and induce immune rejection. Inducing MHC I up-regulation has been the basis of a DFTD vaccine and immunotherapy. Within the restrictions imposed when working with endangered species and with limited access to devils, small-scale vaccination and immunotherapy projects have been undertaken in captivity (*13*). Vaccines consisting of DFT1 cells treated ex vivo with IFN-γ to up-regulate MHC I and inactivated by sonication or ionizing radiation induced production of serum antibodies in devils, indicating activation of an immune response. These serum antibodies were demonstrated to recognize normal DFT1 cells that do not express MHC I in vitro. This crucial finding enabled the next stage of testing: to inoculate the vaccinated devils with live DFT1 cells to determine whether the immunization was protective. Of the six vaccinated devils tested, one did not develop a tumor, and the others had a delayed onset.

The devils that developed tumors were further used to test immunotherapy with IFN-γ injected directly into the tumor (*13*). IFN-γ injection was not successful, most likely because of its short half-life in vivo. However, the injection of live tumor cells treated ex vivo with IFN-γ to up-regulate MHC I caused tumor regression, presumably by activating an allogeneic response. A limitation of using IFN-γ in cancer prevention and treatment is its capacity to up-regulate cell-surface immune inhibitory (checkpoint) molecules. This was demonstrated with DFT1 cells treated with IFN-γ; these cells up-regulated the immune checkpoint molecule programmed cell death 1 ligand 1 (PD-L1), which prevents T cell activation (*14*). To preempt this possibility, and on the basis of successful treatment of some human cancers, monoclonal antibodies that antagonize the programmed cell death protein 1 (PD-1)–PD-L1 pathway in devils have been produced (*14*). These could be incorporated into an immunotherapy protocol, potentially with vaccination by using DFTD tumor cells that express MHC I, to treat or prevent DFTD.

Vaccination of wild Tasmanian devils provides an approach to prevent DFTD transmission. Unlike human cancer "vaccines," which are mostly therapeutic, a vaccine against DFTD could be preventative, analogous to human vaccines that protect against infectious diseases. Tasmanian devils were immunized with DFT1 cancer cells that had undergone prior treatment with IFN-γ to induce MHC I expression. The first cohort included 52 devils that had been bred in a captive environment. These devils were immunized and released into the wild to help restore the population devastated by DFT1. Immune responses, determined by the presence of serum antibodies to DFT1, were detected in 50 devils (*15*). However, preliminary follow-up studies have indicated that protection may only be partial because some devils have developed DFT1.

The long-term presence of CTVT in the dog population suggests a coevolution between the host and the tumor, resulting in a transmissible cancer that is generally not lethal to dogs. Although DFTD currently remains fatal to most infected devils, a similar situation could arise over time, resulting in less aggressive DFTD cancers. In the meantime, interventions against DFT1 and DFT2 are required to protect devils in the wild from these cancers. The challenges associated with delivering a therapy to wild devils are extensive, and a preventative vaccine that can be delivered to trapped devils remains the most viable option for protection from DFTD. This vaccine must provide long-term immunity against DFTD in one "shot," requiring improvements to current vaccines. Research in CTVT and DFTD has determined that MHC I down-regulation is central, but not a sole contributor, to cancer transmissibility. Further research will explore other mechanisms of immune suppression in DFTD, so that these can be jointly targeted to improve DFTD vaccine efficacy. This research is already underway in the studies investigating immune checkpoint molecules (*14*). However, it is possible that other aspects of DFTD survival will need to be elucidated for a robust DFTD vaccine to be developed. Nonetheless, the strong responses to DFTD vaccination that have been produced in preliminary trials are promising, and with ongoing efforts to understand cancer transmission, a vaccination that promotes protective immunity against DFTD is a realistic future expectation. ■

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WILDLIFE CANCER

Cancer cell evolution through the ages

A transmissible dog cancer that has been evolving for 6000 years rapidly reached its optimal state

By **Carlo C. Maley**[1] *and* **Darryl Shibata**[2]

The essential ingredients for evolution are variation, inheritance, selection, and time. Like all of life, cells within human bodies can evolve, because dividing cells accumulate many somatic mutations with age, some of which benefit their survival or reproduction. However, cellular evolution is constrained by the death of the host. But what if somatic cells could outlive their hosts by escaping to another host? On page 464 of this issue, Baez-Ortega *et al.* (*1*) document the remarkable odyssey of the canine transmissible venereal tumor (CTVT) using DNA exome sequencing of 546 CTVT samples from dogs throughout the world. This ancient tumor has been sexually transmitted between dogs for ~6000 years, indicating that there is no inherent limit on the number of mammalian cell divisions.

CTVT probably started from a macrophage (*2*), which evolved into a sexually transmitted parasite that can evade the canine immune system long enough to be transmitted to a new host. It is usually cleared by the host's immune system before it becomes lethal. This rather benign course is common for sexually transmitted infections, the agents of which must rely on relatively healthy hosts to capitalize on infrequent mating to persist (*3*). Baez-Ortega *et al.* found that CTVT originated in Asia ~6000 years ago and started dispersing worldwide ~2000 years ago. They showed that in the past 500 years, CTVT has crisscrossed the globe, with the help of human travel. The same researchers previously sequenced CTVTs from an Australian

