



Review Article

# Canine Transmissible Venereal Tumors: Aspects Related to Programmed Cell Death

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## Abstract

Canine transmissible venereal tumor (CTVT) is a neoplasm transmitted by the physical transfer of viable tumor cells by direct contact with injured skin and/or mucous tissue. These cells can transpose across histocompatibility barriers into unrelated hosts. This review focuses on the biology of apoptosis and the interaction of proteins involved in this process, as well as p53, p63 and the antiapoptotic protein Bcl-2. As such, this disease offers unique opportunity to study the biology of transplantable tumours and the interaction of proteins involved in apoptosis process and the prognosis of CTVT.

**Key Words:** CTVT, Sticker's sarcoma, apoptosis, dog

## Introduction

The canine transmissible venereal tumor (CTVT) is a neoplasia naturally transmitted in susceptible dogs by transplantation of viable tumor cells (11). Also known as Sticker's sarcoma, this tumor was first reported in 1820 by Hüzzard and was then later reported by Delabere-Blaine (1928). However, it was best characterized by Sticker between the years of 1905 – 1906, leading to its designation as Sticker Tumor for many years. Sticker described this neoplasia in detail and found that it was a transmissible neoplasia predominantly localized to the genital region, according to Chiti and Amber (1992) (7).

CTVT tumor cells are classified as round cell neoplasms, mastocytomas, histiocytomas, plasmacytomas and lymphomas (74). Young dogs, stray dogs and sexually active dogs are most frequently affected by this neoplasm (59, 38, 73). It has a worldwide distribution and the incidence is highest in tropical and subtropical regions. This tumor affects dogs (*Canis familiaris*) and can also infect other canids, such as foxes, coyotes and wolves (13, 11).

Recently, another transmissible tumor was described with a pattern similar to CTVT, occurring in carnivorous marsupials such as the Tasmanian devil (*Sarcophilus harrisi*). Unlike CTVT, the neoplasm in Tasmanian devils occurs in the face, neck and oral cavity and often progresses with metastases, causing the death of the animal a few months after the onset of initial symptoms. This neoplasm is causing a general decline in the population of Tasmanian devils (24, 34, 41).

## Transmission and Etiology

CTVT is usually transmitted to genital organs during sexual intercourse but can affect the skin via the direct implantation of tumor cells during contact between skin and tumor masses (12, 13, 39, 48, 37). Transplantation occurs when intact host tumor cells lose the expression of major histocompatibility complex (MHC) class I and II molecules, enabling transposition of the tissue to a healthy animal by contact between skin and damaged mucosa (48, 37). In a study examining the transplantation of CTVT cells into mice,

we observed that this tumor could only be transferred between healthy animals that shared the same MHC or into immunocompromised recipients, as the CTVT cells induce an immune response in healthy recipients (48).

These studies were guided by the transplantation theory and etiology of CTVT. The transplantation theory is based on the observation that experimental tumor transplantation can only occur using living tumor cells (12). Other studies have established that CTVT cells can be derived following mutations induced by viruses, chemicals or radiation of lymphohistiocytic cells and that these clones of tumor cells can then be disseminated by allogeneic transplantation (48).

Studies using immunohistochemical techniques demonstrated that this neoplasm was positive for lysozyme and alpha-1-trypsin and suggested that CTVT is of mesenchymal and histiocytic origin (6, 53, 79). Additionally, there are reports of the detection of naturally-occurring *Leishmania* amastigotes in the cytoplasm of primary, extra-genital CTVT (6).

Cytogenetic studies supporting the theory of clonal transmission are based on dogs having 78 chromosomes, 76 of which are acrocentric. In CTVT cells isolated from animals from different geographic regions, the chromosome number varies from 57 to 59, where 15 to 17 chromosomes are metacentric or submetacentric (76, 30, 49, 48).

In addition to this unique feature, constant and specific chromosomal aberrations, such as the insertion of a LINE-1 element near the c-myc oncogene in the

CTVT genome, are present in most samples collected from various regions of the world. This rearrangement in the genome has not been identified in any other normal tissue of dogs and can be used for CTVT diagnosis (11, 8, 35, 48, 53, 71, 58).

