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Role of Immunohistochemistry in Differentiating Early-Stage Mycosis Fungoides from Its Benign Mimickers

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Abstract

Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphomas (CTCLs), representing almost 50% of all new cases. It affects males nearly twice as often as females in their late fifties. The etiology is still uncertain, but it may be due to chronic antigenic stimulation leading to T-lymphocyte clonal expansion and infiltration of the skin. Histopathologic features of interface dermatitis can occasionally be seen in mycosis fungoides (MF), particularly in early patch-stage disease. Early stage MF is difficult to be differentiated from inflammatory skin disorders(ISDs). CADM1 immunohistochemical staining may be of value in differentiating early stage MF from ISDs. Few studies are available regarding its role in this differentiation.

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Introduction:

Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphomas (CTCLs), which accounts for over 50% of all CTCL cases. The clinical course of MF is defined by the malignant proliferation of CD4+ T cells together with epidermotropism in the skin. Early stages of the disease frequently manifest as erythematous patches and/or plaques, with long course and slow progression from early to advanced-stage disease **(1).**

Some MF patients develop skin malignancies, lymph node involvement, and in rare cases, visceral organ involvement. Clinical manifestation, pathological and immunohistochemical correlation, and occasionally molecular biology study are all used to make a full diagnosis of MF **(1)**.

The identification of a marker that can reliably distinguish early MF from its inflammatory mimickers has been elusive due to the lack of tumor cell-specific markers **(2)**.

CD4 (cluster of differentiation 4)

In molecular biology, CD4 (cluster of differentiation 4) is a glycoprotein that serves as a co-receptor for the T-cell receptor (TCR)**(3,4.)**

CD4 assists the TCR in communicating with antigen-presenting cells (APCs). The receptor complex binds to distinct regions of the antigen-presenting major histocompatibility complex (MHC) molecules. CD4 binds to class II MHC molecule (MHCII). CD4 positive (CD4$^+$) T helper cells' main role is to send signals to other types of immune cells, including CD8$^+$ killer cells. The latter then destroy the pathogens by releasing toxic granules

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containing powerful enzymes which kill the pathogen-infected cells.CD4 continues to be expressed in most neoplasms derived from T helper cells. It is therefore possible to use CD4 immunohistochemistry on tissue biopsy samples to identify most forms of peripheral T cell lymphoma and related malignant conditions. The antigen has also been associated with a number of autoimmune diseases such as vitiligo and type I diabetes mellitus(5).

CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. It was discovered in the late 1970s and was originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984. In humans, the CD4 protein is encoded by the *CD4*gene(3,4).

Normal tissues stained by CD4 include CD4 helper T cells,monocytes, granulocytes, Langerhans cells, dendritic cells and hepatic sinusoidal cells(6, 7).

CD4 is used to identify T-cell lymphomas and leukemias (majority of peripheral T-cell lymphomas are CD4+); with the Main diagnostic use Mycosis fungoides. CD4 is also (+) in monocytic/histiocytic lesions (e.g., monocytic AML) and BPDCN acute myeloid leukemia. Large populations of double-negative or double-positive T cells are typically neoplastic except in the thymus where these phenotypes are normally seen. A small number of circulating CD4/CD8 double-negative T cells is normal(6, 7).

The tumor cells of MF are usually positive for CD4. However, it should be taken into consideration that Langerhans cells and histiocytes are also positive for CD4 so other inflammatory dermatoses are positive for CD4. As the number of tumor cells in the dermis is limited in early MF, the lack of such T-cell markers may be seen in only epidermis in some cases(1).

Thymocyte selection-associated high mobility group box factor (TOX)

Thymocyte selection-associated HMG box protein (TOX) encodes a high-mobility group family (HMG) domain DNA binding nuclear protein, that is able to modify chromatin structure and therefore functions as a transcription factor .It is normally suppressed in peripheral lymphoid tissues. It is a member of a small subfamily of proteins (TOX2, TOX3 and TOX4) that are highly conserved between mice and humans .It regulates the development of CD4+ cell lineages and lymphoid tissue but not typically expressed in mature circulating CD4+ cells (2, 8).

