Review

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Genomic insights into a contagious cancer in Tasmanian devils

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The Tasmanian devil faces extinction due to a contagious cancer. Genetic and genomic technologies revealed that the disease arose in a Schwann cell of a female devil. Instead of dying with the original host, the tumour was passed from animal to animal, slipping under the radar of the immune system. Studying the genomes of the devil and the cancer has driven our understanding of this unique disease. From characterising immune genes and immune responses to studying tumour evolution, we have begun to uncover how a cancer can be 'caught' and are using genomic data to manage an insurance population of disease-free devils for the long-term survival of the species.

Emergence of a new disease in Tasmanian devils

In 1996, a wildlife photographer captured images of wild Tasmanian devils (*Sarcophilus harrisii*) with large ulcerating tumours on their faces in Mt William National Park in the northeastern corner of Tasmania, an island state at the southern tip of Australia [1] (Figure 1). These would become the first records of a newly emerged contagious cancer that has since decimated devil numbers.

Devils are the largest remaining marsupial carnivore in the world [2]. They gained that title following the extinction of the Tasmanian tiger (*Thylacinus cynocephalus*) during the 1930s [3]. Devils were once found across mainland Australia, but went extinct on the mainland 3000–4000 years ago [4]. Devils were isolated in Tasmania approximately 10 000 years ago and, since then, have gone through at least three population crashes [5]. Interestingly, the lack of genetic diversity seen in devils today dates back much further than these recent genetic bottlenecks [6,7]. It appears that devil population declines have coincided with changes in prey abundance associated with increased El Niño-Southern Oscillation activity approximately 2000–4000 years ago, and the Last Glacial Maximum (22 000–48 000 years ago) [4,5]. Devils are now renowned for their low levels of genetic diversity [7–12], which has had a critical role in the spread of a contagious cancer [10,13].

The contagious cancer affecting devils is called devil facial tumour disease (DFTD, see Glossary). We now know that the disease originated in a Schwann cell of a female devil [14] and is transmitted from one animal to another by biting [15]. DFTD cells are undifferentiated neoplasms with highly pleomorphic and anaplastic cells [16]. Tumours result in ulcerating proliferative masses that are prone to secondary infection. Tumours tend to occur around the face and jaw, and masses within the oral cavity can prevent feeding. They can also occlude eyes and damage whisker beds. Tumours are also able to metastasise [16]. They have evolved the capacity to evade the immune system [17] and, to date, have been transmitted through 100 000 devils, resulting in an 85% decline in the species [18,19]. The devil is now listed as endangered on the IUCN Red List of Threatened Species [20]. In this review, we summarise how molecular genetics and genomics have had a critical role in elucidating the origins and mechanisms of transmission of this unique disease.

The cancer is clonal

A pivotal breakthrough in our understanding of this disease came in 2006, when Anne-Maree Pearse and Kate Swift published a paper in *Nature* demonstrating that the DFTD karyotype involved complex chromosomal rearrangements [21]. Subsequent analyses using chromosome painting allowed detailed characterisation of DFTD chromosomes, revealing that a single cataclysmic event, termed 'chromothripsis', likely led to emergence of the contagious cancer [18,22]. Chromothripsis is the shattering of a chromosome or chromosomes, which are then rejoined by nonhomologous end-joining DNA repair mechanisms [22,23]. The DFT karyotype contains massive rearrangement of three autosomes (1, 4, and X) and addition of four marker chromosomes [22]. Marker chromosome 1 contains a rearranged chromosome 1 plus a small amount of X chromosome material; the other copy of chromosome 1 has ended up on marker chromosomes 2 and 3 [22]. Marker chromosome 2 also contains some chromosome 4, 5, and X genes, while marker chromosome 3 also contains X chromosome genes. Marker chromosome 4 contains material from chromosomes 1, 4, and 5 [22]. Chromosome painting enabled chromosomes 1 and 2 to be readily distinguished, resulting in the clarification that chromosome 1, not chromosome 2 as originally suggested by Pearse and Swift [21], was affected in DFTD.

The discovery of chromosomal rearrangements in a cancer karyotype is not that unusual, because most cancers undergo chromosomal changes [24]; the unusual discovery in DFTD was that, despite minor variations among DFTD strains, the basic chromosomal rearrangements observed in eight DFTD samples were essentially identical [21]. This

