MEDICAL UNIVERSITY OF VIENNA

Single-cell RNA sequencing reveals markers of disease progression in primary cutaneous T-cell lymphoma

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Abstract

Background: In early-stage mycosis fungoides (MF), the most common primary cutaneous T-cell lymphoma, limited skin involvement with patches and plaques is associated with a favorable prognosis. Nevertheless, approximately 20-30% of cases progress to tumors or erythroderma, resulting in poor outcome. At present, factors contributing to this switch from indolent to aggressive disease are only insufficiently understood.

Methods: In patients with advanced-stage MF, we compared patches with longstanding history to newly developed plaques and tumors by using single-cell RNA sequencing, and compared results with early-stage MF as well as nonlesional MF and healthy control skin.

Results: Despite considerable inter-individual variability, lesion progression was uniformly associated with downregulation of the tissue residency markers *CXCR4* and *CD69*, the heat shock protein *HSPA1A*, the tumor suppressors and immunoregulatory mediators *ZFP36* and *TXNIP*, and the interleukin 7 receptor (*IL7R)* within the malignant clone, but not in benign T cells. This phenomenon was not only found in conventional TCR-αβ MF, but also in a case of TCR-γδ MF, suggesting a common mechanism across MF subtypes. Conversely, malignant cells in clinically unaffected skin from MF patients showed upregulation of these markers.

Conclusions: Our data reveal a specific panel of biomarkers that might be used for monitoring MF disease progression. Altered expression of these genes may underlie the switch in clinical phenotype observed in advanced-stage MF.

Introduction

Factors associated with lesion progression in mycosis fungoides (MF) are still only insufficiently understood. Especially disease heterogeneity, both on clinical and molecular levels, is a major challenge in this regard. In this study, we profiled patches with longstanding history, and compared them with recently developed plaques or tumors within the same patient to overcome inter-individual variability. We also assessed lesional and nonlesional MF skin within the same patients. Skin cells were isolated from punch biopsies, and characterized using single-cell RNA sequencing (scRNA-seq) combined with T-cell receptor (TCR) sequencing.

Results

In patients suffering from advanced-stage MF, gene expression of *CXCR4, CD69, HSPA1A, ZFP36, IL7R* and *TXNIP* were consistently downregulated in the malignant clone of plaque or tumor vs. patch lesions, a phenomenon that was not observed in polyclonal T cells

