Microbiology Journal Club

- 9:10am alternating Mondays
- Trailer D, next to Flex Labs
- No topic!
- www.mbio.ncsu.edu/MJC
- James_Brown@ncsu.edu
- Bring coffee!
Devil Facial Tumor Disease
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- Endemic in 65% of Tasmania
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- Agent is the cancer cell itself!
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Devil Facial Tumor Disease

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- All sarcoma cells have the same odd karyotype
- Probably originated in ca. 1990
- Is there anything else like it?
Canine Transmissible Venereal Tumor
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- easily treated by chemotherapy
Canine Transmissible Venereal Tumor

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- common worldwide, especially in tropical and subtropical rural areas, where it is the most common tumor found in dogs
- CTVT and devil facial tumor are the only known directly transmissible cancers
Transmissible venereal tumor: A consequence of sex tourism in a dog

A 6-year-old, intact male Pomeranian was examined because of an intermittent preputial discharge of 1-month duration. The dog had returned from a 2-year stay in Venezuela 4 mo prior to examination, and had not been bred since. A polypoid red mass 1 cm in diameter was found on the glans penis. The mass was surgically removed and was histologically identified as a transmissible venereal tumor (TVT).

Transmissible venereal tumor is an allogenic transplant that is transmitted during coitus or socialization (1). The tumor usually affects the external genitalia, although it has also been reported in the nasal and oral cavities, the rectum, and the skin. Metastasis is uncommon, probably occurring in fewer than 5% of cases (2); the most common sites for metastasis are skin, lymph nodes, and subcutis (1). Transmissible venereal tumor is cured through the use of antineoplastic agents and radiation therapy.

Transmissible venereal tumor is a very common canine tumor in tropical and warm temperate areas of the world (1). However, TVT is very rare in Canada, and there is no record of that diagnosis in the archives of the Department of Pathology of the Faculté de médecine vétérinaire. In this case, the dog was presumed to have been exposed to TVT in Venezuela, as it had not been bred or contacted other dogs in the 4 mo since its return to Canada.

The recent increase in the international movement of wild and domestic animals has played an important role in emerging diseases, some of which may have dramatic economic, ecological, or zoonotic implications for Canada (3). In the past, transportation of animals and animal products has resulted in the spread of many diseases, including anthrax, African swine fever, raccoon rabies, and bovine spongiform encephalopathy (3). The purpose of this case report is to illustrate how a pet that has traveled abroad can be exposed to and carry home, rare, exotic, or emerging diseases. As veterinarians in clinical or laboratory practice, we must be aware of the important role that animals can play in disease importation, as we are usually the first line of defense against the spread of such diseases.

References

Igor Mikaelian, Christiane Girard, Département de pathologie et de microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe (Québec) J2S 7C6, Ioan Ivascu, Clinique vétérinaire Boisbriand, 8, 1re avenue, Boisbriand (Québec) J7G 1T6.
Is CTVT really an contagious cancer?
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- All CTVT cells worldwide have the same chromosomal rearrangements & aneuploidies
- All have a LINE-1 (Long Interspersed Nuclear Element) near the c-myc gene
- BUT there have been repeated reports of virus-like particles associated with the tumors. Many originally thought Kaposi’s sarcoma was a contagious cancer for similar reasons!
Clonal Origin and Evolution of a Transmissible Cancer

Claudio Murgia,1,4 Jonathan K. Pritchard,2 Su Yeon Kim,3 Ariberto Fassati,1 and Robin A. Weiss1,*

1 MRC/UCL Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London, 46 Cleveland Street, London W1T 4JF, UK
2 Department of Statistics
University of Chicago, CLSC–507, 920 East 58th Street, Chicago, IL 60637, USA
3 Department of Genetics
4 Present address: Institute of Comparative Medicine, University of Glasgow Veterinary School, 464 Bearsden Road, Glasgow G61 1QH, UK.
*Contact: r.weiss@ucl.ac.uk
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SUMMARY

The transmissible agent causing canine transmissible venereal tumor (CTVT) is thought to be the tumor cell itself. To test this hypothesis, we analyzed genetic markers including major histocompatibility (MHC) genes, microsatellites, and mitochondrial DNA (mtDNA) in naturally occurring tumors and matched blood samples. In each case, the tumor is genetically distinct from its host. Moreover, tumors collected from 40 dogs in 5 continents are derived from a single neoplastic clone that has diverged into two subclades. Phylogenetic analyses indicate that CTVT most likely originated from a wolf or an East Asian breed of dog between 200 and 2500 years ago. Although CTVT is highly aneuploid, it has a remarkably stable genotype. During progressive growth, CTVT downmodulates MHC antigen expression. Our findings have implications for understanding genome instability in cancer, natural transplantation of allografts, and the capacity of a somatic cell to evolve into a transmissible parasite.