Murgia et al. (2009) and Rebbeck et al. (2009) confirmed the clonal transmission of this tumor when they found that the pattern of microsatellite polymorphisms in CTVT from different regions of the world showed evidence of monophyletic origin. Mitochondrial and MHC differences suggest that many modern CTVT clones belong to two groups distributed around the world (48, 58).

The genetic ancestor of CTVT was recently investigated by Rebbeck et al. (2009), who determined that the neoplasia probably arose from a dog or wolf rather than from a distant member of the canid family (58). Additionally, Murgia et al. (2006) used microsatellite polymorphisms to compare CTVT with normal tissues of eighty-five breeds of dogs and eight species of wolves and found that CTVT showed strong identity with wolves. MHC variants found in the tumor cells also showed a significant phylogenetic relationship with wolves (Fig. 1) (48). Rebbeck et al. (2009) used microsatellites to determine the timing of the origin of CTVT (58). This study indicated that CTVT probably arose from a single wolf approximately 7,800 to 78,000 years ago. More recently, a single clone became dominant and then divided into two groups with a worldwide distribution. This evidence indicates that CTVT is the oldest transplantable somatic cell clone known (47).

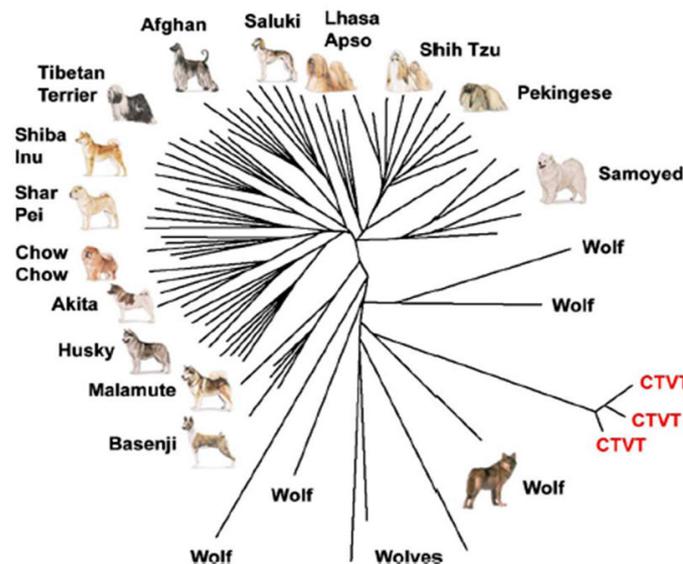


Figure 1: Stages of tumor progression. A) Neoplasia showing high cellularity, mitosis and scarce conjunctive tissue H.E. B) Neoplasia in initial regression phase as shown by the presence of tumour infiltrating lymphocytes (TILs) HE. C) Final regression stage as seen by tumour parenchyma collapse and substitution by fibrous tissue HE. Bar 20µm. Reprinted from Cell, vol. 126, n.3, Claudio Murgia, Jonathan K. Pritchard, Su Yeon Kim, Ariberto Fassati, Robin A. Weiss - Clonal Origin and Evolution of a Transmissible Cancer, p. 477-487, 2006, with permission from Elsevier.

### Clinical and pathological characteristics

CTVT commonly affects the external genitalia in dogs of both sexes. In males, the tumor is commonly located in the caudal part of the penis, the glans, and occasionally in the foreskin. In females, this tumor is often found at the junction of the vestibule and the posterior region of the vagina and occasionally in the urethral opening. (12, 74, 59, 13, 39).

CTVT can occur as a solitary mass or as multiple tumors with pendular, nodular, papillary or multilobular forms presenting a cauliflower-like appearance (5, 23). The tumor is friable and is often ulcerated and inflamed (5). The tumor size can vary from 3 to 12 cm in diameter (53).

Clinical signs of genital CTVT are bloody vaginal or preputial discharge, intermittent or persistent ulcerative skin lesions, poor penile exposure, genital swelling and excessive licking of the genital area (50, 65).

