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Genomics to the rescue of the devil

My studies of marsupial genetics and genomics over decades have revealed many unexpected truths about the human genome. The recent origins (and ignominious decline) of our sex chromosomes; what evolution tells us about the way blood proteins evolved to carry oxygen; our accidental discovery of many new human genes. But many times I have been asked, "But what can genomics do for marsupials?" Now we have an answer.

The Tasmanian devil, *Sarcophilus harissii*, is the biggest marsupial carnivore since we 'extincted' its relative, the Tasmanian tiger, in 1936. The devil has a reputation as a noisy and belligerent carnivore, but it is the icon of Tasmania, and beloved internationally as the cartoon character 'Tas'.

Dr Liz Murchison with the devil

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But in 1996, the first signs of trouble appeared; some animals with a horrible facial tumour were noticed in the northeast of the island. The cancer developed around the mouth of the animal, and within a few months ate up the mouth and sometimes the eyes and the whole face, killing the animal in just a few months.

Alarm bells really started to go off a few years later when it became obvious that the cancer was spreading in the NE of Tasmania. Affected animals were found in increasing numbers further and further away from the site of first description.

But we know that cancer is not infections, so how could this facial tumour spread? Perhaps it was a cancer virus that was passed from animal to animal, and infected the cells of the mouth, changing how growth was controlled? Heroic attempts to identify a virus met with no success.

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The breakthrough came from low-tech work on tumour chromosomes by a persevering cytologist, Anne-Marie Pearse and her assistant Kate Swift. Even the first rather blobby photos that they brought to Canberra to show me in 2005 revealed that the tumour chromosomes were nothing like normal devil chromosomes. This was not surprising, because chromosomes of human solid tumours are very abnormal. They are also very variable, even in the same patient.

What was truly remarkable was that the abnormal chromosomes they showed me were all the same, even in tumours from different animals. This had to mean that the infectious agent had to be the cancer cell itself, a hypothesis proposed in the paper subsequently published in *Nature*[1].

The original animal that had a tumour bit another animal and passed a chunk of the cancer on. It grew in the mouth of the second animal, and was passed to other animals it bit. It started its inexorable march across Tasmania so that now at least 60% of devils have perished. At this rate, extinction is projected in a decade or two.

With the proposal that Devil Facial Tumour Disease (DFTD) was a transmissible tumour, genetic work started in earnest. Hannah Bender in my lab used beautiful new 'chromosome painting' techniques to compare normal and tumour chromosomes, and discover the breakpoints and fusion points that could lead us to some of the genes that are disrupted in the tumour.

A young investigator at Sydney University, Dr Kathy Belov, an expert in the marsupial immune system, turned her attention to this almost unbelievable 'transmissible cancer'. We all know you can't 'catch cancer' from someone who has the disease. Genetic differences between people at the major histocompatibility (MHC) locus evoke antibodies against foreign antigens, and ensure rejection of foreign tissue. This is a problem for kidney transplants, but it saves us from transmissible cancer. Is there something strange about devil MHC? Or has the tumour cell learned some tricks to disguise itself? (And if so, could we use the same tricks to disguise transplanted organs?)

Looking directly at variation at the devil MHC locus, Kathy and her team found that devils all over Tasmania were genetically almost identical[2]. What variation there was enabled them to prove unequivocally that the tumour cells were passed from animal to animal, because the MHC type was always the same, and different from that of the host.

The surprising lack of variation among the population suggested that devils, unbeknown to ecologists, had quite recently suffered several near-extinctions. This supported an important link between genetic variation and endangerment, still much debated among conservation geneticists[3].

But how can genomics help save the beleaguered devil? Scientists in Tasmania are exploring the prospect of immunizing animals against a

protein that is expressed by the tumour but not by normal devils. They are also enacting a desperate strategy of isolating healthy devils, and setting up captive colonies that can restock Tasmania when the last wild devil is dead.