INTRODUCTION

CTVT, also known as Sticker’s sarcoma, is a histiocytic tumor that is usually transmitted among dogs through coitus but may also spread through licking, biting, and sniffing tumor-affected areas (Cohen, 1985; Das and Das, 2000). First characterized 130 years ago (Novinski, 1876), CTVT tumor cells, and not by killed cells or cell filtrates (Cohen, 1985). Second, the tumor karyotype is aneuploid but has characteristic marker chromosomes in tumors collected in different geographic regions (Murray et al., 1969; Oshimura et al., 1973; Weber et al., 1965). Third, a long interspersed nuclear element (LINE-1) insertion near c-myc (Katzir et al., 1985) has been found in all tumors examined so far (Katzir et al., 1987) and can be used as a diagnostic marker to confirm that a tumor is CTVT (Liao et al., 2003). In two animals that had been experimentally inoculated with CTVT, the resulting tumors contained the LINE-1/c-myc insertion, whereas the normal tissues did not (Katzir et al., 1987; Liao et al., 2003). However, in natural transmission, inheritance of a LINE-1 insertion near c-myc in the germline might represent a predisposition to develop CTVT after exposure to an oncogenic agent, similar to the Mendelian LINE-1 insertion in the factor IX gene, which causes mild hemophilia B in dogs (Brooks et al., 2003).

The recent emergence of a tumor transmitted by biting in the endangered marsupial species the Tasmanian devil (Sarcophilus harrisii) (Owen and Pemberton, 2006) has attracted renewed interest in the concept of cellular transmission, for which CTVT is cited as a precedent (Pearse and Swift, 2006). However, authors of reports describing virus-like particles in CTVT (Ajello and Gimbo, 1965; Battistacci and Morriconi, 1974; Lombard and Cabanie, 1967) considered that an oncogenic virus might play a role in tumorigenesis. Although most specialists in the field accept the cellular transmission of CTVT, definitive data that this is the case have been lacking, and the concept of a contagious cancer cell has tended to be greeted with skepticism by many oncologists and immunologists.

Molecular genetic markers have not previously been used to resolve the issue of natural transmission, the breed of origin, or the age of the canine tumor. Here, we compare...
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Tumor and normal (blood) tissues from Sicily were brought to the UK with permission of the Department of Environment, Foods and Rural Affairs and were tested for the absence of rabies by RT-PCR at the Veterinary Laboratory Agency (Weybridge, UK) prior to use. DNA was extracted from tumours and blood samples of Indian and Kenyan specimens on site. The Messina samples of matched tumor and normal tissues were analyzed for the LINE-1/c-myc insertion only. nd = not determined.

Table 1. Sources of CTVT Samples

<table>
<thead>
<tr>
<th>Place</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catania, Italy</td>
<td>5</td>
</tr>
<tr>
<td>Messina, Italy</td>
<td>5</td>
</tr>
<tr>
<td>Kolkata, India</td>
<td>4</td>
</tr>
<tr>
<td>Nairobi, Kenya</td>
<td>2</td>
</tr>
</tbody>
</table>

Paraffin-Embedded Archival Tumors

<table>
<thead>
<tr>
<th>Country</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>4</td>
</tr>
<tr>
<td>Italy</td>
<td>5</td>
</tr>
<tr>
<td>Spain</td>
<td>4</td>
</tr>
<tr>
<td>Turkey</td>
<td>9</td>
</tr>
<tr>
<td>USA</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

Details of age, sex, breed of dog, and site of tumor are in Table S1.

Data sources
Figure 1. Specific LINE-1/c-myc and DLA Haplotype Genetic Markers for CTVT Detected by Specific PCR Amplification
(A) For each of 11 dogs (A–M), fresh normal and tumor samples are indicated as N and T, respectively. The panel is assembled from three separate gels visualized by ethidium bromide. The invariant DLA-88 intron sequence serves as a positive control for each of the 22 specimens.
(B) PCR amplification of DNA using Cy5-labeled forward primers from 21 microdissected tumor cells from paraffin-embedded specimens. The panel is assembled from four separate gels.
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Microsatellite genotyping

simple sequence repeats

Donor
A  B  C  D

Amelogenin  vWA  D7S820  F13A1
Figure 2. Microsatellite DNA Analysis of 11 Fresh Tumors and Matched Host Samples