CTVT may also develop in extra-genital sites such as skin, subcutaneous tissues and around and in the oral and nasal cavities. Extra-genital tumors are well circumscribed and can measure 2-5 cm (13). Metastases are rare in CTVT, yet they can occur, especially in puppies and immunocompromised dogs. These metastases are often considered mechanical extensions

of the primary tumor; however, metastases have been reported in inguinal lymph nodes (1, 40, 53), liver and eye (19).

### Histopathologic characteristics

Histologically, CTVT is composed of round cells, arranged or grouped in strings, interspersed with delicate conjunctival stroma when stained with hematoxylin and eosin. The tumor cells are usually arranged radially around blood and lymphatic vessels and have a high nucleus: cytoplasm ratio with a round nucleus and chromatin ranging from delicate to coarse and prominent nucleoli (13, 61, 53). These cells contain a large amount of cytoplasm that is slightly acidophilic with poorly-defined limits (46).

The tumor can be classified into progression and initial and final regression phases according to developmental stages. The progression phase presents as round cells arranged diffusely, interspersed by delicate conjunctival stroma and the frequent presence of mitotic structures. In the initial phase of regression, tumor-infiltrating lymphocytes (TILs) appear and are widely distributed or associated with the conjunctival stroma (35, 46). The final regression phase involves collapse of the neoplastic tissue and the frequent presence of apoptotic bodies (Fig. 2) (46).

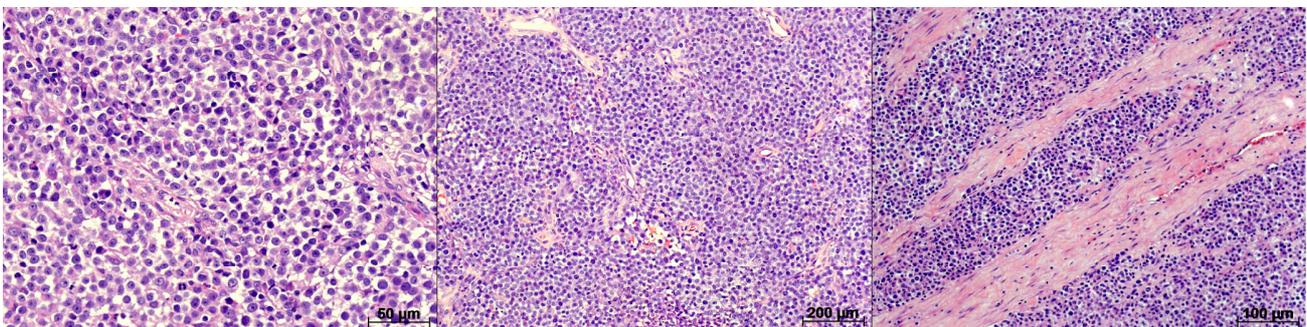


Figure 2: Stages of tumour progression. (A) Neoplasia showing high cellularity, mitosis and scarce conjunctive tissue (haematoxylin and eosin). (B) Neoplasia in initial regression phase as shown by the presence of TILs (haematoxylin and eosin). (C) Final regression stage as seen by tumour parenchyma collapse and substitution by fibrous tissue (haematoxylin and eosin). Bar 20 µm.

### Cytopathological characteristics

Cytological examination is a quick, efficient, inexpensive and relatively simple tool for the diagnosis of CTVT (23). When subjected to Romanovsky staining, both genital and extra-genital neoplasias present characteristic round cells with distinct cytoplasmic borders. The nuclei are oval or round and centrally-located, with delicate chromatin and large nucleoli; the cytoplasm is slightly acidophilic and contains finely granular, delicate vacuoles, and cells do not display anisokaryosis, anisocytosis, hyperchromasia or nuclear macrokaryosis (18, 14). Mitoses are frequent, may be typical or atypical, and are indicative of proliferation of tumor cells (2). Apoptotic bodies of are

also observed by cytological exam and are present in higher quantities in CTVT in the regression phase (61). Inflammatory cells such as lymphocytes, plasma cells, macrophages and neutrophils are observed regardless of the stage of neoplastic development (77, 4, 18, 13, 61).