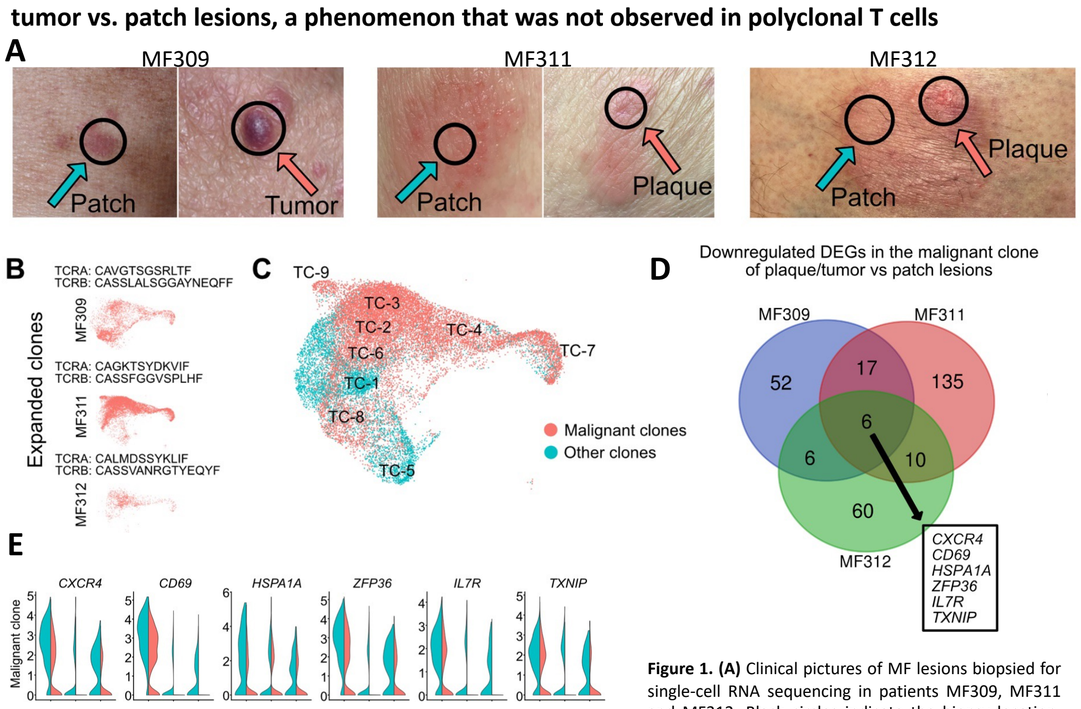

Figure 1. (A) Clinical pictures of MF lesions biopsied for single-cell RNA sequencing in patients MF309, MF311 and MF312. Black circles indicate the biopsy location, turquoise and red arrows indicate patch and plaque/tumor lesions, respectively. **(B)** UMAP plots of the single top expanded clone (according to the most common α and/or β chain CDR3s amino acid sequence) per patient. **(C)** Combined UMAP of T cell clusters colored according to clonality of TCR. Red: Cells with top expanded monoclonal TCR(s) from each sample; turquoise: cells with polyclonal TCR. **(D)** Venn diagram of significantly downregulated genes (DEGs) comparing gene expression in the malignant clone of plaque/tumor vs patch lesions (defined by an adjusted p value<0.05 and a logFCH>|0.3|). **(E)** Violin plots of T-cell clusters showing distribution of normalized gene expression levels of the six genes consistently downregulated in plaque/tumor (red) vs patch lesions (turquoise) in all three patients. These data show consistent downregulation of these 6 markers only in the malignant clone, but not other T cell populations in advancing lesions.

Corroboration of regulated genes in a case of γδ MF

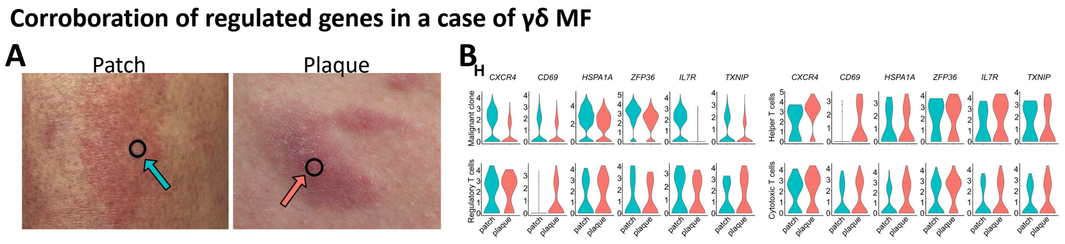

Figure 2. Marker gene expression in patch and plaque lesions of a single γδ TCR+ MF patient. (A) Pictures of patch and plaque lesions; black circles indicate biopsy location. **(B)** Violin plots of T cell clusters showing distribution of normalized gene expression levels of *CXCR4, CD69, HSPA1A, ZFP36, IL7R,* and *TXNIP* in patch (turquoise) and plaque (red) lesions, showing consinstent downregulation only in the malignant γδ clone, but not polyclonal T cell populations. Malignant clone: top expanded γδ clone. Helper T cells: *CD4+ FOXP3-* cells with polyclonal TCRs. Regulatory T cells: *FOXP3+* cells with polyclonal TCRs. Cytotoxic T cells: *CD8A+ FOXP3-* cells with polyclonal TCRs.

***CD69, HSPA1A* and *ZFP36* are further elevated in patches of early-stage MF**

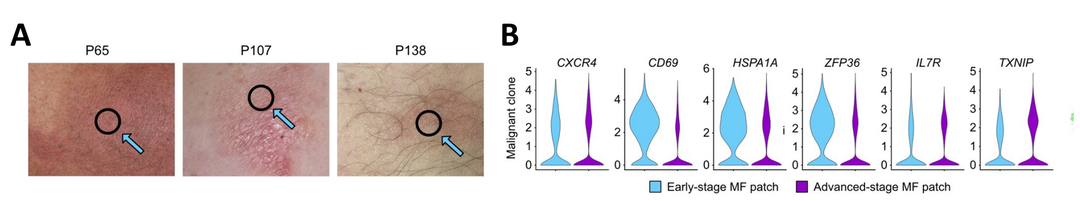

Figure 3. Comparison of patch lesions from advanced-stage vs. early-stage disease. (A) Pictures of patch lesions from 3 patients with longstanding, early-stage MF (patients P65, P107 and P138); black circles indicate biopsy location. **(B)** Violin plots showing distribution of normalized gene expression levels in the top expanded αβ TCR clone in early (light blue) vs advanced-stage MF (purple, 3 patients each). These data show that in patches of early-stage MF, *CD69, HSPA1A* and *ZFP36* are further increased above levels found in patches of advanced-stage MF lesions.