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TOX has multiple roles in the generation of the immune system, including CD4 T cell development as it is transiently upregulated during β-selection and positive selection of developing thymocytes, development of lymph nodes and Peyer's patche(lymph node organogenesis) and formation of NK cells as TOX is also expressed in the NK cell lineage, with highest expression in immature NK (iNK, Lin⁻IL-15Rα⁺NK1.1⁺DX5⁻) and mature NK (mNK, Lin⁻IL-15Rα⁺NK1.1⁺DX5⁺) cells in the bone marrow(9, 10).
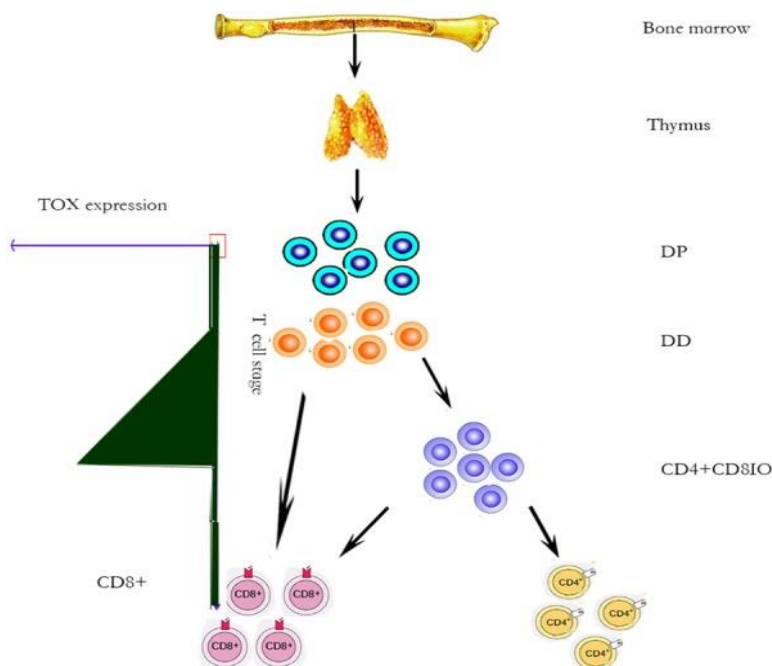
Figure (1): TOX expression in T cell development in the thymus during positive selection**(11)**.

Dysregulation of the gene *TOX* and its protein product, thymocyte selection-associated high mobility group box protein (TOX)occurs in MF .Overexpression of TOX was also found to have prognostic implications in CTCL as it is correlated with thicker lesions, such as patches and plaques as well as disease progression and mortality. TOX was also upregulated in lesional skin from SS patients and in SS peripheral blood mononuclear cells by microarray analysis**(12)**.

Early studies reported TOX to be detected and overexpressed in CD4-positive T cells in MF, and appears to correlate increased risks of progression and disease specific-mortality based on immunohistochemical findings that TOX was expressed in tumor cells of CTCLs but hardly in inflammatory infiltrates of BIDs . However, more recent reports found that TOX was also expressed in infiltrating lymphocytes in ISDs . The same pattern of expression of TOX has been detected in both MF and large plaque parapsoriasis, supporting the contention that these two

processes are closely related, although the frequency was not high. Positive TOX expression was identified in 74% of MF cases and in 32% of BID cases and normal skin . Other group reported that TOX was expressed by more than 50% of tumor cells in 83% of MF cases, whereas only 2% of inflammatory dermatoses cases showed TOX expression in more than 50% infiltrating lymphocytes **(1)**.