Unrooted neighbor-joining tree based on chord distance compiled from 21 microsatellite loci. A similar neighbor-joining tree based on allele sharing is provided in Figure S2.
Figure 3. Analysis of mtDNA in CTVT
Tumor haplotypes from fresh and paraffin-embedded tissues showing two main clusters of mtDNA. Diameter of each circle is proportional to the number of tumor samples. Each branch represents one base pair change, with black dots representing intermediates not found in the tumor samples analyzed. The outlined boxes indicate that tumor samples with homozygous (diploid) and hemizygous (haploid) DQA1 genes coincide with mtDNA clusters, except for tumor 9, which is diploid.
257bp sequence of mtDNA control region

Figure S4. Maximum-Likelihood Tree of mtDNA Sequences

The tree was constructed from 21 CTVT samples and 45 additional dogs, wolves and a coyote outgroup, without assuming that the tumor sequences are monophyletic. Given the short (257bp) amplified region from archival samples, there may be uncertainty about the true position on the tree of the two sequences that group quite separately from the main tumor clusters; moreover they may represent contaminating host mtDNA.
Figure S6. CTVT in Relation to Wolves and 85 Dog Breeds

Data are based on 73 microsatellite loci. Each panel shows the results from a model-based clustering algorithm, Structure, that assigns sampled individuals, based on their genotypes, to a prespecified number, K, of clusters. Each tumor sample, or individual dog, is represented by a vertical bar, with colored segments indicating the proportion of that individual's membership in each cluster. At K=2, the tumors cluster clearly with wolves and 'old' dog breeds; for larger, more stringent K values, the tumors form a distinct group, indicating a common origin.
Figure 4. Relationship of CTVT to Wolves and Dog Breeds

(A) Results of a Structure analysis of the canids that appeared most closely related to CTVT (yellow at K = 2 in Figure S6). The clustering was based on the nontumor samples only, and the three tumor samples with nearly complete data were then assigned to the appropriate clusters.

(B) Neighbor-joining tree based on pairwise differences among the same set of individuals as in (A). The relationship between wolves and CTVT is similar when the tree is constructed using all dogs (Figure S6).

Based on 73 microsatellites - just ‘old’ breeds
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The expected heterozygosity at microsatellite loci in different subsets of canids and CTVT was plotted. Non-CTVT data were derived from Parker et al. (2004). The Australian shepherd represents the most diverse defined breed and the miniature bull terrier the least diverse breed for microsatellite DNA.
MHC expression is repressed in tumor cells

Figure 6. MHC Expression in CTVT
(A) Expression of DLA class I (DLA-88) and class II (DRB1) in the penile tumor of dog C by RT-PCR using tumor-cell-specific primers (T) and primers specific to the alleles of this animal for host stromal cells and infiltrating normal cells (N). M = marker lanes.
(B) Histopathology of a hematoxylin-and-eosin-stained 4 μm section of the same tumor. Scale bar = 30 μm. Cells with large round nuclei are tumor cells, and mitoses are apparent. A stromal cell is indicated with an arrow.
Figure S1. Gene Dosage of DLA Class II Alleles in Fresh and Microdissected Tumor Samples

The copy number of the target DLA genes was compared to the reference gene β-actin using qPCR with SYBR-Green and the kinetic method (standard curve) so that the diploid β-actin gene was calibrated as 1.0. β-actin was compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a second standard for diploid gene dosage. Fresh tumors are denoted by letter and paraffin extracted tumors by number; normal tissues from matched animals A, B, G and M are shown as white bars. Ratios of 0.8 and above were scored as diploid and of 0.6 or below as haploid.
The lineage of mammalian species, including the domestic dog (Canis familiaris), can be traced back to a common ancestor. This ancestral lineage (Canis progenitor) underwent a series of events that led to the development of different subtypes of canine thymic tumors (CTVT). The diagram illustrates the evolution from a single progenitor to various canine species through genetic and environmental factors. Key events include the appearance of the CTVT subtypes and the role of LINE-1 mobile elements in oncogenesis.

- **Coyote** (Canis latrans)
- **Gray wolf** (Canis lupus)
- **Domestic dog** (Canis familiaris)

The diagram also highlights the diversity within each species, showing the evolution of different breeds over time.
A diagram illustrating the evolution of canine tumors, specifically focusing on the transition from an ancestral canine form to domesticated dogs. The diagram shows the lineage from the Canis progenitor to the Coyote (Canis latrans), Gray wolf (Canis lupus), and Domestic dog (Canis familiaris), highlighting the appearance of CTVT (Canine Papillomavirus Type 1) cells. The genetic representation includes LINE-1 and c-myc elements, indicating the genetic markers associated with CTVT subtypes.