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RESEARCH NOTE

No evidence for clonal transmission of urogenital carcinoma in California sea lions (*Zalophus californianus*) [version 1; referees: awaiting peer review]

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Abstract

Urogenital carcinoma is a highly metastatic cancer affecting California sea lions (*Zalophus californianus*). The disease has high prevalence amongst stranded animals, and is one of the most commonly observed cancers in wildlife. The genital localisation of primary tumours suggests the possibility that coital transmission of an infectious agent could underlie this disease. Otarine herpesvirus type 1 has been associated with lesions, however a causative role for this virus has not been confirmed. We investigated the possibility that urogenital carcinoma might be clonally transmissible, spread by the direct transfer of cancer cells. Analysis of sequences at the mitochondrial DNA control region in seven matched tumour and host pairs confirmed that tumour genotypes were identical to those of their matched hosts and did not show similarity with tumours from other individuals. Thus our findings suggest that urogenital carcinoma in California sea lions is not clonally transmitted, but rather arises from transformed host cells.

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Introduction

Urogenital carcinoma (UGC) is the most commonly observed neoplasm in California sea lions (*Zalophus californianus*)[1]. This cancer was first reported in sea lions on the west coast of North America in 1979[2], and over a fifteen-year period, from 1998 to 2012, the disease was found in 26 per cent of adult animals examined post-mortem at The Marine Mammal Center, California[1]. UGC affects both male and female animals, and is most frequently found in sexually mature adults[3,4]. The disease typically presents with extensive multi-organ metastases; however, primary lesions involving the genital epithelium can usually be identified[5].

Three aetiological factors have been proposed for the development of UGC: infection, host genetics, and environmental factors. Otarine herpesvirus type 1 (OtHV-1), a gammaherpesvirus related to Kaposi's sarcoma-linked human herpesvirus-8[5,6] has been associated with UGC[5–7]; however, this virus has not been confirmed as a causative agent. An association between UGC and genital bacterial infection has also been proposed[8]. Genetic studies have indicated that individuals with high parental relatedness[9], homozygosity at the *HSPE2* locus[10], or one or more copies of the *Zaca-DRB.A* MHC class II locus[11] have increased risk of UGC. Environmental contaminants, such as organochlorines, have also been proposed as causative agents in UGC carcinogenesis[12].

Cancer occurs when a somatic cell acquires mutations that drive it towards a program of uncontrolled clonal expansion. Although cancer cells can migrate and invade distant tissues, most cancers remain within the body of the host that spawned them. Rarely, however, cancers can become transmissible such that cancer cells themselves become infectious agents that are transferred between individuals as allogeneic grafts. Only eight examples of naturally occurring contagious cancers are known: canine transmissible venereal tumour (CTVT) found in domestic dogs[13,14], two distinct lineages of Tasmanian devil facial tumour disease[15,16], and five lineages of disseminated neoplasia affecting various species of marine bivalves[17,18]. Tumours derived from clonally transmissible cancers carry the genetic material of the original animal that first gave rise to the cancer; thus, transmissible cancers are characterised by shared genotypes that are distinct from those of their matched hosts.

Several features of UGC are compatible with the possibility that this cancer is clonally transmissible: epidemiological observations of UGC are consistent with an infectious aetiology for the disease[2]; and, in particular, its genital localisation could provide a coital route of transmission[19], as is observed with CTVT, the transmissible cancer in dogs.

We genotyped UGC tumours and their matched hosts to determine if UGC is clonally transmissible. Our results do not show evidence for UGC being a transmissible cancer, but rather confirm that UGC tumours are most likely derived from their hosts.

Methods

Ethics

This study was approved by The Marine Mammal Center Institutional Animal Care and Use Committee (Sausalito, CA) and the National Marine Fisheries Service MMPA (permit number 18786).

Samples

Tissues from seven wild stranded adult California sea lions were collected at The Marine Mammal Center, Sausalito, CA. Complete gross and histopathological examinations were performed on each animal to confirm UGC diagnosis. Tumour (metastasis) and host tissue (liver or muscle) biopsies were collected into RNAlater during post-mortem examination and were stored at −70°C until processing.

DNA extraction

Representative tissue sampled from tumour and host biopsies was used for DNA extraction using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. DNA was quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA).

