Review

Novel Biomarkers in Cutaneous T Cell Lymphomas

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Simple Summary: Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of lymphoproliferative disorders characterized by localization of neoplastic T lymphocytes to the skin. Due to lack of specific markers, diagnosis of CTCL is still a challenge. Frequently CTCL patients are originally misdiagnosed and inadequately treated before a proper diagnosis is made. Recent extensive transcriptome analyses (RNASeq) revealed many dysregulated genes those products: surface antigens, proteins and transcripts, can potentially serve as markers in CTCL. In this review most recurrent and specific biomarkers have been presented that can improve the early diagnosis of CTCL.

Abstract: Cutaneous T cell lymphomas (CTCLs) are caused by malignant clonal proliferation of skin-tropic T cells. Most patients have an indolent disease course managed with skin-directed therapies, while some entities, or in advanced stages of disease, have aggressive progression and poor survival. To efficiently treat CTCL consistent entity markers are needed to prevent the delay in diagnosis and to provide disease specific treatment to patients. Recently, the introduction of high throughput parallel sequencing methods resulted in identification of numerous genetic and epigenetic alterations affecting the transcriptome of CTCL cells. In this review, most relevant genes, including *TOX*, *CADM1*, *PLS3*, *DNM3*, *CXCL13*, *PD-1*, *BCL11B* and *TMEM244* are reported that are specifically expressed in CTCL. Upon verification their specificity and sensitivity in larger studies, their altered expression will be able to be recognized as novel biomarkers, that can improve the early diagnosis of CTCL.

Keywords: CTCL; biomarker; *TOX*; *PLS3*; *CADM1*; *DNM3*; *CXCL13*; *PD-1*; *BCL11B*; *TMEM244*

1. Introduction

In contrast to classical nodal non Hodgkin Lymphomas (NHL) that preferentially develop from B cells, the majority of primary skin lymphomas originate from T cells. Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of lymphoproliferative disorders characterized by localization of neoplastic T lymphocytes to the skin. The incidence of CTCL varies between 0.5-1.5/100 000 [1]. It is most frequent in Asia, followed by Noth America and less frequent in Europe. More than half of CTCL comprise mycosis fungoides (MF) (62%), followed by CD30-positive lymphoproliferative disorders (16%), lymphomatoid papulosis (LyP) (9%), Sézary syndrome (SS) (3%), primary cutaneous small/medium pleomorphic CD4+ T-cell lymphoma (SMTCLPD) (2%) and very rare (<1%) entities: primary cutaneous aggressive epidermotropic CD8 T-cell lymphoma (AECTCL), primary cutaneous γ/δ T-cell lymphoma (PCGD-TCL) and primary cutaneous (extranodal) NK/T-cell lymphoma, nasal-type.

CTCL are manifested by visible skin lesions that are easily recognized by patients, who head either to family doctors or to dermatologists. Unfortunately, most of those changes resemble more frequent benign inflammatory dermatoses (BID). Since, especially at the early stages of the disease, no specific markers and no simple tests exists to diagnose or exclude CTCL, many patients are originally misdiagnosed and not properly treated.

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Currently, CTCL is usually diagnosed based on the flow cytometry using a set of monoclonal antibodies, including: CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD16/56, CD19, CD25, CD26, CD30, CD45, CD45RA, and CD45RO [2]; although dissonant with cutaneous B-cell lymphomas and plasma cell disorders, no dependable protein marker for CTCL has been discovered. The definitive diagnosis of MF, particularly patch/plaque stage disease, is challenging. Many of its clinical and pathologic features are non-specific and overlap with reactive processes as eczema, psoriasis or parapsoriasis. Determination of T-cell receptor genes clonality by PCR and population of CD4+ cells lacking CD2, CD5, and/or CD7 antigen by immunohistochemistry are useful, but frequently do not differentiate between MF and non-malignant T-cell proliferations [2, 3]. On the genomic level CTCL show multiple different lesions (deletions, amplifications, translocations and point mutations) at variable frequencies that make them difficult to use for diagnostic purposes [4]. Therefore, the median time from symptom onset to definitive diagnosis is 4 years, but may last decades [5]. Recently, through the use of high throughput expression analysis as DNA microarrays and RNASeq, altered gene expression has been identified in different CTCL entities. To consider gene expression as a diagnostic marker, its sensitivity and specificity has to be verified in subsequent studies using techniques which can be applied in routine diagnostics.

The aim of this review is to summarize the current knowledge on the most specific gene expression biomarkers that can improve the diagnosis of CTCL. Only biomarkers, whose specific expression in CTCL cells has been confirmed in multiple independent studies, were selected.

