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Original Contribution

Heterogeneity of T-cell receptor expression at transformation in mycosis fungoides/Sezary Syndrome (MF/SS)

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1. Introduction

Cutaneous T cell lymphoma (CTCL), a subcategory of Non-Hodgkin lymphoma, is a neoplasm of skin-homing T cells with significant mortality in its advanced stages. The most common CTCL is mycosis fungoides (MF), which in its early stages presents with plaques and patches in sun-protected areas. In advanced stages, cutaneous progression to nodules and erythroderma may occur as well as dissemination to the viscera, blood, and lymph nodes.

In 8% of MF cases, it is possible for the disease to undergo large cell transformation (LCT) which has been shown to have a poorer prognosis [1]. It is currently unknown what causes LCT to occur, but large cell transformed mycosis fungoides (LCT-MF) is associated with a higher likelihood of disease progression, metastasis, and poorer survival [2]. Cases are classified as LCT if 25% of the T cell infiltrate in the biopsy are large cells or if there are accumulations of large cells create microscopic nodules [3]. Clinically, at transformation, the lesions usually become nodular and may ulcerate [4]. The transformed cells are invariably large T cells and characteristically have cerebriform or indented nuclei. Immunohistochemically LCT-MF cells are CD3+ and CD4+ with loss of CD 5 and/or CD7similar to the untransformed MF component. Furthermore, about 30% of cases exhibit CD30 expression as shown in larger French series [2,5].

One set of immunohistochemical markers' expression that has not been examined in cases of LCT-MF are T cell receptors. Every T cell that matures in the thymus undergoes T cell rearrangement via VDJ rearrangement to either create an α/β T cell receptor or γ/δ T cell receptor [6]. α/β T cells localize in secondary lymphoid tissue, and recognize peptide ligands in the context of MHC I and MHC II molecules. γ/δ T cells localize to epidermal surfaces and do not recognize ligands in an MHC-dependent manner [7]. The majority of T cells in the body are shown to express α/β T cell receptors [8], including MF which is malignancy of TCR α/β receptor derivation. Given the aggressive nature of γ/δ T-cell lymphomas, we sought to investigate the phenotype of large cell transformed-MF (LCT-MF) to determine the patterns of immunophenotypic expression of TCRβ and TCRγ within the large cell transformed component of tumor stage lesions of MF.

Keywords

Mycosis fungoides
TCR expression
TCRγ
Heterogeneity

2. Materials and methods

2.1. Case selection

Nine skin biopsies of cutaneous lesions representing histologic LCT were selected for inclusion in this study based on clinical, pathological and follow up data from the Section of Dermatology, at our University. These biopsies were reviewed with diagnosis confirmation of LCT by two dermatopathologists and a hematopathologist (> 25% large cells or presence of multifocal clusters of > 10 large cells were the histologic criteria used to assign transformation). Eight of these biopsies were LCT-MF and one biopsy was LCT occurring in the context of Sezary Sydrome after bone marrow transplant. Patients were staged using the WHO-EORTC staging system [9].

2.2. Histology and immunohistochemistry studies

Formalin-fixed, paraffin-embedded biopsies of skin lesions were stained with hematoxylin and eosin. Each of these biopsies was reviewed by one dermatopathologist and one hematopathologist at our University for presence of large cells, epidermotropism, and density of infiltrate for inclusion in the study. Immunohistochemical studies on paraffin sections were performed on the nine biopsies with a three-stage

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streptavidin peroxidase procedure using the following antibodies anti-CD3, CD4, CD8, CD20 (L26), CD30, TCRβ (BetaF1 clone), and TCRγ (γ3.2 clone). Staining was performed on the Leica Bond III instrument or the Ventana Benchmark XT for all stains. Positive controls including normal tonsils were run in tandem to ensure that the antibodies functioned appropriately. Singly scattered lymphoid cells were noted on tonsils for TCRγ while TCRβ stained all paracortical T-cells with negative follicular structures rich in B-cells. In the neoplastic tissues, TCRβ and TCRγ expression were examined in the transformed large cells and the background smaller neoplastic untransformed T-cells. The expression of all antigens was scored ordinally from 0 to 4 based on numbers of positive cells as: 0% (score 0), 1–25% + cells (score 1), 26%–50% + cells (score 2), 51–75% + cells (score 3) 76%–100% + cells (score 4). Expression of TCR status in prior patch/plaque biopsies was not performed however.