PCR

We amplified a 1289 bp fragment of the mitochondrial DNA (mtDNA) control region using primers described by Wolf *et al*[20]. PCR was performed using an Eppendorf Mastercycler Nexus GSX1 (Eppendorf, Hamburg, Germany) with conditions as follows: 40 ng of genomic DNA was amplified in a total volume of 20 μl containing 0.5 μM of each primer, 0.2 mM of each dNTP and 0.02 units of Taq DNA polymerase (Qiagen, Hilden, Germany) per reaction. Cycling conditions were 95°C for 3 min, 30 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 45 s and a final extension step at 72°C for 5 min. PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Purified PCR products were capillary sequenced at Source BioScience LifeSciences Genomic Services (Source BioScience LifeSciences, Nottingham, United Kingdom).

Alignment and variant calling

Sequences were aligned to the California sea lion mtDNA reference genome (accession number NC_008416)[21] using Sequencher DNA Sequence Analysis Software v5.4.6 (Gene Codes, Ann Arbor, MI, USA). Alignment errors were inspected manually and corrected. Variant positions were identified by viewing alignments, as well as by manual assessment of sequence chromatograms using FinchTV v1.4.0 (Geospiza Inc., Seattle, WA, USA). Variants were only assessed within a 397 bp region of the product, comprising region 15490–15886 in NC_008416.

Results

We assessed 397 base pairs of the mtDNA control region in seven UGC tumours and their matched hosts. The analysis identified nine polymorphic sites characterising four unique genotypes within the sampled sea lion population (Table 1). In all cases, the alleles present in tumours were identical to those found in matched host tissue (Table 1). Chromatograms were closely examined at

Table 1. Mitochondrial DNA (mtDNA) genotypes at nine polymorphic sites in sea lion hosts and matched tumours. Coordinates are relative to the sea lion mtDNA reference genome, NC_008416[21]. Individual California sea lions (CSLs) are labelled numerically and matched hosts and tumours are represented side-by-side. Alleles, represented by nucleotide code (A,C,G,T), are shown. Alleles that differ from the reference are shaded in grey.

Individual:	Reference	CSL 1		CSL 2		CSL 3		CSL 4		CSL 5		CSL 6		CSL 7	
Tissue:		Host	Tumour	Host	Tumour	Host	Tumour	Host	Tumour	Host	Tumour	Host	Tumour	Host	Tumour
15524	T	T	T	T	T	T	T	T	T	T	T	C	C	T	T
15527	T	T	T	T	T	T	T	C	C	C	C	C	C	T	T
15528	T	T	T	T	T	T	T	C	C	C	C	C	C	T	T
15550	G	G	G	G	G	G	G	A	A	A	A	A	A	G	G
15551	A	A	A	A	A	A	A	G	G	G	G	A	A	A	A
15629	C	C	C	C	C	C	C	C	C	C	C	C	C	T	T
15652	A	A	A	A	A	A	A	A	A	A	A	G	G	A	A
15660	T	T	T	T	T	T	T	C	C	C	C	C	C	T	T
15812	G	G	G	G	G	G	G	A	A	A	A	A	A	G	G

mtDNA coordinate

polymorphic sites, but no evidence for amplification of additional alleles in tumour tissues was found[22].

Discussion

Our study does not support the hypothesis that UGC is clonally transmitted, but rather further confirms that UGC arises from host cells. Importantly, however, we cannot exclude the possibility that some UGCs are clonally transmitted. Given the genital localisation of this cancer, and likely accessibility of UGC cancer cells to other individuals during coitus, UGC tumours may pose a particular risk for the emergence of a transmissible cancer clone.

In this analysis we only examined genetic variation at one mtDNA locus. It is worth noting that at least one transmissible cancer – CTVT in dogs - has been observed to periodically capture mtDNA from its hosts[23]; thus, mtDNA may not be considered the most reliable marker for testing clonality in transmissible cancers. However, mtDNA horizontal transfer events were detected only five times in a cohort of 449 CTVT tumours[24]; thus even if mtDNA capture had occurred, it would not be expected that tumours would genetically match their hosts as frequently as we have observed in UGC.

Given that transmissible cancers are clonal lineages, tumour cell morphology and tissue architecture is generally very similar between tumours[25,26]. However, previous research has shown that UGCs appear to develop through histologically distinct stages[5], which further supports the idea of step-wise oncogenic transformation of host tissue rather than direct transmission of a cancer lineage.