2. Potential novel diagnostic gene expression biomarkers in CTCL

2.1. Thymocyte selection associated high mobility group box (TOX)

TOX is an evolutionarily conserved DNA-binding protein, a member of the high-motility group box (HMG) protein superfamily, that functions as a transcription factor. TOX is required for the development of CD4+ T cells, natural killer (NK) cells and innate lymphoid cells (ILCs), as well as the autoimmunity mediated by CD8+ T cells. Emerging evidence supports role for TOX in the induction of T cell exhaustion in the setting of tumor or chronic viral infection by mediating transcriptional and epigenetic changes [6].

In 2012, for the first time, *TOX* was reported to discriminate between early MF lesions and biopsies from BID [7], however, this has not been confirmed by some later studies [8]. Other reports revealed that TOX was also expressed in infiltrating lymphocytes in BID, although the frequency of positive cells was not as high as in MF. Positive *TOX* expression was identified in 74% of MF cases and in 32% of BID cases and normal skin [9]. Other group reported that the discrimating factor is the percentage of cells expressing the marker. *TOX* was expressed by more than 50% of tumor cells in 83% of MF cases, whereas only 2% of BID cases showed *TOX* expression in the majority of infiltrating lymphocytes [10]. The latest study reported very strong association between TOX expression and early-stage MF ($p < 0.001$); TOX had the highest sensitivity of 96.77% and accuracy of 85.71% in histopathological diagnosis of MF, outperforming CD4, GATA3 and FOXP3 [11]. *TOX* overexpression was also reported by us and others in SS cells that immunohenotypically resamble MF cells [12-14], and very recently two groups, using a novel highthrouput method of single-cell RNA sequencing, have shown overexpression of *TOX* in individual SS cells [15, 16]. Mechansitically, TOX was shown to downregulate a tumor suppressor gene *RUNX3* in a novel TOX-RUNX3 pathway, suggesting its role in CTCL pathogenesis . Furthermore, high *TOX* expression was shown to correlate with increased disease-specific mortality in SS [12]. Taking together, *TOX* can not be 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 and SS.

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2.2. Plastin 3; T-Plastin (PLS3)

Plastins are highly conserved proteins belonging to a family of F-actin-binding and -bundling proteins that are conserved throughout eukaryote evolution. In humans three plastin genes, located on different chromosomes, are expressed: *PLS1* (Plastin-I), *PLS2* (Plastin-L) and *PLS3* (Plastin-T) [17]. *PLS1* is specifically expressed in the small intestine, colon and kidney, *PLS2* is predominantly expressed in hematopietic cells, and PLS3 is expressed at low levels in most tissues and solid organs, except hematopietic cells. Recent research has shown that PLS3 is involved in many cellular processes, signaling pathways, and diseases. Actin-dynamics, regulated by PLS3 among others, are crucial in a lot of cellular processes including endocytosis, cell migration, axonal growth, neurotransmission, translation, and others. Also, PLS3 levels influence the infection with different bacteria, mycosis, and other pathogens. PLS3 is localized on the X-chromosome, and when escaping X-inactivation, PLS3 triggers different types of cancers [18].

In the original study published in 2003, Kari et al. showed that *PLS1* was not expressed in any of hematological samples tested, and *PLS2* was expressed at simmilar levels in normal and malignant T cells. Interetingly, unlike T-helper cells from healthy individuals or patients with nonmalignant dermatoses, Sézary cells from most patients with SS aberrantly express *PLS3* mRNA and protein [19]. The same year, those resutls were confirmed by Su et al., who further proposed using the expression of *PLS3* to differentiate between SS and MF [20]. Also further studies reported that *PLS3* is a valuable marker to differentiate MF from SS and can be used for following transformation/progression. [21] [22]. *PLS3* expression was increased in PBMC in SS compared with earlier stages of MF and with psoriasis, Furthermore, *PLS3* expression was correlated to disease extent in a patient who developed SS. In SS PBMC, *PLS3* expression was greater than 400-fold compared with normal. It suggests that *PLS3* is a sensitive marker to distinguish SS from other stages of MF and inflammatory skin diseases.,

2.3 Cell adhesion molecule 1 (CADM1)

CADM1 belongs to the immunoglobulin superfamily and was initially identified as a tumor regulator in small cell lung cancer (SCLC). It participates in the formation of epithelial cell morphology and polarity, along with intracellular signal transduction. Furthermore, CADM1 mediates adhesion with neighboring cells through trans-homophilic interactions. Furthermore, it forms a multiprotein complex that activates the PI3K pathway, which results in actin cytoskeleton reorganization and formation of the epithelial cell structure. CADM1 is a well-known tumor suppressor gene in a variety of human cancers, including respiratory and digestive systems [23].