Data on TCR expression in both components were plotted using the open-source graphing library Plotly 2.4 in Python 3.6.

2.3. Institutional board review approval

The study protocol was approved by the ethics committee at our University (IRB: 15–1249).

3. Results

3.1. Immunohistochemical analysis

After two separate reviews of the immunohistochemical stains by two pathologists, the expression of CD3, CD4, CD5, CD7, CD30, TCRβ, and TCR γ/δ of the lymphoid untransformed small T-cell and concurrent transformed components are shown in Figs. 1 and 2. Within the small lymphoid T-cell component, neoplastic T-cells exhibit nuclear convolutions while reactive T-cells exhibit round nuclear contours and this was used to differentiate between the two components. The small neoplastic T cells in all nine biopsies had a TCRβ+/TCRγ- signature although there was variability in the expression of TCRβ+, with one biopsy displaying dual expression of TCRβ and TCRγ (Fig. 3; patient 8 in Fig. 1). As for TCRγ expression, two biopsies showed gain of expression in the large cell component in comparison with the corresponding small T-cell component. The remaining seven biopsies showed

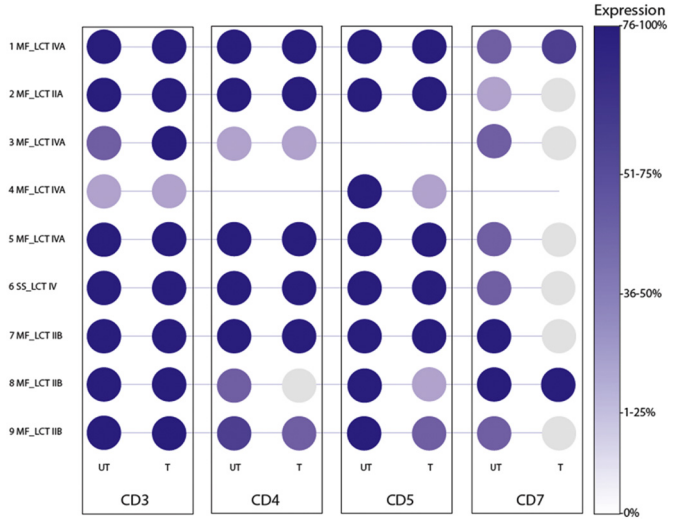

Fig. 1. Results of immunohistochemical stains for T-cell antigens in all 9 cases in the transformed and untransformed components. All untransformed (UT) cases had CD3+/CD4+ phenotype with variable loss of CD3 and CD4 in a small subset of untransformed cases. There was variable loss of CD5 and CD7 in 3 and 7 cases in the transformed (T) component. Only two of nine cases were positive for CD30 at transformation (> 50%).