Upon cytopathological exam, it is possible to classify the CTVT tumor based on the predominant cell type as lymphoid, plasmacytoid or mixed. The lymphoid type of tumor predominantly includes cells with a rounded morphology, scant and finely granular cytoplasm, the presence of vacuoles, and round nuclei with coarse chromatin and the presence of one or two evident nucleoli. In plasmacytoid tumors, most cells have an ovoid morphology, a smaller relative nucleus: cytoplasm ratio and eccentrically-located nuclei,

whereas the mixed type of tumor exhibits mixed cellularity (23, 2).

### The immune system and CTVT

The development of CTVT is mediated by the immune system, where the outbreak of disease represents the success of the neoplasia in overcoming the host immune system. Puppies born to females exposed to CTVT are less likely to contract this cancer (46). In immunocompromised animals experimentally infected with viable CTVT cells, disease progression and metastasis was observed; however, those dogs who quickly recovered acquired immunity against subsequent implantations (46).

In healthy animals, CTVT regresses spontaneously. Regression is associated with the infiltration of lymphocytes and plasma cells and with necrosis (54) and apoptosis (61).

The transition between the progression and regression phases of CTVT is accompanied by a significant increase in the infiltration of TILs (54, 22, 10, 27, 45, 46). The major histocompatibility complex (MHC) class I and II molecules are either not expressed or are present on only a small subset of neoplastic cells during the progressive phase (12, 78, 43, 54, 27, 48). Interestingly, a significantly greater proportion of CTVT cells express MHC class I and II in the regression phase (78, 54, 27).

Liao et al. (2003) showed that the proportion of B lymphocytes in the peripheral blood decreased dramatically with CTVT growth. The destruction of B lymphocytes was caused by substances released by the tumor cells, such as cytotoxic proteins and other circulating substances. These cytotoxic substances cause B lymphocyte apoptosis during the neoplastic progression phase (35).

Hsiao et al. (2004) showed that cells from CTVT produce transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), and that this cytokine inhibits the activity of natural killer cells (NK) and the infiltration of cytotoxic lymphocytes. The suppressive effect of TGF  $\beta$ 1 on NK cell activity can be balanced by the effect of interleukin 6 (IL-6), which is a proinflammatory cytokine secreted by tumor lymphocytes (26)

The expression of MHC by CTVT cells *in vitro* can be induced by synergy between interferon- $\gamma$  and IL-6 (26). IL-6 can induce the expression of MHC by CTVT cells *in vitro* and *in vivo*. In the latter case, MHC is induced by the presence of IL-15 (9).

CTVT does not induce immune recognition regulated by MHC class I and II and by NK cells in the progression phase because it suppresses the secretion of IL-6 during this phase. When the level of IL-6 secreted by tumor lymphocytes reaches a threshold level, it begins to act in concert with interferon- $\gamma$ , allowing the infiltration of lymphocytes into the tumor, activating the expression of MHC class I and II by the CTVT cells, thus beginning the regression of CTVT (28, 26).

It should be noted that the presence of apoptosis, mitosis, cell proliferation, fibrosis and

infiltration of TIL are good indicators of the CTVT developmental stage. Mast cells also play an important role as their numbers can be used to predict the stage of tumor evolution (45).

### The p53 family

*TP53* is one of the most important tumor suppressor genes involved in the development of neoplasias (68, 72). Given the important role of this gene, its functional polymorphisms can profoundly affect the development of tumors (51, 67). *TP53* encodes a 393 amino acid nuclear protein, p53, able to bind to specific DNA sequences and act as a transcription factor (31). The p53 maintains genomic integrity and controls cell growth (57). The cellular expression and activity of p53 are closely related. Additionally, p53 has a short half-life (20 minutes) and is therefore present at low levels within cells. The activation of this protein occurs in response to stress or agents that damage cellular DNA, causing cell cycle arrest and the induction of senescence or apoptosis (75). More than 10 mutations in the *TP53* gene have been described in canine neoplasias (51, 64). Recently, *TP53* mutations have also been observed in CTVT (8, 60, 69).

