High-throughput TCR Sequencing Provides Added Value in the Diagnosis of Cutaneous T-cell Lymphoma

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INTRODUCTION

Diagnosis of early-stage CTCL can be challenging, because skin lesions contain a mixture of both benign and clonal malignant T cells. Currently, clonality is assessed by TCR PCR but this method is qualitative, often fails to detect minimal clonal disease present at low numbers, and cannot be used to identify malignant T cells for subsequent study by flow cytometry or immunohistochemistry. High throughput sequencing (HTS) of the TCR gamma (TCRG) and TCR beta (TCRB) CDR3 regions provide comprehensive analysis of the total repertoire of T cell clones in a specimen, the breadth of repertoire diversity, and an exact quantitation of individual T cell clones.

OBJECTIVE

In this study, we investigated whether the Adaptive Biotechnologies ImmunoSEQ assay of TCRG and TCRB could discriminate between benign and malignant T cell dyscrasias.

PATIENTS

This study involved a total of 132 patients. We analyzed skin biopsies from 39 patients with histopathologically and clinically suspicious or diagnostic CTCL (over 80% Stage I or Stage II), 9 patients with Lymphoma-like Papulosis, 4 treated CTCL patients in partial or complete remission, non-lesional biopsies from 3 CTCL patients, 6 biopsies from individuals without skin disease, 9 patients with atopic dermatitis, 12 patients with eczematous dermatitis, 24 patients with psoriasis, 15 patients with psoriasis after treatment with adalimumab and clincept, and non-lesional biopsies from 11 patients with psoriasis.

METHODS

Genomic DNA was extracted from FFPE or OCT sections of skin biopsy specimens of patients. T cell TCRG and TCRB chain sequences were then independently amplified using multiplex PCR with optimized primer sets. Following HTS, a bioinformatics pipeline clusters the sequences into distinct clonotypes to determine overall frequencies and to identify diagnostic clonotypes (V (A), D (B), and J genes are also identified for each clonotype).

The assays defined the dominant clonal sequences in every case and distinguished likely alpha/beta from alpha/beta T cell lymphoma. Using the fraction of the top two TCRG sequences as a fraction of the total nucleated cell population defined a cut off of approximately 1/1000 above which the biopsy approached 100% specificity for malignant disease, and below which the assay approached 100% specificity for non-malignant or treated malignant disease. TCR PCR was negative in 10 out of 39 biopsies suspicious of CTCL—all of these were among those determined to be clonal by HTS. While there was a strong correlation between analyses using the top two TCRG sequences (because both TCRG alleles are VJ rearranged in most T cells), the TCRG analysis was more powerful in terms of discrimination and separation of disease categories.

RESULTS

The most frequent T cell clone was expressed as the fraction of total nucleated cells that expressed this sequence and the sum of the top two benign clones. The frequency of the top two clones, as well as the total clonal population, were compared to the known total clonal population (indicated by the sum of all top 10 benign clonal populations in each sample).

Skin samples were subjected to deep sequencing of both the TCRG and TCRB CDR3 regions. The top TCRB sequence and the sum of the top two benign clonal sequences were compared to the total nucleated cell population defined a cut off of 0.001 for all groups. The most frequent T cell clone expressed as a fraction of total nucleated cells (Fig. 2). The most frequent T cell clone expressed as a fraction of total nucleated cells (Fig. 3).

Skin of patients with CTCL, psoriasis, or Stage III MF were shown. Figure 4 shows the top TCRB and TCRG sequences obtained from the skin biopsy of a patient with stage III CTCL. The top two TCRG sequences (A, B) and the top two TCRB sequences (A, B) are shown. The top TCRB clone is discriminated from benign cells on the basis of the known total clonal population (indicated by the sum of all top 10 benign clonal populations in each sample).

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REFERENCES

High throughput sequencing is a powerful tool that can provide novel insights into the biology of CTCL.

CONCLUSIONS

HTS analyses of DNA extracted from skin biopsies of patients with skin disorders can provide important, quantitative data of T cell number, clonality, repertoire, and frequency and in so doing provide a useful discriminator of benign versus malignant disease. This same methodology may prove useful for other lymphoid malignancies in which the diagnosis is based on clonal pathophysiological and functional characteristics. The criteria for the high throughput sequencing of TCRG and TCRB is provided.

Figure 6. HTS is a valuable research tool that can provide novel insights into the biology of CTCL.

(A) The TCRB V HST profile is shown for patient 514; both the malignant clone and benign infiltrating T cells in the skin lesions are explained by this technique. (B) CDR3 length analysis provides a rapid way of identifying T cells by malignant clones in biopsies from a patient with stage III CTCL. The CDR3 lengths of all T cells (left panel), including the malignant clone and benign infiltrating T cells, are shown. The top two malignant TCRB clones are indicated by asterisks. A healthy diversity of benign infiltrating T cells was present. (C) HTS provides evidence that CTCL is a malignancy of mature T cells. Prior studies have demonstrated that mature T cells have an average 1.8 rearranged TCRb alleles. We studied 33 CTCL patients with malignant T cell clones by TCRb HTS. 27 patients had two rearranged TCRb alleles, as expected with the presence of two similarly frequent TCRb sequences. 6 patients had a single rearranged TCRb allele, as evidenced by only a single high-frequency TCRb sequence. TCRb T cells therefore had on average 1.8 rearranged TCRb alleles, a proportion characteristic of mature T cells (A). The presence of clonality and repertoire by TCRb HTS provides an unparalleled opportunity to validate and study malignant T cell clones. (D) Identification of the TCRb V chain by HTS and subsequent immunostaining using commercially available TCRb antibodies allows study of clonal malignant T cell clones by immunostaining of malignant T cells with TCRb V specific antibodies in two patients with stage IIIs MF were shown. The top two clones are shown. The top two malignant TCRb clones are indicated by asterisks. A healthy diversity of benign infiltrating T cells was present. (E) TCRG high throughput sequencing of TCRG and TCRB is provided. The criteria for the high throughput sequencing of TCRG and TCRB is provided. The top two TCRG sequences (A, B) are shown. The top two benign TCRG clones are indicated by asterisks. A healthy diversity of benign T cells was present. (F) TCRD high throughput sequencing of TCRG and TCRB is provided. The criteria for the high throughput sequencing of TCRG and TCRB is provided. The top two TCRD sequences (A, B) are shown. The top two benign TCRD clones are indicated by asterisks.