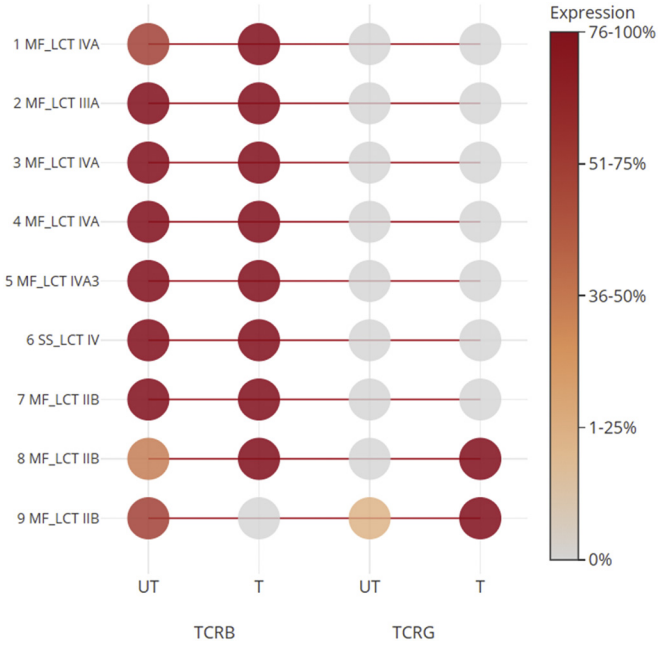

Fig. 2. Results of TCR expression in both untransformed (UT) and transformed (T) components for all 9 cases. In the TCR analysis, the untransformed component showed variable loss of TCR beta in 3 cases while one of these cases (case 9) showed TCR gamma expression in the untransformed component. This case lost the TCR beta expression at transformation with upregulation of TCR gamma at transformation in 100% of cells. Levels of expression are indicated by opacity.

no change in expression. CD3 expression was maintained in both small and LCT components in eight of nine biopsies. CD4 expression was maintained in small and LCT components in seven of nine biopsies with two biopsies showing decreased expression in the large cell transformed component. CD5 expression was maintained both small and LCT components in six of nine biopsies with three biopsies showing loss of expression. CD7 was frequently lost in the untransformed and transformed components.

These biopsies contained variable numbers of large cells intermingled with untransformed small T-cell component. Six of the nine biopsies contained large cells that were CD30+. On examination of the large cells, two of nine biopsies showed large cells that were variably double negative for both TCRβ and TCRγ. The cells were spatially ascertained to be CD3+/CD20- cells confirming T-cell derivation. Large cells in the five biopsies were uniformly TCRβ+/TCRγ-. One case of large cell transformation showed complete loss of TCRβ in the large cell component with new acquisition of TCRγ (Fig. 4; patient 9 Fig. 1). In this case, the untransformed component showed loss of TCRβ and gain of expression of TCRγ. Lastly, one case showed dual expression of TCRβ and TCRγ in the large cell transformed component and loss of TCRβ expression in the untransformed component (Fig. 2; patient 8 Fig. 1).

3.2. Clinical analysis

Clinical data for all patients was obtained. All the cases were found to be in advanced stages of MF ranging from stage IIB to stage IVA, with five of the nine biopsies obtained in stage IV disease. There were four females and five males, with the average age of 56.2 years. Patients had no significant past medical or family history of any other dermatologic or hematologic issues. Clinical information on the patients showed that for the four patients that passed away, the average time to death after biopsy confirming transformation was 26.5 months. Of the patients who did not lose TCRβ in the LCT component, one died after 51 months and the other after 4 months. The other four patients that did not lose TCRβ

