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Tough times for biotech
BIO 2009 PREVIEW

Patent on the gene
Who owns the rights?

HOT ARABIDOPSIS and its triplet trouble

Cell biology, stem cells, pathology & diagnostics

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Last year, Australian biotech Genetic Technologies announced it would enforce its rights to testing for the BRCA1 and BRCA2 cancer mutations, and all hell broke loose. The decision has since been reversed by founder Dr Mervyn Jacobson, who spoke at length to Graeme O'Neill. We also take a look at a triplet repeat expansion in Arabidopsis, and the complex genetic interactions responsible for two rare metabolic disorders.

20 BIO 2009 – state of the nation

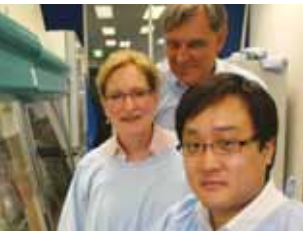
The biotech industry, along with the global financial industry, is in crisis. While there are many strong biotech companies in Australia, some are destined to fold. We talk to some of the movers and shakers of the biotech industry and talk to Senator Kim Carr and industry analysts on what can be done to shepherd biotechnology through the economic fallout.

28 BIO 2009 – profiles

In the second part of our BIO 2009 preview, we profile some of the best and most promising biotechs in the country. These include Hexima, Australia's first agbiotech that recently signed a big deal with global giant DuPont; Novogen, which is in Phase III trials with an isoflavones based anti-cancer compound; Implicit Bioscience, which has taken a different slant on the biotech business, and a brief look at what else has been happening in biotech recently. We also look at who is exhibiting on the Australian Pavilion at BIO.

44 Cell biology

Fiona Wylie talks to Jenny Gamble, a speaker at the Hunter Cellular Biology meeting, about the role of senescence in vascular cells in their twilight years, and to Dominique Soldati-Favre about a little host-parasite interaction. We also look at developments in malaria, and in high content screening.



56 Stem cells

In our stem cells feature, we muscle up with chemo-resistant stem cells and Peter Gunning, who is involved in a team that recently re-grew muscle fibres using adult stem cells. We also look at Australia's very own line of induced pluripotent stem (iPS) cells with Paul Verma; and at how Ben Herbert and his team are using mesenchymal stem cells to treat dogs with dodgy hips.

63 Pathology and diagnostics

Australian researchers have developed a highly specific PCR-based technique to distinguish between species of intestinal and liver flukes. We talk to Rebecca Traub about her work on these widespread and often dangerous parasites, and about her main interest, canine parasitic zoonoses.

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Muscling up with chemo-resistant stem cells

An Australian team has been able to re-grow skeletal muscle in a mouse model using a combination of chemo-ablation and chemo-resistant stem cells. Using what team member Peter Gunning calls a very simple idea, clinical trials are not far away, as Kate McDonald reports.

TEN YEARS AGO, an Australian oncologist called Dr Geoff McCowage arrived back in Sydney from a stint in the US announcing he wanted to investigate a new treatment for brain cancer, in which the bone marrow is genetically manipulated to be chemo-resistant to allow more aggressive treatment of the tumours.

The idea was that by introducing a mutant version of a DNA repair gene that was chemo-resistant, you could use alkylating chemotherapy to destroy endogenous cells while the transplanted cells remained unaffected.

While it sounds a rather radical concept, it is currently being used in haematopoietic stem cell transplantation, a strategy primarily developed by Stan Gerson's lab at Case Western Reserve University in the US.

Now, an Australian team has taken the idea much further and has managed to grow new muscle fibres in a mouse model from adult stem cells using the chemo strategy.

McCowage works at the Children's Hospital at Westmead in Sydney, where Professor Peter Gunning was previously head of the oncology research unit. About four years ago, Gunning was shanghaied into attending a public lecture during Medical Research Week.

Dr Geraldine O'Neill, group leader of the focal adhesion biology group at Westmead, asked Gunning and the muscle biology expert Professor Edna Hardeman to attend a series of lectures to make up the numbers.

One lecture was on stem cell therapies. Gunning says both he and Hardeman had the same thought at the same time – they could theoretically apply chemo-resistance to drive stem cell engraftment in any tissue.

