Clonally transmissible cancers in dogs and Tasmanian devils

Elizabeth Murchison, Wellcome Trust Sanger Institute, Cambridge, UK
Clonal evolution of cancer

Clonally transmissible cancer

- Tasmanian devil facial tumour disease (DFTD)
- Canine transmissible venereal tumour (CTVT)
Transmissible cancers in devils and dogs

To use genomics to understand

- Origin
- Diversity
- History
- Evolution
Tasmanian devil
Sarcophilus harrisii

Photo: Wayne McLean
1996

2000

2003

Adapted from Hawkins et al, Biol Conserv 131:307-24, 2006
2004


95% population decline

>80% population decline
“Tasmanian Devil Facial Tumour Disease” (DFTD)
Devil cancer chromosomes

Normal devil

Anne-Maree Pearse, Unpublished
Predictions....

• If the devil’s cancer is spread by living cancer cells then
  – All the cancers should be genetically identical
  – The cancers should be genetically different to their hosts

Photo: Hannah Bender
Microsatellite genotyping
Microsatellite genotyping

Genotype

- Green: 170/170
- Blue: 170/174
- Orange: 170/172
- Purple: 174/174
Devil tumours are genetically identical

DFTD founder devil
DFTD founder devil
DFTD founder devil
DFTD founder devil
Tasmanian devil reference genome

“Salem”: Female Tasmanian devil

Illumina paired end sequencing

De novo assembly

Photo: Taronga Zoo
Tasmanian devil (Sarcophilus harrisii)

Assembly

This site displays the DEVIL7.0 assembly (RCA_00219685.1) of the Tasmanian devil (Sarcophilus harrisii) genome. The genome sequence and assembly were provided by the Wellcome Trust Sanger Institute and Illumina. The N50 size is the length such that 50% of the assembled genome lies in blocks of the N50 size or longer. The N50 length for supercontigs is 1947.19 kb and is 20.13 kb for contigs. The total number of bases in supercontigs is 3.17 Gb and in contigs is 2.93 Gb.

Download Tasmanian devil genome sequence (FASTA)

Annotation

The Ensembl genome annotation pipeline was used to identify genes. Models built from Tasmanian devil proteins and cDNAs have been given priority over predictions from other vertebrate species. 5,663 transcript models made from paired end Illumina RNA-Seq were added into the gene set where they added a novel model or splice variant. RNA-Seq data was also used to add UTR to non species specific models. The total gene set contains 20,419 genes including pseudogenes and ncRNAs.

http://www.ensembl.org/Sarcophilus_harrisi
Sequencing the DFTD genome
The DFTD genome

- Germline variants
- DFTD founder devil
- Somatic variants
The DFTD genome

DFTD founder devil

Germline variants

Phylogeography

Somatic variants
How many somatic mutations are present in DFTD?
Tasmanian devil cancer genome sequencing
DFTD genome sequencing

~2 \times 10^9 \text{ paired end sequence reads}
DFTD genome analysis

Devil reference sequence

DFTD sequence reads
DFTD variant calling

Devil reference sequence

DFTD sequence reads
DFTD variant calling

Devil reference sequence
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG

Narawntapu DFTD sequence reads
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG

Forestier DFTD sequence reads
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
ATCTTCGGAGTACTTCTAGTACTAAGCGTAAGATACAACAG
Tasmanian devil cancer genomes