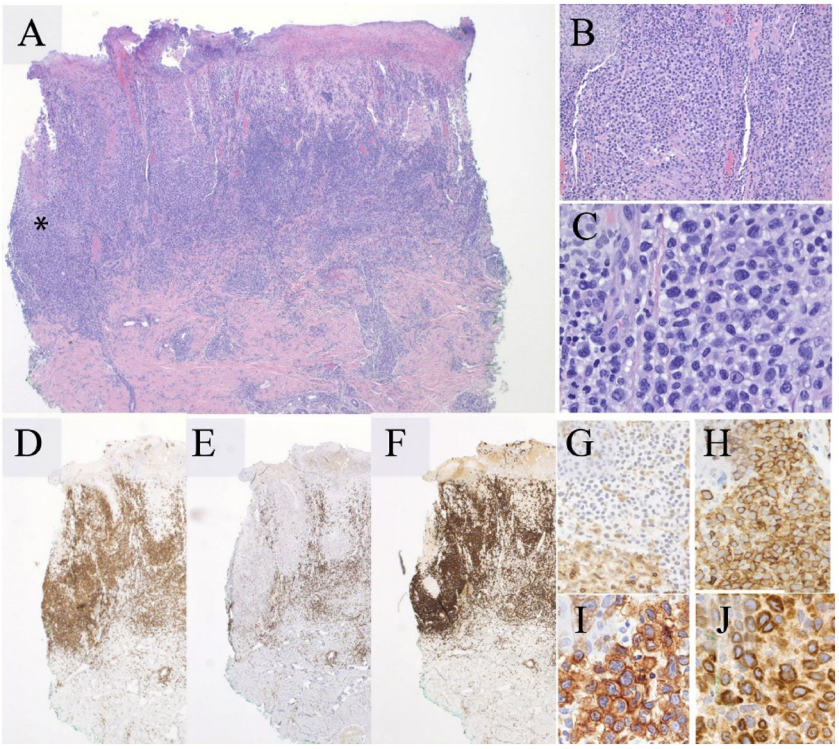

Fig. 3. Dual expression of TCR beta and TCR gamma noted at transformation (patient 8). A. low power view of the biopsy at transformation showing extensive surface ulceration with dense dermal infiltrate with patchy transformed areas (*) shown at higher power in panels B&C. Panels D, E and F show that the transformed component is positive for CD3 and CD7 with loss of CD5 (panel E). These transformed cells are CD4- (G) and CD8+ (H) (shown at low power) as well as TCRβ (I) and TCRγ (J). The small cell component in the background at transformation lacked TCRγ expression.

expression in the LCT component remain alive and well after their diagnosis with LCT. In the cases of the three patients who had a loss of TCRβ in the large cell component, one died within five months of LCT, one died at four months, and the last which gained expression of TCRγ in the large cell component while losing TCRβ remains alive with disease as of the time of this study.

4. Discussion

Primary cutaneous γ/δ T cell lymphomas are regarded as considerably more aggressive cytotoxic T cell lymphomas opposed to α/β T cell lymphomas and hence distinction of the two entities is critical from a prognostic and therapeutic standpoint. This is particularly notable with regard to subcutaneous panniculitis-like T cell lymphoma (SPTCL)