Future research exploring the role of viral agents, host genetics and environmental factors, as well as somatic genetics, will be important for understanding the carcinogenic processes that cause UGC. It is interesting to note that an OtHV-1-associated UGC has recently been reported in a South American fur seal (*Arctocephalus australis*)[27], indicating that other pinnipeds are susceptible to UGC, and further implicating OtHV-1 as a causative agent. Furthermore, a recent analysis of cytological smears collected from California sea lions in the Gulf of California revealed that transformation of the genital epithelium may be relatively common in this species[28].

Wildlife models of cancer can provide novel insights into general mechanisms of cancer development[29]. Furthermore, an understanding of the aetiological factors underlying commonly observed cancers in wildlife is important for conservation and biomonitoring. In this study, we have found no evidence that UGC, one of the few "cancer epidemics" in wildlife[1,29], is clonally transmitted. Ruling out this mode of carcinogenesis is an important step in our understanding of UGC, and paves the way towards further research investigating the processes underlying this aggressive disease in sea lions.

Data availability

Data associated with this study are available in GenBank with accession numbers: MF000998 - MF001011.

References

1. Browning HM, Gulland FM, Hammond JA, *et al.*: **Common cancer in a wild animal: the California sea lion (*Zalophus californianus*) as an emerging model for carcinogenesis.** *Philos Trans R Soc Lond B Biol Sci.* 2015; **370**(1673): 20140228.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

2. Gulland FM, Trupkiewicz JG, Spraker TR, *et al.*: **Metastatic carcinoma of probable transitional cell origin in 66 free-living California sea lions (*Zalophus californianus*), 1979 to 1994.** *J Wildl Dis.* 1996; **32**(2): 250–258.
   PubMed Abstract | **Publisher Full Text**

3. Colegrove KM, Gulland FM, Naydan DK, *et al.*: **Tumor morphology and immunohistochemical expression of estrogen receptor, progesterone receptor,** p53 and Ki67 in urogenital carcinomas of California sea lions (*Zalophus californianus*). *Vet Pathol.* 2009; **46**(4): 642–655.
   PubMed Abstract | **Publisher Full Text**

4. Lipscomb TP, Scott DP, Schulman FY: **Primary Site of Sea Lion Carcinomas.** *Vet Pathol.* 2010; **47**(1): 185.
   PubMed Abstract | **Publisher Full Text**

5. Lipscomb TP, Scott DP, Garber RL, *et al.*: **Common metastatic carcinoma of California sea lions (*Zalophus californianus*): evidence of genital origin and association with novel gammaherpesvirus.** *Vet Pathol.* 2000; **37**(6): 609–617.
   PubMed Abstract | **Publisher Full Text**

6. King DP, Hure MC, Goldstein T, *et al.*: **Otarine herpesvirus-1: a novel**

gammaherpesvirus associated with urogenital carcinoma in California sea lions (*Zalophus californianus*). *Vet Microbiol.* 2002; **86**(1–2): 131–137.
PubMed Abstract | **Publisher Full Text**

7. Buckles EL, Lowenstine LJ, Funke C, *et al.*: **Otarine Herpesvirus-1, not papillomavirus, is associated with endemic tumours in California sea lions (*Zalophus californianus*).** *J Comp Pathol.* 2006; **135**(4): 183–189.
PubMed Abstract | **Publisher Full Text**

8. Johnson S, Lowenstine L, Gulland F, *et al.*: **Aerobic bacterial flora of the vagina and prepuce of California sea lions (*Zalophus californianus*) and investigation of associations with urogenital carcinoma.** *Vet Microbiol.* 2006; **114**(1–2): 94–103.
PubMed Abstract | **Publisher Full Text**

9. Acevedo-Whitehouse K, Gulland F, Greig D, *et al.*: **Inbreeding: Disease susceptibility in California sea lions.** *Nature.* 2003; **422**(6927): 35.
PubMed Abstract | **Publisher Full Text**

10. Browning HM, Acevedo-Whitehouse K, Gulland FM, *et al.*: **Evidence for a genetic basis of urogenital carcinoma in the wild California sea lion.** *Proc Biol Sci.* 2014; **281**(1796): 20140240.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

11. Bowen L, Aldridge BM, Delong R, *et al.*: **An immunogenetic basis for the high prevalence of urogenital cancer in a free-ranging population of California sea lions (*Zalophus californianus*).** *Immunogenetics.* 2005; **56**(11): 846–848.
PubMed Abstract | **Publisher Full Text**