~17,000 somatic mutations

Narawntapu, 2007

Forestier Peninsula, 2007
Tasmanian devil cancer genomes

Narawntapu, 2007

< 20,000 somatic mutations

Forestier Peninsula, 2007
DFTD founder devil

DFTD founder devil

DFTD founder devil
Gender of the founder devil

Gender of the founder devil

Y chromosome

Gender of the founder devil


**Y chromosome**

- SRY
- GAPDH

**X chromosome**

- Female
- Male
- 87T DFTD
- 53T DFTD

X chromosome variants

- Female
- Male
- DFTD (87T)
- DFTD (53T)
Summary

DFTD founder devil

Cancer phylogeography

DFTD founder devil
Devil samples

Tumour

Host

Samples

Devil samples

Tumour

Host

Samples

Devil samples

Tumour

Host

Samples
MT:3297 G>A

Tumour

Host

Legend

● G

● A

○ Unconfirmed

Narawntapu

West Pencil Pine

Captive/Unknown

Narawntapu

West Pencil Pine

Captive/Unknown

Forestier Peninsula

Forestier Peninsula

Legend

● G

● A

○ Unconfirmed

Kilometres
MT:3297 G>A

Tumour

[Map showing locations labeled as Narawntapu, West Pencil Pine, and Forestier Peninsula with coordinates and distances marked.]
Chromosome 1_supercontig_000000246_95466 T>C
Tumour

Host

Legend
- T/T
- T/C
- C/C
- Failed
Chromosome 1_supercontig_000000246:95466 T>C Tumour
Summary
What’s next for the Tasmanian devil?

• Captive breeding programs
• Restrict disease spread in the wild
• Future research
  – Monitor DFTD genetic diversity and evolution
  – DFTD host interaction
  – DFTD vaccines and therapies

Photo: Save the Tasmanian Devil Program
Canine transmissible venereal tumour

Photo: Ted Donelan
Canine transmissible venereal tumour (CTVT)
CTVT worldwide distribution

Andrea Strakova
CTVT is a single somatic cell lineage with a global distribution

- Transplantation experiments
  (Nowinsky, 1876; Sticker, 1904)
- Karyotype
- Genetics
  - LINE-1 insertion upstream of MYC
    (Katzir et al, 1987)
  - Microsatellites, MHC
    (Murgia et al, 2006; Rebbeck et al, 2009)
ON THE
DISEASES
OF
HORSES AND DOGS;
so conducted as to
ENABLE PERSONS TO PRACTISE WITH EASE AND SUCCESS
ON
THEIR OWN ANIMALS,
WITHOUT THE ASSISTANCE OF A FARRIER:
Including likewise the Natural Management, as Stabling,
Feeding, Exercise, &c.: together with the Outlines of
a Plan for the Establishment of Genuine Medicines for
these Animals throughout the Kingdom.

BY DELABERE BLAINE,
PROFESSOR OF ANIMAL MEDICINE;
Author of "The Anatomy of the Horse," "Outlines of the Veterinary

FOURTH EDITION,
WITH VERY LARGE ADDITIONS.

LONDON:
PRINTED FOR T. BOOSEY, 4, OLD BROAD STREET,
ROYAL EXCHANGE.
1810.
habitants of mountainous countries, and has been supposed to be dependant on some particular quality of the water in those vicinities. But in dogs no such peculiarity takes place: it does not appear in them indigenous to any particular soil, but almost peculiar to some particular species of dogs, though other dogs sometimes have it, as terriers; but it is much less frequent, and in the larger tribes is hardly ever seen. It comes on generally while very young, and continues to increase to a certain size, when it becomes stationary, seldom increasing to such a degree as to prove fatal. It is however troublesome, and in some measure hurtful, from the pressure it occasions on the surrounding parts. If an ointment is made with equal parts of mercurial and blistering ointment, and the swelling rubbed with it every day, avoiding salivation, it commonly lessens and frequently wholly removes it. But it is necessary also at the same time to give internal alteratives: four, five, six, or seven grains of burnt sponge, with half the quantity of nitre given every morning, will be found useful.

CANCER.