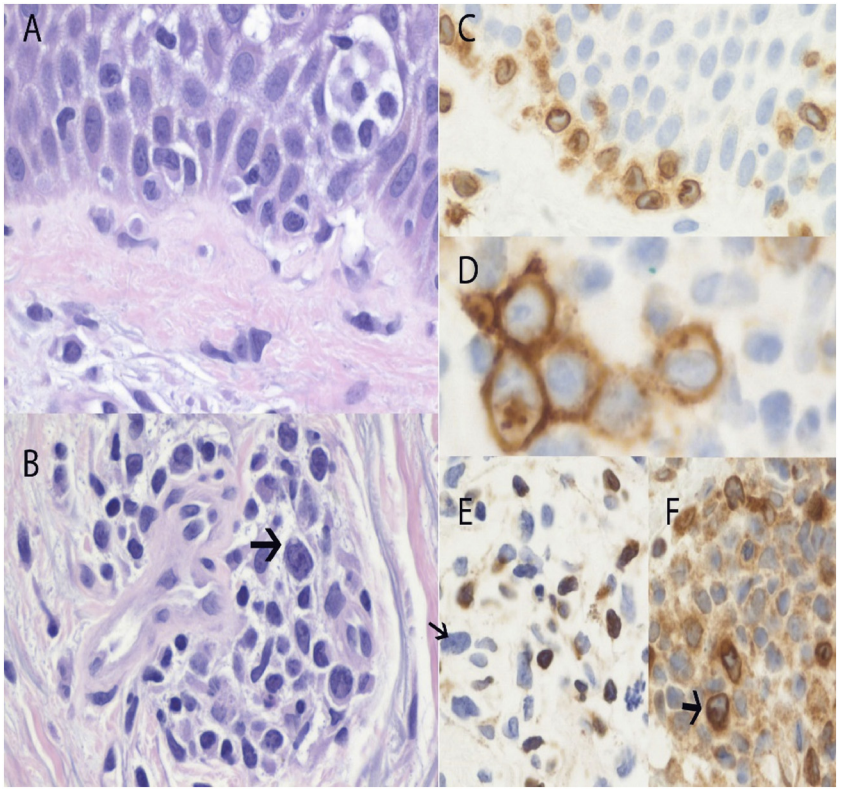

Fig. 4. Abnormal TCRγ acquisition with loss of TCRβ in LCT-MF (patient 9) A. Some areas of the biopsy depicting typical MF cytomorphology with Pautrier microabscesses B. Other areas in the dermis show increased numbers of large cells but overall accounting for < 25% of all cells C. CD3 immunostain depicts epidermotropic atypical T-cell infiltrate lining the basal epidermis impacting a "string of pearls" appearance. D. CD30 is positive in the large cell component E. TCRβ immunostain is positive in some of the small T-cells while it is negative in the all the large cells (arrow). F TCRγ immunostain is acquired at transformation in the large cell component. Also, at transformation, the small epidermotropic component expressed TCRγ At least visually, a significant double negative component could be identified.

in which the discovery of differences in prognosis between α/β SPTCL and γ/δ SPTCL led to reclassification of the disease by the WHO-EORTC [9]. While the limited published literature on MF in the patch and plaque stages that expressed TCR γ/δ were shown to have indolent courses [10,11] these papers had small sample sizes, and all patch and plaque stage MF are considered indolent. Furthermore, loss of both TCRs (TCR null phenotype) has been noted in aggressive CD30 + T-cell lymphomas such as systemic anaplastic large cell lymphoma, which has a more aggressive clinical course than primary anaplastic large cell lymphoma, leading to disruption of downstream intracellular signaling cascades related to T-cell activation [12]. Thus it seems plausible that loss of surface TCR expression may herald aggressive biology although this may be a mechanism that operates uniquely in systemic as opposed to cutaneous lymphomas.

This is the first paper in which TCR expression in LCT-MF was exclusively examined. We analyzed the clinical data from eight biopsies of LCT-MF and one biopsy of Sezary Syndrome with LCT post-transplant. The immunohistochemical analysis showed two of the MF biopsies has loss of 51–75% loss of TCRβ and one MF biopsy showed 100% loss of TCRβ and 100% gain of expression of TCR γ on the large cells. Five biopsies were 100% positive for TCRβ expression and had no expression of TCR γ. The remaining biopsy showed 50% gain of expression of TCRβ and 100% gain of expression of TCR γ on the large cells. This dual expression of TCR has been seen in previous cases of MF before [11,13] though its clinical significance is unclear.

This exploratory study showed that there is in fact a significant variation in TCR expression in cells that have undergone LCT although the nosologic significance of this finding as it related to the disease progression and biology remains unclear. Nevertheless, it is plausible that changes of surface TCR expression might herald aggressive behavior in MF although it is unclear as to how changes of surface TCR expression might provide survival advantage. Whether such loss or gain of TCR expression stems from an acquisition of a genetic mutation at the level of the TCR or represents a post-translational event remains to be clarified. While the average survival rate of 25.5 months after decreased expression of α/β and γ/δ in our study falls within the range of the median survival rate of 2 and 36 months seen after LCT in other studies a [14], our sample size is small.

Despite the small size of our study, a couple of findings merit consideration. The acquisition of TCRγ at transformation in two cases with dual expression of TCRβ and TCRγ in one is rather notable. While both patients continue to have active disease with chronic progressive skin lesions typical of the disease course of MF, the dual expression in the other case is explained by the loss of allelic exclusion principles and leading to functional rearrangements of TCRG and TCRB on different alleles. Nevertheless, the disease biology does not reflect a primary TCRγ derived malignancy despite this feature. We believe that cases of untransformed MF that exhibit some loss of TCRβ should be tested for TCRγ additionally in such cases to identify possible early cases of transformation. Hence, our study underscores the need to test both antigens routinely in serial biopsies of MF patients. With the availability of the newer TCRδ (H-41) monoclonal antibody that is superior to the TCRγ3.2 clone we used, we believe that this antibody should be used instead. A larger, prospective multi-institutional study will clarify the prognostic relevance of these phenotypic shifts in TCR expression in the context of MF.

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