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Glossary

β2-microglobulin (B2m): a protein that stabilises the heavy chain of MHC I molecules.
Allograft: transplant of biological tissue from one individual to another of the same species. DFTD is transmitted among Tasmanian devils as an allograft.
Amplicon: a product of a PCR reaction, which may be barcoded and sequenced.
Chromosome painting: a technique in which chromosome-specific probes, labelled with coloured fluorescent dyes, are hybridised with a cellular karyotype to identify individual chromosomes, rearrangements, duplications, and other cytogenetic mutations.
Chromothripsis: massive genomic restructuring that occurs via the cataclysmic fragmentation of the chromosomes of a cell and the rearrangement of nonhomologous fragments that occurs by end-joining DNA repair mechanisms.
Devil facial tumour (disease) DFT(D): a highly contagious, fatal, transmissible cancer, limited to Tasmanian devils, which is thought to have arisen during the 1990s and now threatens the species with extinction in the wild.
Free-range enclosure (FRE): a large, semi-wild enclosure where captive Tasmanian devils are housed in groups, not only facilitating maintenance of natural behaviour, but also resulting in challenges for pedigree reconstruction.
Hayflick limit: the number of times a cell population can divide before telomeres shorten to a critical length and cell division ceases.
Karyotype: the arrangement of chromosomal material in a cell. Rearrangements, deletions, duplications, and other chromosomal mutations may be observed by examining the karyotype of a cell.
Kinship: the genetic relationship, either based on a known pedigree or molecular data, between one animal and another.
Major histocompatibility complex (MHC): a set of molecules primarily involved in presentation of antigenic peptides to the immune system; first discovered for their role in graft rejection.
Methyl-CpG binding domain 2/4 (MBD2, MBD4): enzymes that cause active demethylation.
Methylation: an epigenetic modification of DNA that may lead to changes in gene expression. Demethylation refers to a decrease in methylation patterns, resulting in a genome that may be said to be hypomethylated.
Microsatellite: also known as simple sequence repeats or short tandem repeats; regions of highly repetitive DNA (with a repeat unit of usually two to five base pairs in length) widely used for many ecological genetics applications, such as diversity surveys, quantifying population differentiation, or parentage analysis.
miRNA: small noncoding RNA molecules that regulate gene expression (including miR-21, mi-R-24, miR-19b, and miR-222, which are of interest in DFTD genetics).
Myelin basic protein (MBP): a protein encoding by a gene that is typically expressed in Schwann cells and an indicator that DFTD originally arose in this tissue.
Natural killer (NK) cells: cytotoxic lymphocytes of the innate immune system that can kill cells lacking cell surface MHC.
Periaxin: a protein that can be detected in DFTD cells with antibodies against Schwann cell specific myelin protein.
Ploidy (also tetraploidy, polyploidisation): the number of sets of chromosomes in a cell; mammalian somatic cells are normally diploid (have two sets of chromosomes). Polyploidisation refers to the increase in ploidy that may be seen in tumour cells, such as an abnormal transition to tetraploidy (four sets of chromosomes).
Reverse transcriptase-polymerase chain reaction (RT-PCR): a laboratory technique for studying gene expression through generating complementary DNA transcripts from RNA.
Schwann cell: cell of the peripheral nervous system; progenitor of DFTD.
Sex-determining region (*SRY*): a gene found on the Y chromosome that is involved in mammalian sex determination.
Single nucleotide polymorphism (SNP): a sequence variant that occurs as a substitution in the alignment of two or more sequences.
Toll-like receptors (TLR): a family of pattern recognition molecules that forms part of the innate immune system of many organisms.
Transporter (TAP): a heterodimer comprising *TAP1* and *TAP2* gene products, which pumps peptides from the cytoplasm into the lumen of the endoplasmic reticulum to be loaded onto MHC.

result indicated that the karyotypic changes seen in each tumour did not occur independently in each individual, instead suggesting that the chromosomal rearrangements occurred once, early in the evolutionary history of the cancer, and that this cell line was then transmitted from animal to animal as an allograft [21].

The allograft transmission hypothesis has now been confirmed by several independent lines of evidence. Siddle *et al.* [13] showed that DFTD cells from different individuals were identical at microsatellite markers and MHC markers. Murchison *et al.* [25] also confirmed the clonal origin of DFTD through microsatellite genotyping and mitochondrial genome analysis. In both of these studies, tumour genotypes were identical to each other regardless of sex, age, or location, and differed from those of the hosts.

Continuous replication and division of a cell like this should eventually lead to erosion of the telomeres at the ends of the chromosomes, with the cell ultimately hitting the 'Hayflick limit' (i.e., replicative senescence) [26]. However, this has not happened in DFTD, which has evolved to maintain telomeres at a constant length [27]. The telomeres of DFTD cells are short but protected by increased activity of genes associated with telomerase (which increases telomere length) and the shelterin complex (which increases stability and protects against extensive elongation) [27]. These changes have enabled the tumour cell line to continue to be passed from animal to animal and remain immortal.

The cancer arose from a Schwann cell

A second major breakthrough in our understanding of DFTD came from a study by Murchinson *et al.* [14] that involved sequencing the DFTD transcriptome and its miRNAs. Based on the gene expression profile of the tumour, the authors determined that the disease was of Schwann cell origin, because they noted high expression of *MBP*, a gene that encodes myelin basic protein [14]. Nine of the 20 investigated highly expressed genes were involved in the myelination pathway, and DFTD gene expression profiles matched most closely those of peripheral nerves [14]. Taken together, these observations indicated that the tumour may be of Schwann cell origin. To confirm this, the authors stained tumour cells with an antibody against Schwann cell specific myelin protein, periaxin, confirming the likely origin of the tumour [14]. A large number of sampled primary tumours and metastases as well as DFTD cell lines were all positive for periaxin with minimal nonspecific labelling [14,28], and it was determined that periaxin was the most specific and sensitive marker for identifying DFTD cells [28]. Murchison *et al.* [14] also analysed DFTD miRNAs and identified several miRNAs that had previously been found to be upregulated in tumours [29,30].

Schwann cells are the primary glial cells within the peripheral nervous system; there are numerous subtypes, two of which are myelinating and nonmyelinating Schwann cells [31,32]. Myelinating Schwann cells coat the axons of neurons to form the myelin sheath, participate in nerve repair, and modulate local immune reactions [33,34]. The original functional roles of these cells may have provided DFTs with the capacity to modulate immune reactions and evade immune responses [35,36]. This aspect of DFTD biology needs to be explored further.

The cancer can evade the immune response and pass between individuals as an allograft

DFTD is unusual in that the allograft cell passes from animal to animal without invoking any immune responses