On the contrary, CADM1 is overexpressed and involved in cancerogenesis in adult T-cell leukemia/lymphoma (ATLL) [24]. As CADM1 is not expressed on normal T cells, it can be a diagnostic marker for ATLL. Recently, CADM1 was reported to be a potential diagnostic marker also in MF. *CADM1* expression, defined as more than 5% of infiltrating lymphocytes, was observed in 55 of 58 (94.8%) MF cases, including 33 of 34 (97.0%) early cases, while no expression was detected in all 50 BID cases [25]. Increased percentage of CD4+ cells expressing CADM1 were also reported in SS [26]. Circulating CADM1+ cells were significantly increased in 7 out of 10 patients with SS, ranging from 7.9% to 74.5% of the CD3+CD4+ fractions. The percentages of CADM1+ cells were usually less than those of circulating Sézary cells. CADM1 was expressed, to various degrees, in six of nine T-cell lines derived from SS, MF, ATLL, and ALCL, but negative in B-cell lymphoma-derived cell lines. CADM1+ cells were present in the skin infiltrates of MF, SS, ATLL and ALCL. Serum levels of soluble CADM1 were not significantly elevated in SS/MF. Three major splicing variants of CADM1 expressed by neoplastic T-cells contained a putative oncogenic variant composed of exons 7-8-9-11. In conclusion, CADM1 is frequently

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expressed in Sézary cells and cell lines from CTCL. Although further validation from other groups is required, *CADM1* can be a potential diagnostic marker for MF, including early stages of the disease.

2.4. Dynamin-3 (DNM3)

Dynamin-3, encoded by the *DNM3* gene, is a member of the dynamin family which belongs to the guanylate triphos-phatases superfamily. It possess mechanochemical properties involved in actin-membrane processes, predominantly in membrane budding [27]. The dynamin family is involved in the pathogenesis of a variety of carcinomas. Dynamin 1 and 2 can promote the proliferation and metastasis of cancer cells, whereas dynamin-3 (*DNM3*) is generally considered as a candidate tumor suppressor [28]. The *DNM3* promoter is hypermethylated in hepatocellular carcinoma, and *DNM3* is expressed at significantly lower levels in hepatocellular carcinoma, cervical carcinoma and lung cancer. On the other hand, *DNM3* was found up-regulated in glioblastoma multiforme [29] and CTCL [16, 30, 31].

Booken et al, by analyzing 10 SS patients usind DNA microarrays, found increased *DNM3* expression, as compared to peripheral blood mononuclear cells (PBMC) of 10 healthy individuals [30]. The results were confirmed using qRT-PCR. Further comparison of PBMC and skin samples of SS versus MF revealed *DNM3* overexpression exclusively in SS. In a subsequent study, Boonk et al analyzed 59 patients with SS and 19 patients with erythrodermic inflammatory dermatoses usindg flow cytometry and qRT-PCR [31]. In sorted CD4+ T cells they found *DNM3* overexpression in 75% of SS. In a recent high throughput single-cell RNASeq analysis of SS [15], Borcherding et al have found *DNM3* among top ten overlap with the study of Booken et al [30].

2.5 Chemokine (C-X-C motif) ligand 13 (CXCL13)

CXCL13 is a small chemokine belonging to the CXC chemokine family. It is selectively chemotactic for B cells, and elicits its effects by interacting with chemokine receptor CXCR5 [32]. CXCL13 and its receptor CXCR5 control the organization of B cells within follicles of lymphoid tissues and is expressed highly in the liver, spleen, lymph nodes, and gut.

In T lymphocytes, CXCL13 expression is thought to reflect a germinal center origin of the T cell, particularly a subset of T cells called follicular B helper T cells (or TFH cells) [33]. Primary cutaneous peripheral T-cell lymphomas with a T-follicular helper phenotype (pcTFH-PTCL), expressing CXCL13, are poorly characterized, and yet have not been recognized by WHO as a distinct entity. They include the majority of MF, SS, SMTCLPD and skin manifestations of AITL [34]. Early studies showed that besides T follicular helper (Tfh) PTCL, CXCL13 is also strongly expressed in skin lesions, lymph nodes and in plasma of SS patients [35]. Another study showed that CXCL13 expression is very common in MF and CD4+SMTCLPD, but it can be observed rarely also in other types of CTCL [36]. Therefore, expression of Tfh markers should not be used for classification of any entity of CTCL, but together with other immunohistochemical stainings it might be used for a more accurate characterization of T cell lymphomas.