Downregulation of *CXCR4, CD69, HSPA1A, ZFP36, IL7R* and *TXNIP* is reverted in malignant clones of clinically unaffected skin

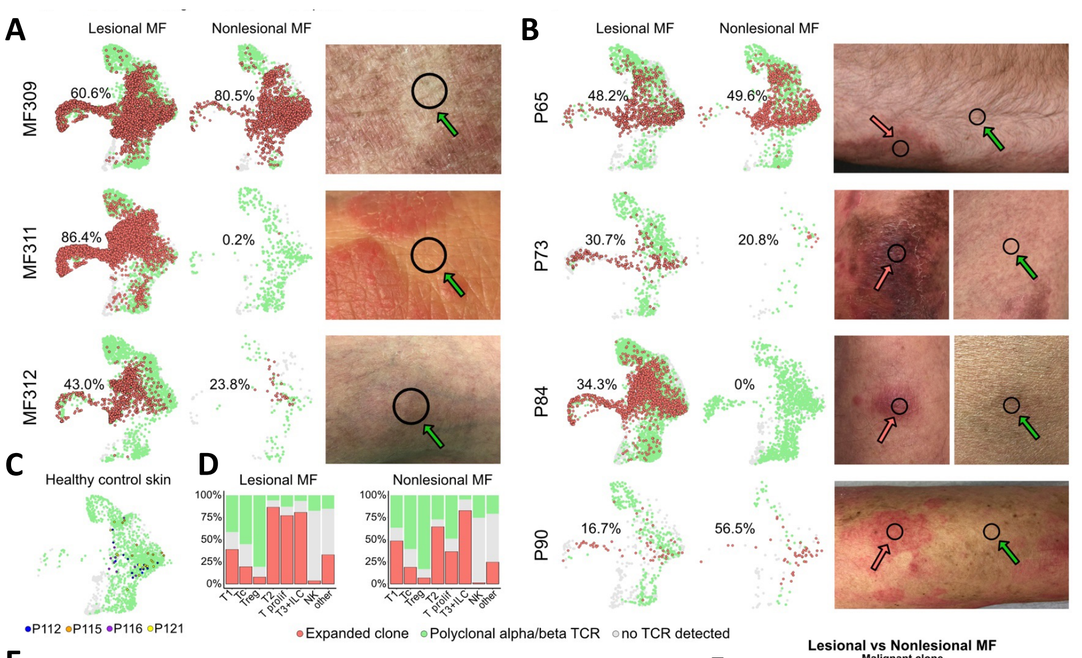

Figure 4. (A-B) UMAP plots of T cell clusters of individual patients colored according to top expanded monoclonal (red) and polyclonal (green) αβ TCR; cells without TCR are displayed in grey; photographs of biopsy sites for nonlesional (green arrow) and lesional (red arrow) skin. Percentages denote frequencies of malignant cells (colored in red) among all TCR+ cells for each plot. These data show that in most patients, malignant clones were also present in clinically normal appearing skin. **(C)** UMAP of four healthy control biopsies; the single most frequent clone for each sample is depicted in blue (P112), orange (P115), purple (P116) or yellow (P121); all other TCR+ cells are shown in green, and cells without detectable TCR in grey. These data show an overall polyclonal pattern of T-cell receptors in healthy control individuals. **(D)** Distribution of the malignant clone (red), polyclonal TCR+ cells (green), and cells without detectable TCR, as percentages for each cluster in lesional and nonlesional biopsies, showing a decrease in proliferating malignant cells in nonlesional MF lesions when compared to lesional MF. **(E)** Violin plots showing distribution of normalized gene expression levels in nonlesional (green), lesional (red) and healthy control skin (blue) for the top expanded αβ TCR clone, as well as helper T cells, regulatory T cells, and cytotoxic T cells. These data demonstrate that in malignant T cells of clinically normal appearing MF skin, *CXCR4, CD69, HSPA1A, IL7R* and *TXNIP* are increased compared to malignant cells of lesional skin. This phenomenon was not observed in polyclonal T cell populations such as helper T cells, regulatory T cells, or cytotoxic T cells. **(F)** Volcano plot showing differentially expressed genes (DEGs) of the malignant clone, as well as polyclonal helper, cytotoxic and regulatory T cells between lesional and nonlesional MF biopsies.

Conclusion

Using single-cell RNA sequencing combined with αβ and γδ TCR sequencing, we identified a panel of markers that were consistently downregulated in the malignant clone of progressing MF skin lesions. The markers that we identified (*CXCR4, CD69, HSPA1A, ZFP36, IL7R* and *TXNIP*) have all been previously described to be either involved in skin homing, cell growth or cancer development. Much to our surprise, five out of seven MF patients harbored substantial numbers of malignant cells also in clinically uninvolved skin. In line with a potentially more “silenced” phenotype, these nonlesional tumor cells harbored elevated levels of *CXCR4, CD69, HSPA1A, ZFP36, IL7R* and *TXNIP* when compared to matched lesional skin. This finding sheds a new light on future curative CTCL treatment approaches, that will need to take into account a tumor cell burden well beyond merely visibly involved skin. In contrast to the expanded clone, we did not find consistent transcriptomic regulation within the tumor microenvironment across patients (data not shown).

Taken together, we identified a characteristic panel of markers associated with cutaneous disease progression in MF. Such potential drivers of disease might constitute ideal targets for future drug therapy with a new treatment strategy of preventing disease progression by preserving a more indolent cancer biology.