TOX can be a potential diagnostic marker differentiating hypopigmented MF from early active vitiligo .TOX expression was found in 93% of hypopigmented MF, while only 7% of vitiligo was weakly positive for TOX. Unfortunately, TOX is not considered as a tumor cell-specific marker, but TOX expression can be an adjunctive diagnostic marker, similar to loss of pan T-cell markers, and might be added in the diagnostic algorithm for early MF**(1)**

TOX, also known as KIAA0808, is located on q12.1 of human chromosome 8 and has one transcript. The length of the *TOX* mRNA is 4076 bp, and it contains nine

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exons that translate into a protein consisting of 526 amino acids. TOX is expressed in many human tissues, including hematopoietic and immune tissues, such as the tonsils, thymus, spleen, and bone marrow, and non-hematopoietic tissues, such as the intestines, lung, kidney, ovary, pancreas, breast and testis TOX expression is relatively different in different tissues. For example, TOX is highly expressed in the thymus but has low expression in the spleen .It has been found that both TOX and TOX2 are related to CD8+ T cell exhaustion **(13)**

Cell adhesion molecule 1 (CADM1)

Cell adhesion molecule 1 (CADM1), a member of the immunoglobulin superfamily of cell adhesion molecules (IgCAM) encoded on chromosome 11q23.2, has been designated with a variety of different names because of its multiple functions: TSLC1 (tumor suppressor in non-small cell lung cancer 1), which was found as a favorable clinical factor in malignancies of solid tumors, IGSF4 (immunoglobulin superfamily 4), RA175 mRNA, SynCAM (synaptic cell adhesion molecule), and Necl-2 (nectin-like molecules). CADM1 expression has been observed in the human lung, brain, testis, and various epithelial tissues including skin, and has been shown to function in cell-cell adhesion through the homophilic binding of its ectodomains between the adjacent cells**(14)**.

CADM1 encodes an immunoglobulin-like cell adhesion molecule consisting of three loops of immunoglobulin .The CADM1 ectodomain acts as an intercellular adhesion mediated by homophilic or heterophilic trans-interaction in each cell . On the other hand, the CADM1 cytoplasmic domain consists two conserved protein-interaction modules, the submembranous protein 4.1-binding motif (protein 4.1-BM), and the type II PDZ-binding motif (PDZ-BM) . Protein 4.1-BM combines CADM1 to the intracellular actin cytoskeleton structures . PDZ-BM is a membrane-associated guanylate kinase homolog (MAGUK) that interacts through PDZ (PSD-95, Discs large and ZO-1) domains , and induces various cellular functions. For instance, TIAM1 (T-lymphoma invasion and metastasis 1) bears a type II PDZ domain and CADM1 promotes Tiam1-mediated Rac activation, which are involved in cell migration . CADM1 contributes to the interaction in each individual cell and other types of cells. In addition, CADM1 is known to act as a scaffolding molecule for various cells to support cell motility. CADM1 involving cell functions in the skin. CADM1 on keratinocytes acts as a scaffolding molecule for immune cells. CADM1 on vascular endothelial cells promotes the repair of the endothelial barrier. CADM1 on immune cells, such as dendritic cells (DC), T cells, NK cells and mast cells, contributes to the development of their immune functions**(15)**.
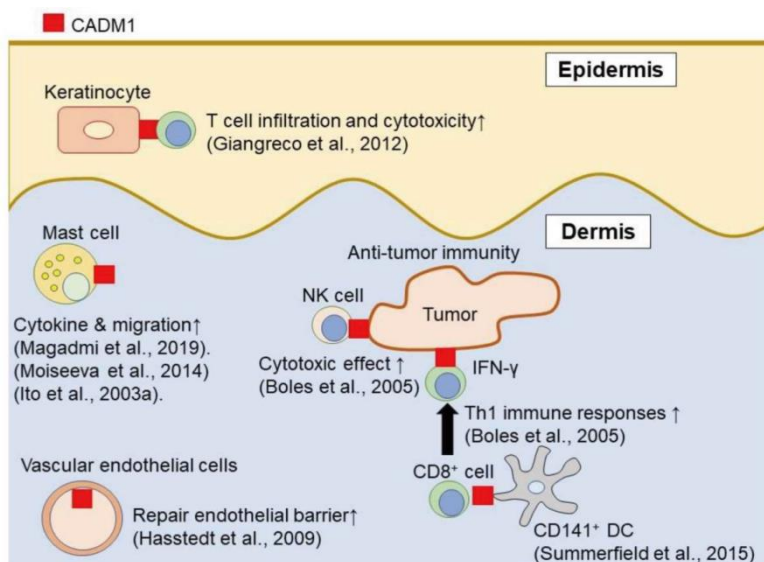
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Figure (2): The involvement of cell adhesion molecule 1 (CADM1) in cutaneous cell function**(15)**.