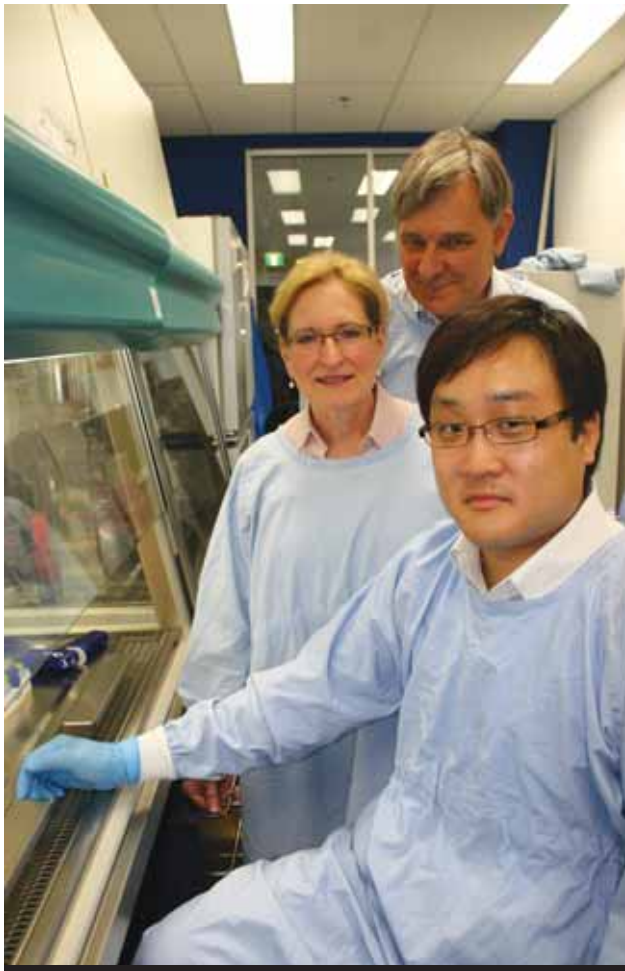
"Edna's the muscle guru so we thought; why don't we try for muscle?" Gunning says. "It's not that hard a concept, and Edna's lab does this stuff all the time. So we thought about how we'd mimic a bone-marrow transplant, except do it in muscle."

The result, four years down the track, is spectacular. In a paper published online in *Stem Cells* in February, Gunning and Hardeman, now both at the University of NSW, their colleagues Dr Antonio Lee and Prathibha Kahatapitiya, and McCowage and colleagues at Westmead, reported growing a new muscle in a mouse, something that has been tried without much success for decades.

While the idea of regenerating a whole new muscle, particularly in muscular dystrophy patients, using stem cells has been attempted for 30 years, it has never worked. The main problem is that the stem cells usually die off within an hour or so – they simply cannot compete with existing cells.

"So, get rid of the existing cells by chemo ablation, inject stem cells that are chemo-resistant, give a few more blasts of chemo and Bob's your uncle," Gunning says.

"It is that simple. It's the same as with bone marrow – you activate the bone marrow stem cells and hit them with chemo, and you can do the same thing with muscle. The bottom line is that you need to deal with getting the stem cells into the stem cell niche and you need to get rid of the competitors."



Antonio Lee, Edna Hardeman and Peter Gunning

Enrichment strategy

Every cell in the body contains MGMT, a gene which codes for O^6-methylguanine-DNA methyltransferase, a DNA repair enzyme which basically demethylates DNA. MGMT has been studied for many years because of its relation to cancer therapy – it has the ability to repair chemotherapy-induced DNA alkylation, and thereby reduces the effectiveness of alkylating chemo.

When administering alkylating agents, which prevent the cell from dividing, the idea is to also include an analogue of MGMT called O^6benzylguanine (O^6BG), which inhibits normal MGMT activity. This renders the enzyme non-functional and allows the alkylating agents to do their job.

However, there is also a mutant form of the gene called MGMT(P140K), which is not inhibited by O^6BG. In the strategy designed by Gerson and used to great effect in haematopoietic stem cell transplants, alkylating chemotherapy and O^6BG are used on the host's endogenous stem cells, while MGMT(P140K) is added to the donor stem cells.

This enrichment strategy has seen donor cell engraftment of between 75 and 100 per cent after repeated rounds of selection, Gunning says. "It is phenomenally effective in haematopoietic stem cell transplants. You can

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start with a very low level of transplantation and if you've got chemo-resistant stem cells you can drive them up to 70, 80 or 90 per cent of the total bone marrow just by using multiple rounds of chemo-therapy."

Gunning believes that a big problem in previous muscle stem cell research has been the difficulty in ejecting endogenous stem cells to make room for incoming stem cells. "It has become increasingly clear that the specific environment in which the stem cell finds itself is incredibly important," he says.

As for the muscle stem cells, the Australian researchers are working with a team from the Pasteur Institute, led by Didier Montarras, that is doing pioneering work in identifying how to select the appropriate muscle stem cells for transplantation.