Moro et al. (2010) found more cells that expressed p53 protein in transplanted CTVT in the regression phase compared with naturally occurring CTVT. These findings suggested that there may be functional abnormalities in the *TP53* gene and its transcripts in these tumors (44, 66). The loss of p53 function is a common event in many human and animal tumors (20, 63). Inactivation of p53 occurs not only through mutation and deletion of p53 itself, but also by disruption of pathways that regulate its activity, such as via Mdm2 (15).

Almost a decade after the discovery of the *TP53* gene, two other related genes were identified: *P63* and *P73* (29, 62, 52; 70, 78, 80). Similar to p53, p63 is expressed at higher levels in cells with DNA damage (55).

The p63 protein is expressed by normal epithelial stem cells, protecting these cells from apoptosis and coordinating their differentiation (15). This protein is also necessary for the development of somatic tissues, as has been observed in mammary glands (21). It has at least six isoforms, including a full  $\alpha$  isoform, a truncated  $\beta$  isoform and a  $\gamma$  isoform. These isoforms (TAp63 $\alpha$ , TAp63 $\beta$  and TAp63 $\gamma$ ) have the ability to activate p53 and induce apoptosis and block the cell cycle (17). However, the other three isoforms ( $\Delta$ Np63 $\alpha$ ,  $\Delta$ Np63 $\beta$  and  $\Delta$ Np63 $\gamma$ ) act as dominant negatives to suppress the activity of both p53 and the TAp63 isoform (17). Despite structural homology, there is no evidence that *P63* is a tumor suppressor gene like *TP53* (20). P63 may also play a role in replicative cellular senescence, by competing with P53 to bind DNA or through a direct interaction with p53 bound to DNA (16).

### The Bcl-2 family

The Bcl-2 family is a group of proteins that induce or inhibit cell death by apoptosis (3). Some members of the Bcl-2 family, including Bcl-2 and Bcl-xL, are anti-apoptotic regulators that inhibit apoptosis, preventing the release of cytochrome c from mitochondria. (32). Other members of this family, Bax, Bid and Bak, are pro-apoptotic proteins (25).

Overexpression of Bcl-2 has been associated with mutant p53 (33). The p53 can induce apoptosis, leading to mitochondrial outer membrane permeabilization, and form complexes with Bcl-2 proteins, resulting in the release of cytochrome c from mitochondria (42). Cells of TVTC showed overexpression of Bcl-2 protein independent of the stage of tumour development (66). Bcl-2 and its family members participate in carcinogenesis, but their contributions remain puzzling because their expression can be associated with resistance to drugs and

radiotherapy on the one hand and with a low-malignancy phenotype and a favorable prognosis on the other. The association of Bcl-2 expression with tumour survival suggests that the overexpression of this protein promotes the acquisition of functions associated with tumour progression (5).

Cellular homeostasis is maintained by controlling the presence of anti-apoptotic and pro-apoptotic proteins. Stimuli such as DNA damage lead to increased expression of pro-apoptotic proteins, inducing apoptosis (56).

Among the well-studied proteins in this family are Bax (pro-apoptotic) and Bcl-2 (antiapoptotic), which is overexpressed in colorectal adenomas and carcinomas in humans (56). The Bax and Bcl-2 proteins are capable of forming homodimers (Bax-Bax and Bcl-2-Bcl-2) and heterodimers (Bax-Bcl-2), and equilibrium between these dimers can set the cellular pro-apoptotic or antiapoptotic balance (Fig. 3) (25).