2.6. Programmed cell death protein 1 (PD-1; CD279)

PD-1 is an inhibitory protein receptor related to apoptosis. It is regarded as a sign of T cell unresponsiveness or exhaustion (Ishida et al., 1992). It is mainly expressed on the surface of T lymphocytes, B lymphocytes, dendritic cells (DC), NK cells and other cells (Calles et al., 2015), involved in autoimmune tolerance. It has a role in regulating the immune system's response to the cells by down-regulating the immune system and promoting self-

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tolerance by suppressing T cell inflammatory activity. This prevents autoimmune diseases, but it can also prevent the immune system from killing cancer cells.[5] After engagement with its ligands, mainly PD-L1, PD-1 is activated and recruits the phosphatase SHP-2 in proximity to T cell receptor (TCR) and CD28 signaling. This event results in dephosphorylation and attenuation of key molecules in TCR and CD28 pathway, leading to inhibition of T cell proliferation, activation, cytokine production, altered metabolism and cytotoxic T lymphocytes (CTLs) killer functions, and eventual death of activated T cells.

Although expression of PD-1 is observed in skin biopsies from eczema, psoriasis, drug-induced erythroderma and erythrodermic MF [37, 38], in SS PD-1 is expressed significantly higher than in other entities [37]. Using flow cytometry, two studies showed that PD-1 is commonly overexpressed in circulating Sézary cells and significantly contributes to accurate Sézary cell detection by FACS in the context of pan-T cell antigens and loss of CD26 [39, 40]. PD-1 overexpression quickly and efficiently indicated the presence of an abnormal population, helped distinguish normal T cell subsets with differential expression of the other markers from a truly abnormal population, and provided better separation of abnormal from normal populations for improved gating and quantification when used in conjunction with the remaining antigens. Indeed, in 12% of cases, PD-1 was critical for accurate Sézary cell identification, without which accurate gating and quantification were significantly affected or nearly impossible. Therefore increased PD-1 expression on T cells in IHC or FC might suggest the diagnosis of SS.

2.7. B-cell lymphoma/leukemia 11B gene (*BCL11B*)

BCL11B is a Kruppel-like C2H2-type zinc finger transcription factor, which is a key player in T-cell development [41], but its role in T-cell malignancies is still unclear. While some research, reporting inactivation mutations in T-ALL and radiation induced T cell lymphomas, suggest that *BCL11B* acts as a tumor suppressor gene, other suggest its oncogenic function. We found a high expression of *BCL11B* in T-ALL [42]. In our subsequent studies we showed that the in vitro suppression of *BCL11B* leads to massive apoptosis in malignant, but not in normal T-cells [43], and that the oposite, overexpression of *BCL11B*, results in chemoresistance of malignant T cells [44]. This strongly supports the role of BCL11B in the development of some T cell mailgnancies.

Later, other group demonstrated significant upregulation of *BCL11B* in all stages of MF, compared with BID, in both mRNA expression level and protein level [45]. In addition, *BCL11B* expression increased with advancing lesion tumor stage and overall disease stage. In subsequen they found a positive correlation between *BCL11B* expression and sensitivity to HDACi , and the physical interaction and shared downstream genes between BCL11B and HDAC1/2 [46]. Very recently another group reported that all 23 MF cases and all 8 CSMTLC cases analyzed stained histochemicaly positive for BCL11B, and the staining intensity was higher than that of reactive T-cells [47]. In conclusion, if confirmed in further studies, *BCL11B* may be used as marker in CTCL diagnosis and serve as a therapeutic target to improve HDACi efficacy in advanced CTCL.

2.8. Transmembrane protein gene 244 (*TMEM244*)

TMEM244, identified by *in silico* analysis of human genome, has been included in a large family of genes encoding proteins embedded in the cell membrane, and span both the intracellular and extracellular environments. To date neither the biological function, nor even the presence of the TMEM244 protein has been reported.

