Letter to the Editor

Characterization of a novel canine T-cell line established from a dog with cutaneous T-cell lymphoma

Cutaneous T-cell lymphoma (CTCL) has been recognized in dogs, which accounts for 11.84% of all canine lymphomas or 33.49% of canine T cell lymphoma [1]. Canine CTCL is characterized by proliferation of epidermotropic cells and rapid progression with multiple patches, plaques, and ulcerations. Immunophenotypically, the neoplastic T cells express CD8 in 82.6% of dogs with CTCL [2]. A previous study also indicated that transcription levels of perforin and granzyme B in the lesional skin of canine CTCL were significantly higher than those in normal skin, suggesting that neoplastic cells are most likely categorized as cytotoxic T cells [3]. The prognosis of canine CTCL is very poor, with a median survival of 6 months [4]. Interestingly, clinical, histopathological, and molecular features in canine CTCL are similar to those in primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma (CD8+PCECTL). Thus, we consider that canine CTCL has the potential to be used as a model animal for CD8+PCECTL. In the present study, we established a novel cell line derived from a dog with CTCL, termed as EO-1, and evaluated its morphological, immunophenotypical, and transcriptional characteristics.

A six-year-old female English bulldog was referred to Gifu University Animal Medical Center with a generalized skin disorder characterized by a diffuse cutaneous erythema, plaques, alopecia, and ulceration (Fig. 1a). A dermatohistopathological examination revealed an infiltration of lymphoid cells into the dermis (Fig. 1b). An immunohistochemical examination indicated that the majority of neoplastic cells were positive for CD3 (Fig. 1c) but negative for CD20 (data not shown). No atypical lymphoid cells were identified on the peripheral blood smear, making the final diagnosis CTCL. An aseptic biopsy of the enlarged popliteal lymph nodes was performed for cell isolation after informed consent from the owner. The collected tissue was cultured in 75 cm² flasks in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Biological Industries, Beit HaEmek, Israel), 1% penicillin/streptomycin (Sigma), and 2 mM L-glutamine (Sigma). The cells, termed as EO-1, were maintained at 37 °C in a humidified atmosphere of 5% CO₂ without any growth factors or feeder cells. The culture medium was changed 3 times a week.

![Fig. 1](http://dx.doi.org/10.1016/j.jdermsci.2017.05.017)

*Fig. 1.* Gross and histopathological findings of the dog with canine CTCL.

(a) The dog shows multiple erythematous, plaques, crusts, alopecia, and ulcerations. A section from the skin lesion stained with (b) HE or (c) anti-canine CD3 monoclonal antibody. At 200× magnification.


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The EO-1 cells were maintained for over 2 years with a doubling time of 28.4 h (Fig. S2). Morphological analysis with a phase-contrast microscopy indicated pseudopod formations (Fig. 2a). The EO-1 cells were generally bleb-like in shape with diameters of approximately 20 μm. Multiple nucleoli were observed to be horseshoe-shaped or irregular and located eccentrically. The cytoplasm was stained blue and had a perinuclear halo containing fine or coarse azurophilic granules (Fig. 2b).

The immunophenotypical analysis demonstrated that the EO-1 cells were positive for CD3, CD8, CD18, CD45RA, and MHC II, but negative for CD1c, CD4, and CD11b (Fig. 2c), suggesting that the EO-1 cells share the immunophenotype of mature T cells. Transcription levels of mRNA (Table S3) are shown by word cloud visualization (Fig. 2d), in which the word size is proportional to the transcription level compared with the mean value of those in other CD8-positive canine lymphoma cell lines (UL-1 and CLK). The results of the transcription analysis indicated that transcription levels of cytokines (IL-2, IL-8, IL-13, IL-17F, IL-22, IL-34, and IFN-γ), chemokine receptors (CCR4, CCR7, and CXCR4), and others (FUT7 and S100A8) in EO-1 were higher than the mean values of UL-1 and CLK. Flow cytometric analysis indicated co-expression of CCR4 and CD8 on the EO-1 cells (Fig. 2e). A sequence analysis demonstrated that both reads for complementary and genomic DNA corresponded perfectly to the reference sequence of canine CCR4. The results of the transwell chemotaxis assay showed that the average number of migrated EO-1 cells significantly increased at the concentration of 100 and 500 nM recombinant human (Fig. 2f) or mouse (Fig. 2g) TARC. The results of the transwell chemokinesis assay revealed that the motility of the EO-1 cells occurred mainly by chemotaxis (Fig. S3).