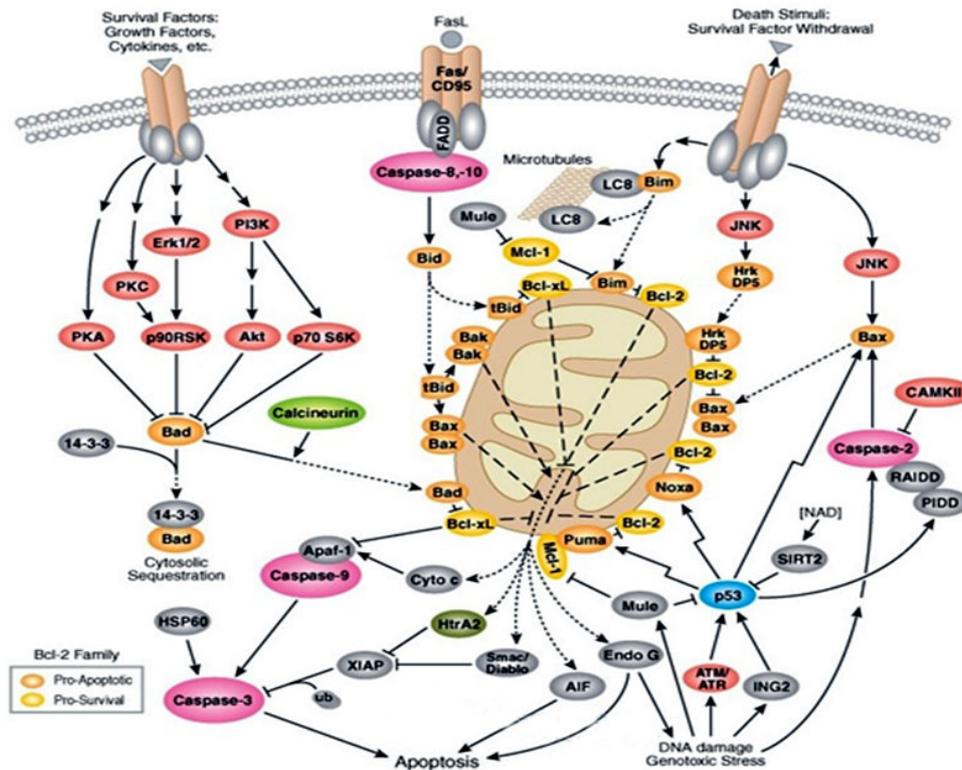


Figure 3: Diagram representing the Bcl-2 family of proteins in the apoptotic process controlled by mitochondrial permeability. Bcl-2 and Bcl-xL are anti-apoptotic proteins residing in the outer mitochondrial wall that inhibit the release of cytochrome c. The pro-apoptotic proteins Bad, Bid, Bax and Bim may be located in the cytosol and translocate to the mitochondria after a cell death signal, where they promote the release of cytochrome c. Bad translocates to mitochondria and forms a complex with Bcl-xL. This translocation is inhibited by survival factors that induce Bad phosphorylation. The Bim and Bax proteins also undergo mitochondrial translocation in response to death stimuli including withdrawal of survival factors. DNA damages activate p53, which induces the transcription of Bax, Noxa and PUMA. After cytochrome c is released from mitochondria, it binds to Apaf-1 and activates caspase-9. Although the mechanisms that regulate mitochondrial permeability and cytochrome c release during apoptosis are not fully understood, Bcl-xL, Bcl-2 and Bax may influence the voltage-dependent anion channel (VDAC), which may play a role in regulating the release of cytochrome c. Mule/ARF-BP1 is E3 ubiquitin ligase involved in DNA damage-induced apoptosis that is activated by p53 and Mcl-1 and is an anti-apoptotic Bcl-2 family member (adapted from Cell Signaling Technology).

After a cell death stimulus, Bcl-2 inhibits the permeabilization of the outer membrane of the mitochondria by sequestering Bax or by competing with sites that would be occupied by Bax. Bax can also promote apoptosis through interaction with mitochondria, independent of interaction with anti-apoptotic proteins (32). Bcl-2 overexpression is observed in neoplasias that are significantly associated with hormone receptors (36).

### Closing remarks

Although some apoptotic aspects related to CTVT have been described in the last years, little information is still available in order to understand the mechanism involved in the regression phase of these cells. Developing molecular approaches associated to neoplasia pathology evaluation is therefore important and can elucidate new strategies for oncolytic therapy in near future.

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