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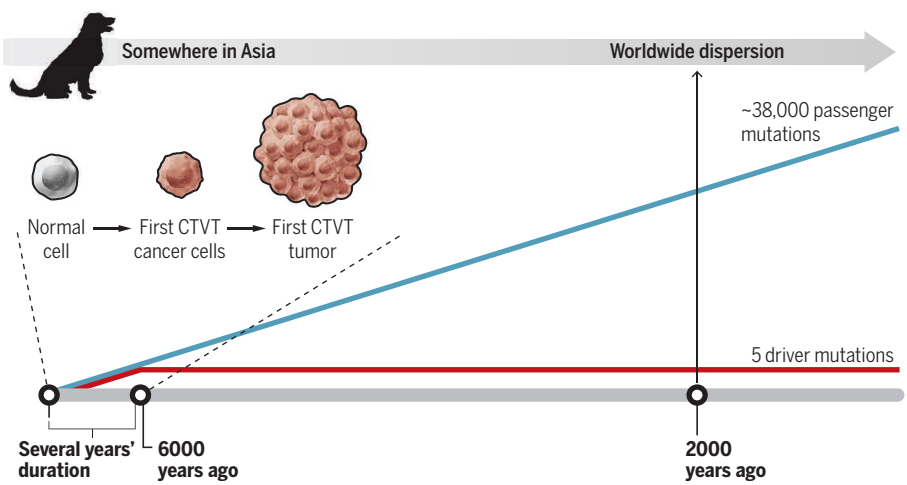
and a Brazilian dog and found a pronounced stability in the CTVT genomes, despite considerable divergence from the original dog genome (*4*).

The generation of a cancer cell requires mutations in genes that confer growth advantages and immortality to the cell, called driver mutations. An open question in cancer biology is whether cancers ever reach a peak of fitness: a locally optimal strategy for surviving and reproducing in their environment. Approximately two-thirds of human cancers show evidence of ongoing natural selection, even after they accumulate sufficient mutations to make them cancerous (*5*). Current CTVT cells have had thousands of years to optimize their fitness, and their exomes (DNA sequences that encode proteins) are riddled with somatic mutations: ~38,000 per sample compared to ~100 per human cancer sample.

The evolution of transmissible tumors in dogs

Canine transmissible venereal tumor (CTVT) arose in a normal dog ~6000 years ago in Asia. The five driver mutations identified in CTVTs today were likely present in the first CTVT cell. CTVT evolution has been neutral for most of its history, accumulating large numbers of passenger mutations.



GRAPHIC: A. KITTERMAN/*SCIENCE*

Most genes in the CTVT genome had at least one protein-altering mutation among the 546 tumors. There are more than 200 known driver genes in humans that, when mutated, can increase cellular fitness. However, only five such driver genes were found to be mutated in the CTVT cells, which is approximately the same number of driver mutations estimated to occur in many human cancers (*6*). These driver mutations were found in all the CTVT samples, and therefore arose very early in CTVT evolution. Perhaps they were even present in the founder cancer cells (see the figure). The lack of further driver mutations suggests that there has been little ongoing selection since the CTVT line developed.

The absence of ongoing selection was also assessed by Baez-Ortega *et al*. by measuring the ratio of mutations that change proteins to those that do not. The data suggest that CTVT has been evolving neutrally, accumulating mutations that do not change cell fitness. This lack of selection is in marked contrast to long-term cultures of the bacteria *Escherichia coli*, which continue to adapt over thousands of generations (*7*). CTVT appears to be on a fitness peak, or more accurately, a fitness plateau.

Only ~2000 genes in CTVT have been conserved. Mutations that changed those genes were probably detrimental to the CTVT cells and were quickly weeded out by natural selection. This implies that most genes in the mammalian genome are not needed by cancer cells, and only a handful of genes need to be tweaked to reach a fitness plateau. Once reached, there is little advantage in having a high mutation rate, and indeed CTVTs show no signs of high rates of DNA mutation or chromosomal instability that are common in human cancers. It is unclear if CTVTs were always genetically stable, or like human cancers, went through a period of genetic instability. The key to longevity may be the avoidance of declining fitness caused by the accumulation of deleterious mutations in essential genes, a problem known as Muller's ratchet (*8*).

The lack of ongoing natural selection suggests that CTVT has not had much of an effect on dog survival and reproduction, which is consistent with the typical benign course of the disease. If CTVT had a strong selective effect on dogs, there would be evidence of a coevolutionary arms race, with dogs evolving defenses to CTVT and CTVT evolving adaptations to overcome those defenses.

Genome sequences also reveal mechanisms of mutagenesis, which produce characteristic base changes (*9*). CTVT mutations appear to be caused by many of the same mechanisms that cause mutations in human cancers, including the signature of aging or cell division. Another common signature was caused by ultraviolet (UV) light. The closer the dogs were to the equator, the more UV-induced mutations accumulated in their tumors. This suggests that the mutations were likely caused by sunlight striking CTVT cells on exposed genitalia.

The remarkable evolution documented by CTVT genome sequencing provides evidence that neoplasia is not inherently progressive. This gives hope that some relatively indolent human cancers, including many prostate cancers, could also be controlled for long periods of time when cure is not possible. The idea of such evolutionary or adaptive therapy (*10*) is to limit tumor growth rather than inevitably selecting for more aggressive or lethal subclones with attempts at curative therapy.

Mapping CTVT somatic cell evolution and its spread throughout the world has many parallels to mapping how mutant cells evolve and spread within a human body. Although the scales are different, the genomes that are increasingly being sequenced from human cells can also record what those cells do, how they spread, and what they were exposed to. This is valuable because it is difficult to observe the life and death of most human cells over time. Understanding how mammalian cancers can evolve over long periods of time will likely be important in future attempts to manage that evolution to prevent mortality and morbidity due to cancer. ■

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