Two parts only in dogs are subject to a cancerous affection, and both these are organs concerned in generation. The teats of bitches become at times indurated, and swelled with a schirrous indolent tumour, which more commonly remains indolent and without ulcer: but now and then, when such a tumour has increased to a very considerable size, a small ulcer bursts out, which slowly increases to a very considerable surface. In the worst of these cases there is not present the virulent and horrible spreading of the human cancer, nor does it appear to give much pain, or to injure the health; and dogs who are suffered to remain with it, live for a great length of time without much inconvenience to themselves. This state admits of only one cure, which is the complete removal of the whole tumour. The vagina or rather the womb of bitches also frequently takes on an ulcerous state, accompanied with a fungous excrescence, which is brought on oftentimes from the horrible brutality of boys who force dogs from bitches in the act of copulation. This complaint admits of no cure, that I have witnessed. In the penis of dogs also a similar fungous excrescence sometimes forms, but it does not appear to erode the neighbouring parts much: it increases rather than diminishes the size, till its offensiveness obliges the animal to be made away with.
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obliges the animal to be made away with.
CTVT is the oldest known mammalian somatic cell lineage

• CTVT lineage is at least 200 years old

• CTVT may be much older than this

Murgia et al, Cell, 126:477-87, 2006; Rebbeck et al Evolution, 63(9):2340-9, 2009
CTVT genome sequencing
The CTVT genome

CTVT Founder animal

Germline variants

Somatic variants

Identity of the CTVT founder animal
Principal component analysis of dogs and wolves

Principal component analysis of dogs and wolves

Principal component analysis of dogs and wolves

Principal component analysis of dogs and wolves

CTVT founder phenotype

The CTVT founder

The CTVT founder

Modern breeds

The CTVT founder

The CTVT founder

The CTVT founder

Modern breeds

Coyotes

Wolves

“Ancient breeds”

The CTVT founder was an “ancient breed” dog

# Founder dog phenotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>CTVT genotype</th>
<th>CTVT phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>FGF5</em></td>
<td>32:7,473,337G&gt;T</td>
<td>G/G</td>
<td>Short hair</td>
<td>Cadieu et al, 2009</td>
</tr>
<tr>
<td><em>KRT71</em></td>
<td>27:5,542,806C&gt;T</td>
<td>C/C</td>
<td>Straight hair</td>
<td>Cadieu et al, 2009</td>
</tr>
<tr>
<td>-</td>
<td>10:11,072,007C&gt;T</td>
<td>T/T</td>
<td>Prick ears</td>
<td>Vaysse et al, 2011</td>
</tr>
<tr>
<td>-</td>
<td>10:11,169,956C&gt;T</td>
<td>C/C</td>
<td>Medium to large size</td>
<td>Vaysse et al, 2011</td>
</tr>
<tr>
<td><em>BMP3</em></td>
<td>32:8,196,098C&gt;A</td>
<td>C/C</td>
<td>Pointy snout</td>
<td>Schoenebeck et al, 2012</td>
</tr>
<tr>
<td><em>CDH2</em></td>
<td>7:63,867,472</td>
<td>C/C</td>
<td>Lower risk of OCD</td>
<td>Dodman et al, 2010</td>
</tr>
</tbody>
</table>
LETTER

The genomic signature of dog domestication reveals adaptation to a starch-rich diet

Erik Axelsson¹, Abhirami Ratnakumar¹, Maja-Louise Arendt¹, Khurram Maqbool¹, Matthew T. Webster¹, Michele Perloski², Olof Liberg³, Jon M. Arnemo⁴,⁵, Åke Hedhammar⁶ & Kerstin Lindblad-Toh¹,²

MGAM chr16:10,135,196C>T  V1001I

C: Adaptation to starch-rich diet
T: Meat-lover
The genomic signature of dog domestication reveals adaptation to a starch-rich diet

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MGAM chr16:10,135,196C>T  V1001I

C: Adaptation to starch-rich diet
T: Meat-lover

CTVT: T/T
The CTVT genome

Germline variants


Somatic variants
Using Loss of Heterozygosity (LOH) to identify somatic mutations

Loss of heterozygosity (LOH) → Duplication → Somatic mutations

Mutation fraction

Genomic position

100% Germline (& early somatic)
50% Somatic
Number of somatic mutations in CTVT

Number of somatic mutations in CTVT

2 – 2.5 million SOMATIC MUTATIONS

Variants in CTVT genomes
Variants in CTVT genomes

- 90% (~2.2 million mutations)
- 10% (~200,000 mutations)

Brazil and Australia flags
90% of mutations occurred before divergence of modern tumours
Age of CTVT

Most recent common ancestor of modern CTVTs

~200 – 2,000 years

Murgia et al, Cell, 126:477-87, 2006; Rebbeck et al Evolution, 63(9):2340-9, 2009
Age of CTVT

Founder lived 2,000 to 20,000 years ago

Most recent common ancestor of modern CTVTs

~200 – 2,000 years

For most of its history, CTVT was probably confined to an isolated dog population.

