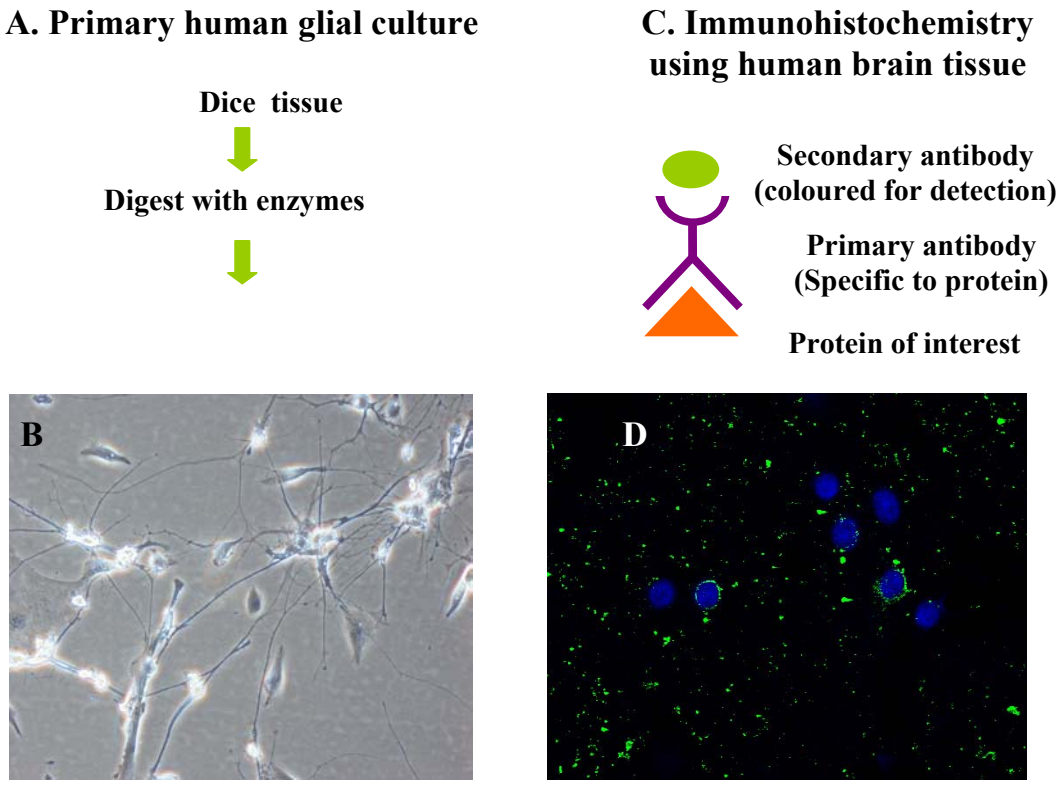

Figure 1. Cutting edge methods

1A. Primary human glial culture: flow chart of primary culture procedures
1B. Primary human glial culture: brightfield micrograph of E135 (temporal lobe post-operative tissue from epilepsy surgery) Day 14, 200x magnification.
1C. Immunohistochemistry using human brain tissue: diagram of immuno-histochemical procedure.
1D. Immunohistochemistry using human brain tissue: confocal micrograph of H129 (normal cingulate gyrus) blue stain is Hoechst which detects DNA, green stain is porin complexed to Alexa488, a mitochondrial marker, magnification 1200x

Tikanga and Science

Melanie realised that undertaking research on human brain tissue was culturally challenging;, so discussions were held with her kaumātua and whānau about the project before beginning. Much of this discussion about tikanga comes from kōrero kanohi ki te kanohi (face to face) with her Māori friends, whānau and academics. A very helpful definition of tikanga is that of Hirini Moko Mead who described it as a body of knowledge and customary practices carried out characteristically by communities (Mead, 2003). In considering this definition of tikanga and how it applies to science, the following questions arise:

- What are the Māori cultural implications of working with human brain tissue?