Genomics can be used to sharpen both these strategies. Direct studies of devil populations are identifying devils with MHC variants, which are

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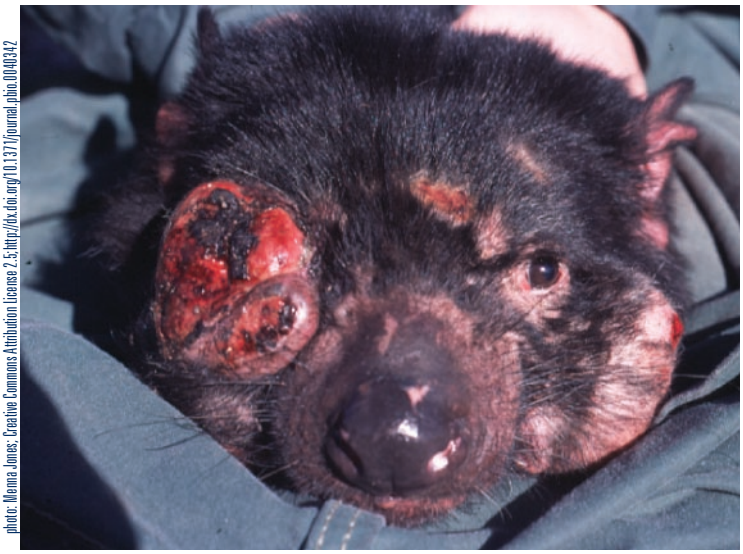
used to build healthy colonies that retain maximum genetic variation.

Genomics, too, can potentially identify tumour markers that tell us how the tumour originated, and can be used to identify the early stages of the tumour, and even to vaccinate animals. For some years, a young Tasmanian scientist, Liz Murchison, then completing her PhD at Cold Spring Harbor in New York, struggled to gain support for genomic studies of the devil. She eventually persuaded Roche to fund sequencing of the RNA transcribed from all the genes that are active in the tumour and in normal devil tissue.

Liz (by then a part of my lab in Canberra), with two of our Centre collaborators at the Walter and Eliza Hall Institute, bioinformaticians Tony Papenfuss and Arthur Hsu, analysed the torrent of genomic data. They identified about 14,000 genes that are active in the tumour and/or normal tissues, and discovered that the tumour expressed some unusual



proteins that were typical of Schwann cells, and not shared with the normal devil tissues that were sequenced. Schwann cells make folds of cell membrane to wrap up and insulate bundles of nerve cells. To do this specialised job, they make large amounts of some specific proteins found almost nowhere else in the body, so their expression by the tumour implies a Schwann cell origin.

The paper, recently published in Science[4] offers new ways to know, and hopefully outwit, the rogue cancer clone. Identifying marker genes

should help to identify new target proteins to design vaccines. Staining for one protein that is highly expressed in the tumour is easily detected under the microscope, even when the tumour is very small. It will help us to study the earliest stages of tumour growth and help to establish a

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quarantine period for selecting healthy animals for insurance colonies.

The next step will be to completely sequence the devil genome. Some sequencing is already being undertaken in a project led by Dr Vanessa Hayes of the Children's Cancer Institute Australia in Sydney. Even light coverage of genomic sequence will provide more detailed information on variation at regions all around the genome, and should help us identify other unique regions that could be targeted.

So, genomics and molecular cytogenetics has allowed us to progress from baffling mystery to a deep understanding of Devil Facial Tumour Disease at the genetic level in just a few years. It tells us that the tumour originated some 15 years ago when a single Schwann cell in a single animal underwent mutations that interfered with the regulation of its growth. It multiplied to form a tumour mass, then bits were injected into other animals by biting. Because all the animals were genetically so similar, the tumour cells grew and flourished, and in turn were passed on to other animals. As in some Sci-Fi movie, this devilish cell clone now threatens the entire species. Now genomics is contributing to practical efforts to make sure the devil does not go the way of the Tasmanian tiger.

[1]Pearse AM, Swift K (2006) Nature 439, 549; [2]Siddle HV et al (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 16221; [3]O'Brien SJ et al (1983) Science 327, 459; [4]Murchison EP et al (2010) Science 327, 84;

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