Founder lived 2,000 to 20,000 years ago.

~200 – 2,000 years

What caused the mutations in CTVT?
Mutation spectrum

Type of mutation can give clues as to processes that caused cancer

Mutation spectrum in CTVT

Australian CTVT

Brazilian CTVT

Mutations are represented by the number of somatic mutations for each mutation type in Australian and Brazilian CTVT. The x-axis represents the mutation type, and the y-axis represents the number of somatic mutations. The mutations are C>A, C>G, G>T, G>A, T>Y, A>T, T>C, and A>C.
Clonally transmissible cancers

DFTD
- Recent (<20 years)
- Metastasis common
- Transmitted by biting
- Facial tumour
- Neural crest origin
- Unresponsive to therapy

CTVT
- Old (>1,000 years)
- Metastasis rare
- Sexually transmitted
- Genital tumour
- Histiocytic origin
- Very sensitive to therapy
Clonally transmissible cancers
Genetic Analysis of a Sarcoma Accidentally Transplanted from a Patient to a Surgeon

Hermine-Valeria Gärtner, M.D., Christian Seidl, M.D., Christine Luckenbach, Ph.D., Georg Schumm, M.D., Erhard Seifried, M.D., Horst Ritter, M.D., and Burkhard Büttmann, M.D.

Isolation of DNA
Genomic DNA from peripheral-blood samples was isolated by the “taking-out” method. DNA from paraffin-embedded tumor and tissue samples was extracted according to a modification of the method of Goetz et al.

Analysis of Short Tandem-Repeat Polymorphisms
Short tandem-repeat polymorphisms of the loci HUMTHO1, HUMCYF04, and HUMACFP2 were amplified by the polymerase chain reaction (PCR) with fluorescence-labeled primers. Primer sequences were chosen from published sequences. The 5' primer for HUMCYF04 was labeled with 5-carboxyfluorescein, whereas...
Wellcome Trust Sanger Institute

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Bronwen Aken
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Sarah Peck
Anne-Maree Pearse
Chris Boland
Trapping teams and field assistants

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Willem Rens
Malcolm Ferguson-Smith

Hamish McCallum (Griffith University)
Kathy Belov (University of Sydney)
David Obendorf (Veterinary pathologist)
Matthew Breen (North Carolina State University)
Albert Vilella (European Bioinformatics Institute)
Thank you…

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Michelle Morters
Malcolm Ferguson-Smith
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Austin Burt
Robin Weiss
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Jan Allen
Ted Donelan
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Karen Allum
Simon Martinez
Oscar Rarieya
Laura Delgadillo
Scott Moroff
Jack Reece

Collaborating Organisations

Vets Beyond Borders
World Vets
SCAD Thailand
AMRRIC (Australia)
IFAW
Animal Care Association, The Gambia
St George’s University, Grenada
Colorado State University
University of Messina
University of Yucatan
Bons Amigos (Cape Verde)
University of Cambridge, Vet School
European Bioinformatics Institute
Imperial College London
University College London
University of Franca
Sao Paulo State University
Animal Protection Society Samoa
Soi Dog Foundation (Thailand)
Worldwide Veterinary Service
PAWS (Mauritius)
University of Ibadan
University of Nairobi
Usmanu Danfodiyo University
KSPCA (Kenya)
Help in Suffering (India)
Thank you!

Photo: Sarah Peck
Immune evasion

Canine transmissible venereal tumour (CTVT)

NK cells

T cells

Antigen presenting cells

Complement

Tasmanian devil facial tumour disease (DFTD)

??