"Didier gave us access to his information prior to publication, so that allowed us to move quickly to identify what stem cells to use," Gunning says.

"The second French connection is a group in Paris that is developing a stem cell trial for oculo-pharyngeal muscular dystrophy. This dystrophy weakens the muscle in the throat,

> **"Rather than just having a tissue that is self-sufficient, what it is indicating is that there are ways in which injured tissue can in fact draw stem cells from additional sites in the body."**

Other researchers have thought about ablating the stem cell muscle bed by using radiation, in order to create a niche for the new stem cells. The problem is that the toxicity associated with radiation and its severe effects on the kidney rules it out.

"The great thing about the chemotherapy approach is that it actually doesn't result in kidney failure," Gunning says. "I was at a stem cell conference about two and half years ago, talking to Terry Partridge, who did some of the very first experiments on [muscle stem cell] transplantation, and he told me they had considered using chemotherapy as a means of getting rid of competitors, but he just hadn't pursued it."

To the clinic

When Gunning, Hardeman and co were thinking about using this strategy for muscle stem cells, they had the foresight to create a transgenic mouse model that ubiquitously expresses the chemo-resistance gene. At the same time, a new research facility was built at the Children's Hospital at Westmead for gene therapy, which included a vector production laboratory.

Dr Ian Alexander, head of the gene therapy research unit, is responsible for producing the vector to deliver the mutant gene. The vector, a standard MFG first developed by Richard Mulligan from Harvard Medical School, has been validated and is awaiting approval from the TGA. Then, hopefully, it is on to the clinic for the original brain tumour trial.

and our approach is ideally suited to treatment of a single muscle. We are at the point now where we want to move to testing human cells in the mouse model, in collaboration with that group in France.

"If that works out then the plan would be to then move it into clinical applications, piggybacking on what they are already doing. It's one of those stories where things come together in the most unexpected way and you look at it and think: I can't believe it. It's also a good example of good ideas colliding by accident and leading to a new way of thinking about an old problem."

Calling on the mesenchyme

In the meantime, there is something else extremely interesting about the mouse muscle cell experiments. Upon transplantation of the donor stem cells isolated from regenerating skeletal muscle into injured mice, donor cells showed enhanced engraftment after seven days. After 14 days, donor-derived new muscle fibre formation was identified.

Then the researchers noticed something else very interesting. Not only were new muscle fibres growing from donor stem cells, but host cells also seemed to be contributing to regeneration, even after chemo-ablation.

Gunning doesn't want to discuss this finding in too much detail, as it is the focus of current work, but he admits it is extremely exciting. Satellite cells, the stem cells already resident in skeletal muscles, seem to be significantly contributing to the regeneration of the chemo-ablated muscle.

As the researchers point out in their paper, in the absence of transplanted chemo-resistant cells, the ablated area is not able to regenerate. But in this case, endogenous cells seem to be helping in some way. There are a couple of hypotheses as to why this should be happening, including the possibility that the donor-derived myofibres are attracting some other cells.

This could be due to the chemokine stromal-derived factor 1 (SDF-1), which is a chemo-attractant for cells expressing its G-protein coupled receptor CXCR4.

"It is well-established that SDF-1 is released from regenerating muscles, but more importantly, that undifferentiated muscle satellite cells express CXCR4 and respond strongly to the chemo-attractant gradient," the researchers write.

If not that, then "it is possible that one or a small number of satellite cells within the recipient and/or neighbouring muscles was spared from alkylation, responded to signals from engrafted donor cells and contributed the significant endogenous regeneration".

Or it could be something else: mesenchymal stem cells. These bone-marrow derived stem cells that circulate in the peripheral blood are able to differentiate into a number of different tissues, including bone, muscle, cartilage, tendon and fat and are the subject of a great deal of both basic and clinical research.

Is it possible that the destruction of skeletal muscle sends out a signal to mesenchymal stem cells to get into action?

Gunning is coy in his response, but admits he thinks mesenchymal stem cells are the number one candidate. "It could be from the surrounding muscle," he says. "Forty per cent of your body is muscle, so nearby muscles might actually be doing it themselves. But my bet is that it's probably blood-borne."

What this research shows is that not only is the stem cell niche probably the most important factor in successful tissue regeneration, but that there are mechanisms in the body that go beyond even this. "Rather than just having a tissue that is self-sufficient, what it is indicating is that there are ways in which injured tissue can in fact draw stem cells from additional sites in the body.

"Where that is coming from, we don't know, but we've advanced further down that path and it's a very real and incredibly exciting observation." ALS