Cell adhesion ability is one of the components to establish cell organization and shows a great contribution to human body construction consisting of various types of cells mixture to orchestrate tissue specific function. The cell adhesion molecule 1 (CADM1) is a molecule of cell adhesion with multiple functions and has been identified as a tumor suppressor gene. CADM1 has multifunctions on the pathogenesis of malignancies, and other normal cells such as immune cells**(15)**.

In general, as the first step of invasion and metastasis of the solid tumors, the fragility of adhesion ability in the tumor is necessary for release from the primary sites. Therefore, it is assumed that the invasion and metastasis is closely related with the loss of CADM1 expression. Actually, CADM1 expression in lung cancer tissues is inversely correlated with clinical stage progression . On the contrary to the solid malignancies, CADM1 exhibits the opposite clinical behavior in bone marrow-derived tumors. Adult T cell leukemia/lymphoma (ATLL) tumor cells highly upregulate CADM1 and involved in oncogenesis . As CADM1 is not expressed on normal T cells, it can be a diagnostic marker for ATLL . As one of the mechanisms, the expression of CADM1 on ATLL cells contributes to infiltration and the adhesion ability to vessels and the skin to form nodules and tumors **(1, 15)**.

Solid cutaneous malignancies, such as cutaneous squamous cell carcinoma and malignant melanoma, reduce their CADM1 expression to promote the invasion and metastasis of the tumor. On the contrary to these cutaneous solid tumors except for Merkel cell carcinoma, cutaneous lymphomas, such as adult-T cell leukemia/lymphoma, mycosis fungoides, and Sézary syndrome, increase their CADM1 expression for the development of tumor environment. Based on the role of CADM1 in the etiology of tumor development, the theory of CADM1 contribution will desirably be applied to skin tumors according to other organ malignancies, however, the characteristics of skin as a multicomponent peripheral organ should be kept in mind to conclude their prognoses**(15)**.

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CADM1 is positive in a subset of B cells, monocytes, and neutrophils and epidermis but normal T lymphocytes are negative for CADM1. CADM1 was reported to be a potential diagnostic marker also in MF. Yuki et al. revealed that 55 of 58 MF cases including 34 early cases showed CADM1 expression in more than 5% of infiltrating lymphocytes, while CADM1 expression was found in less than 5% of infiltrating lymphocytes in all 50 ISD cases. 94.8% of MF cases were positive while no reactive dermatitis showed positivity for CADM1 by immunohistochemistry. CADM1 mRNA expression was found in the intradermal lymphocytes of the patients with MF, but not in those of the patients with an ISD **(2, 16)**.

Regarding the contribution of CADM1 on the pathogenesis of mycosis fungoides, the survival rate in mycosis fungoides is significantly lower in patients with high CADM1-expressed groups. Therefore, CADM1 expression in mycosis fungoid tumor cells is negatively related to the prognosis of mycosis fungoides. CADM1 expression in mycosis fungoides tumor cells was investigated to identify its utility as a diagnostic marker for mycosis fungoides. CADM1 is expressed in mRNA in infiltrating lymphocytes into the dermis in patients with mycosis fungoides, although no CADM1 expression could be detected in those of patients with inflammatory skin diseases, suggesting it as a distinguished tool for an early stage of mycosis fungoides compared with inflammatory skin diseases**(15)**

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