Recently, our group reported highly specific *TMEM244* mRNA expression is all SS patients and SS derived cell lines [14, 48]. In subsequent studies we showed that quantitative

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analysis of *TMEM244* mRNA expression is significantly higher in SS patients compared not only to healthy individuals but what's more important to diseases with similar clinical presentation: MF and erythroderma of non-malignant origin. Our study also revealed that higher expression of *TMEM244* was observed either in CD4+ or in CD8+ subsets of memory cells (CD4RO+), which is in line with the immunophenotype of Sézary cells [49]. Furthermore, collaborating with us Chinese group showed that high expression of *TMEM244* is associated with poor overall survival of patients with different forms of T-cell lymphoma, including CTCL [50]. Mechanistically, we showed that *TMEM244* expression in SS cells is driven by specific hypomethylation of its promoter [48]. Samples with *TMEM244* expression, among them mostly SS and a few other T-cell leukaemia/lymphoma cases, had promoter region hypomethylated, while in all samples not expressing the gene, the promoter was methylated. If confirmed by other groups, measuring *TMEM244* expression using qRT-PCR could be used as an easy and cheap blood diagnostic marker to distinguish SS from diseases with similar clinical presentation.

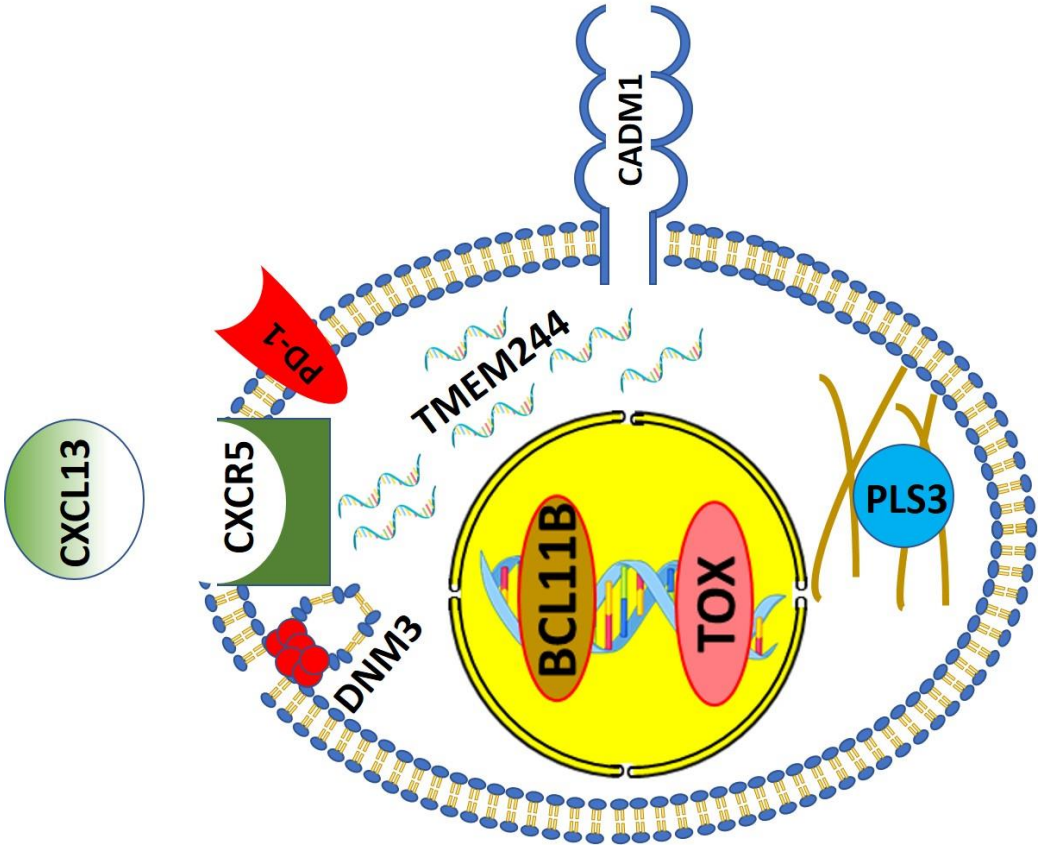

Figure 1. Gene expression products that can be used as biomarkers in CTCL.

3. Conclusions

Due to lack of specific markers, frequently CTCL patients are originally misdiagnosed and inadequately treated, what worsens the disease outcome. Recent research using high throughput methods, as DNA microarrays and next generation RNA sequencing (RNASeq), revealed several genes, as *TOX, PLS3, CADM1, DNM3, CXCL13, PD-1, BCL11B and TMEM244*, selectively expressed in CTCL. To date these results have been confirmed in few independent studies. Upon verification their specificity and sensitivity in larger studies, an algorithm using a combination of their expression pattern and currently used immunophenotypic markers shall improve the diagnosis of CTCL.

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