12. Ylitalo GM, Stein JE, Hom T, *et al.*: **The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*).** *Mar Pollut Bull.* 2005; **50**(1): 30–39.
PubMed Abstract | **Publisher Full Text**

13. Murgia C, Pritchard JK, Kim SY, *et al.*: **Clonal origin and evolution of a transmissible cancer.** *Cell.* 2006; **126**(3): 477–487.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Murchison EP, Wedge DC, Alexandrov LB, *et al.*: **Transmissible [corrected] dog cancer genome reveals the origin and history of an ancient cell lineage.** *Science.* 2014; **343**(6169): 437–440.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

15. Pearse AM, Swift K: **Allograft theory: transmission of devil facial-tumour disease.** *Nature.* 2006; **439**(7076): 549.
PubMed Abstract | **Publisher Full Text**

16. Pye RJ, Pemberton D, Tovar C, *et al.*: **A second transmissible cancer in Tasmanian devils.** *Proc Natl Acad Sci U S A.* 2016; **113**(2): 374–379.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

17. Metzger MJ, Reinisch C, Sherry J, *et al.*: **Horizontal transmission of clonal cancer cells causes leukemia in soft-shell clams.** *Cell.* 2015; **161**(2): 255–263.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

18. Metzger MJ, Villalba A, Carballal MJ, *et al.*: **Widespread transmission of**

independent cancer lineages within multiple bivalve species. *Nature.* 2016; **534**(7609): 705–709.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Buckles EL, Lowenstine LJ, DeLong RL, *et al.*: **Age-prevalence of Otarine Herpesvirus-1, a tumor-associated virus, and possibility of its sexual transmission in California sea lions.** *Vet Microbiol.* 2007; **120**(1–2): 1–8.
PubMed Abstract | **Publisher Full Text**

20. Wolf JB, Tautz D, Trillmich F: **Galápagos and Californian sea lions are separate species: Genetic analysis of the genus *Zalophus* and its implications for conservation management.** *Front Zool.* 2007; **4**: 20.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

21. Arnason U, Gullberg A, Janke A, *et al.*: **Pinniped phylogeny and a new hypothesis for their origin and dispersal.** *Mol Phylogenet Evol.* 2006; **41**(2): 345–354.
PubMed Abstract | **Publisher Full Text**

22. Riquet F, Simon A, Bierne N: **Weird genotypes? Don't discard them, transmissible cancer could be an explanation.** *Evol Appl.* 2017; **10**(2): 140–145.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

23. Rebbeck CA, Leroi AM, Burt A: **Mitochondrial capture by a transmissible cancer.** *Science.* 2011; **331**(6015): 303.
PubMed Abstract | **Publisher Full Text**

24. Strakova A, Ní Leathlobhair M, Wang GD, *et al.*: **Mitochondrial genetic diversity, selection and recombination in a canine transmissible cancer.** *eLife.* 2016; **5**: pii: e14552.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

25. Loh R, Bergfeld J, Hayes D, *et al.*: **The pathology of devil facial tumor disease (DFTD) in Tasmanian Devils (*Sarcophilus harrisii*).** *Vet Pathol.* 2006; **43**(6): 890–895.
PubMed Abstract | **Publisher Full Text**

26. Karlson AG, Mann FC: **The transmissible venereal tumor of dogs: observations on forty generations of experimental transfers.** *Ann N Y Acad Sci.* 1952; **54**(6): 1197–1213.
PubMed Abstract | **Publisher Full Text**

27. Dagleish MP, Barrows M, Maley M, *et al.*: **The first report of otarine herpesvirus-1-associated urogenital carcinoma in a South American fur seal (*Arctocephalus australis*).** *J Comp Pathol.* 2013; **149**(1): 119–125.
PubMed Abstract | **Publisher Full Text**

28. Barragán-Vargas C, Montano-Frías J, Ávila Rosales G, *et al.*: **Transformation of the genital epithelial tract occurs early in California sea lion development.** *R Soc Open Sci.* 2016; **3**(3): 150419.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

29. McAloose D, Newton AL: **Wildlife cancer: a conservation perspective.** *Nat Rev Cancer.* 2009; **9**(7): 517–526.
PubMed Abstract | **Publisher Full Text**