CD8+PCECTL is characterized by rapid progression with aggressive skin lesions, such as ulcerations, and has a poor overall prognosis. Effective therapeutic strategies should be established based on the pathogenesis, which remains largely unknown due to a lack of suitable animal models and cell lines. Since canine CTCL shares characteristic clinical features with those of CD8+PCECTL, canine CTCL could be regarded as a possible animal model for humans. In the present study, therefore, we established a novel cell line derived from a dog with CTCL, and evaluated its morphological, immunophenotypical, and transcriptional characteristics.

The distinctive feature of the EO-1 cells is a high expression of a functional CCR4. In human CTCL, the neoplastic cells often express CCR4 [5], which is a chemokine receptor that plays a crucial role in T-cell homing to skin [6]. Thus, CCR4 is thought to be involved in the aggregation of CCR4+ malignant T cells in the skin, leading to the development of Pautrier’s microabscesses or clusters of neoplastic lymphoid cells that are characteristic of human CTCL [5]. Furthermore, it was reported that the transcriptional factor of CCR4 was involved in the cell growth and up-regulation of several proto-oncogenes, such as MYB, MDM2, and BCL6, in CTCL and adult T-cell leukemia/lymphoma (ATLL) cell lines [7,8]. In canine CTCL, we reported that the transcription level of CCR4 in lesional skin

![Fig. 2](image-url)  
Fig. 2. Characteristics of canine CTCL cell line.  
(a) Phase-contrast micrograph and (b) May–Grünwald–Giemsa staining. (c) At passages of 200, EO-1 cells were positive for CD3, CD8, CD18, CD45RA, and MHC II, but negative for CD1c, CD4, and CD11b. (d) A word cloud visualization of mRNA transcription levels in EO-1. (e) The surface expression of CCR4 and CD8 on EO-1. The chemotaxis assays using recombinant human (f) or mouse (g) TARC/CCL17. The results represent the means ± SD of three independent experiments. *: P < 0.05, **: P < 0.01, ***: P < 0.001.
was higher than that in normal skin [3], suggesting that CCR4 may play an important role in the pathophysiology of canine CTCL, as it does in humans.

EO-1, a novel canine CTCL cell line established in the present study, was shown to express CCR4 that was bioologically active to both human and mouse TARC. Thus, EO-1 may be a useful tool in the research for not only canine CTCL but also human CD8\(^+\) T cells.

Conflict of interest

No conflicts of interest have been declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2017.06.001.

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Letter to the Editor

PLACK syndrome resulting from a new homozygous insertion mutation in CAST

Inherited abnormalities of proteases or protease inhibitors have been shown to underlie a spectrum of skin barrier genodermatoses that lead to variable degrees of fragility, scaling and inflammation (reviewed in de Veer et al. [1]). One recent addition to this group is the autosomal recessive disorder, PLACK syndrome (OMIM616295). In 2015, Lin et al. [2] reported homozygous loss-of-function mutations in CAST (encoding calpastatin) in three families from different ethnic backgrounds (Chinese, Nepalese and European). Clinically, the signs comprised peeling skin, leukonychia, acral keratoses, cheilitis, and knuckle pads, hence the derivation of the acronym, PLACK syndrome. Calpastatin is an endogenous specific inhibitor of calpain, a calcium-dependent cysteine protease [3]. It is expressed in most tissues (except brain) with high expression of CAST noted in stratified squamous epithelia, including skin [3]. Loss of calpastatin in the affected individuals led to defective keratinocyte adhesion as well as increased keratinocyte apoptosis [2]. Three different homozygous mutations were reported: c.607dup (p.Ile203Asnf8), c.424A>T (p.Lys142*), and c.1750delG (p.Val584Trpfs*37) [2]. However, no further reports of PLACK syndrome have been documented thereafter.

Here, we outline the case of a 10 year old Tunisian boy, born to consanguineous parents, who presented with fragile blistering skin since birth. The parents described superficial post-traumatic or sometimes spontaneous superficial blisters mainly on his limbs. Typically these lesions healed completely within few days with mild superficial desquamation but without scarring. Furthermore, he also had chronic fissuring of the lips and around the mouth, as well as increasing pallor affecting all 20 nails. He has a younger sister with normal skin but three third-degree cousins have been noted to have similar skin blistering (no further details available).

Although the history raised the possibility of an intra-epidermal form of epidermolysis bullosa simplex [4], clinical examination demonstrated the full characteristic features of PLACK syndrome (Fig. 1). The punctate keratoses were most noticeable along the margins of the feet (Fig. 1c), but were also seen embedded within the knuckle pads on the fingers (Fig. 1d). No mucous membrane involvement or hair abnormality was noted. The only other clinical