- What are the ethical implications of working with human brain tissue?
- How might tikanga apply to cutting edge scientific methods?
- How can we develop appropriate tikanga for use in the laboratory?

The answers to these questions are not always clear, because trying to apply tikanga to the laboratory is something our tupuna didn't necessarily have to deal with. However, the process of searching for the answers has been rewarding.

In struggling to answer these questions, we realised that it is only in contemplating the values and principles that underlie tikanga that we are truly able to engage questions about tikanga (Mead, 2003). There are a number of Māori core values outlined by Mead (2003) and Henry (1999) which inform tikanga. These values are held in high esteem and form a cornerstone of our cultural ideals. They are tapu, mana, take-utu-ea, mauri, whānaungatanga, manaakitanga, tika, noa, wairuatanga, kotahitanga and kaitiakitanga; these values enable us to engage these questions in a meaningful way (Mead, 2003; Henry, 1999).

What are the (Māori) cultural implications of working with human brain tissue?

Because the head is tapu, working with human brain tissue can be seen as a breach of tapu. Therefore, whakanoa should be used. To do this we use karakia, waiata and wai. We must also ask, if the benefits outweigh the breach of tapu (Mead, 2003). Since HD is a genetic disease, it's about whānau, and there are whānau who benefit from the perceived breach of tapu. Thus the value of whānaungatanga is upheld. He aha ngā mea nui o te ao? He tangata! He tangata! He tangata! We have also been building relationships with Māori Huntington's whānau. In particular, we have been working closely with a Māori whānau who bequeathed their father/husband's roro. They say that regardless of any cultural reservations that they might have had, because their daughter is an at-risk individual, they were committed to donating the brain of their loved one for their daughter's future. We also want to keep the kaupapa of our work with Māori clear; i.e. we are not working with and talking to Māori to seek tissue, but rather to learn more about how Māori whānau are dealing with HD and how we can assist them. Ultimately, we want to build and nurture reciprocal and respectful relationships with Māori about our research and about tikanga. We are also aware that there is a kaitiaki role and responsibility that we have to all whānau that have bequeathed their loved ones roro. We take that role very seriously in terms of being respectful of the tissue.

What are the ethical implications of working with human brain tissue?

The ethical issues involve consent, justice, respect, social responsibility, privacy and confidentiality (Hudson, 2004). In terms of consent, when an individual bequeaths their tissue, the whānau is always actively involved and we only accept the bequest if all the whānau are supportive and in agreement. This recognises that for Māori, the collective decision making process is an important one. Our sense of social responsibility is one of the reasons why we have a lot of community involvement, especially with community organisations and families.

How might tikanga apply to cutting edge scientific methods? How can we develop appropriate tikanga for use in the laboratory?

We believe that using Māori core values to inform the decision making processes will guide this process. We recognise that the development of appropriate tikanga in the laboratory will be an ongoing process. We have sought the guidance of kaumātua, kuia, whānau, hapü, iwi and tikanga scholars to inform the tikanga used in the laboratory. Our first hui with Melanie's iwi, Ngāti Rangitihi, has been a milestone in our research. To have the blessing of Melanie's iwi for our research is the first step in applying tikanga to our work. We had the most wonderful enriching day of manaaki, aroha, titiro, whakarongo, me korero and look forward to our next hui in November 2007.

Figure 2. Visit with Ngāti Rangitihi - Räkauheketara, June 2006

In conclusion, we believe there is a way to remain true to Māoritanga, yet move forward with science. Part of that process is acknowledging that there is not just one Māori view, but that there are many. We must not marginalise our own voices as we search for answers. We have found that it is only in contemplating the values and principles that underlie tikanga that we are truly able to appreciate tikanga Maori.

It is particularly apt to end with the beautiful whakataukī which is the overarching theme of this conference:

Mā te rongo, ka mōhio	*Through resonance comes cognisance*
Mā te mōhio, ka mārama	*Through cognisance comes understanding*
Mā te mārama, ka mātau	*Through understanding comes knowledge*
Mā te mātau, ka ora	*Through knowledge comes life and well-being*

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Author Notes

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