High variability of TIGIT expression in Hodgkin's lymphoma

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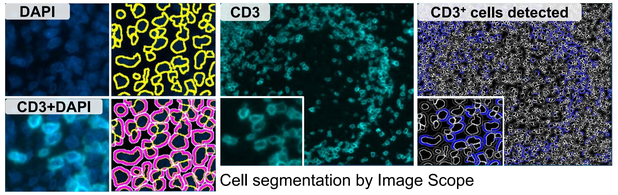
Introduction and Objectives

TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an immune checkpoint protein that has a comparable function to PD-1 and is frequently expressed on subsets of T lymphocytes. Immune checkpoint inhibiting drugs targeting TIGIT are currently under development. The purpose of this study was to analyze patterns of TIGIT and PD-1 expression in the characteristic lymphocytic background of Hodgkin's lymphoma (HL).

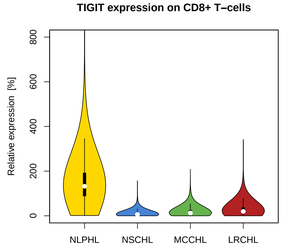
Materials & Methods

Monoclonal mouse antibodies were used for immunohistochemical TIGIT (Dianova, Hamburg, Germany) and PD-1 (Abcam, Cambridge, UK, ab52587) analysis of formalin-fixed tissue samples from Hodgkin's lymphoma lymph nodes (n=40) and normal human tonsils (n=2). To study patterns of TIGIT expression in the T-cell background surrounding malignant cells, including Hodgkin cells, Reed-Sternberg cells and histiocytic cells, a microenvironment (ME) tissue microarray (TMA) was constructed from tissue punches measuring 2 mm in diameter. Fluorescence images were taken and analyzed with a Leica Aperio Versa 8 automated microscope system equipped with Leica Image Scope analysis software.



Cell segmentation by Image Scope

Measuring fluorescence intensity using relative expression (RE)

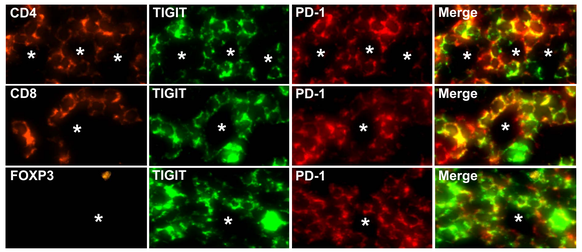


Comparison of relative[1] TIGIT expression levels in individual patients with different subtypes of HL. The colored area corresponds to the number of cells with the same intensity level.

[1]Compared to the average fluorescence level in germinal centers of normal human tonsil.

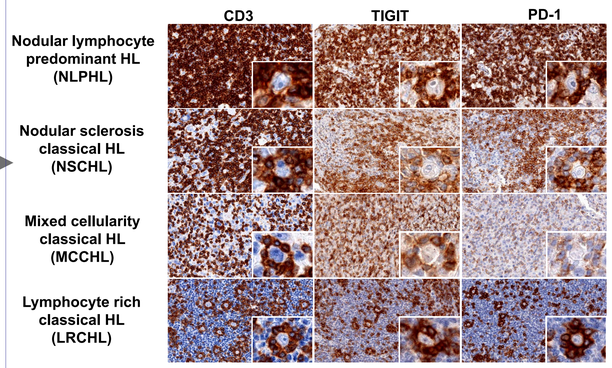
RESULTS

High level co-expression of TIGIT and PD-1 in CD3⁺ T-cells surrounding the malignant cells

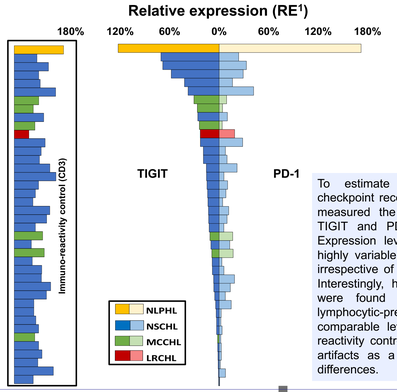


IHC based identification of T cell subtypes revealed TIGIT and PD-1 expression on all analyzed T-cell subtypes, including CD4 positive helper T-cells, CD8 positive cytotoxic T cells and FOXP3+ regulatory T-cells. Particularly high TIGIT and PD-1 (co-)expression was seen in T-cells surrounding the malignant cells (indicated by asterisks) of Hodgkin's lymphoma.

TIGIT and PD-1 are frequently expressed in HL subtypes

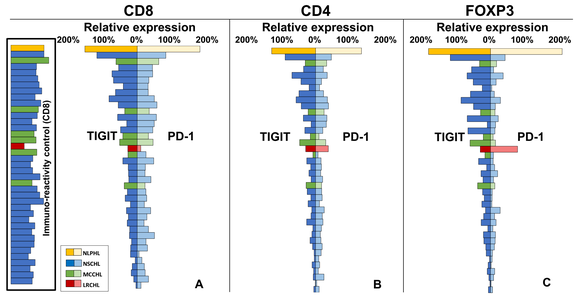


Variable expression of TIGIT and PD-1 in 40 HL patients



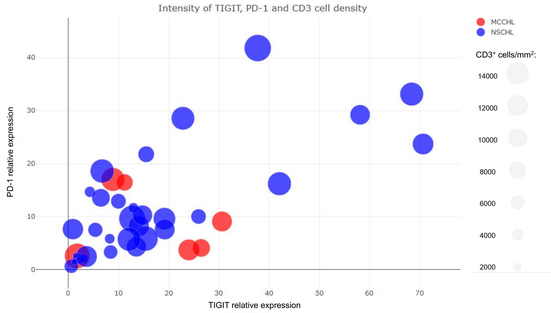
To estimate the variability of immune checkpoint receptors across individual HL, we measured the relative expression levels of TIGIT and PD-1 on CD3 positive T-cells. Expression levels of TIGIT and PD-1 were highly variable among the analyzed samples irrespective of the histological subtype of HL. Interestingly, highest levels of both proteins were found in one sample of nodular lymphocytic-predominant HL (NLPHL). The comparable level of CD3 staining (immuno-reactivity control) largely excludes processing artifacts as a potential reason for intensity differences.

T-cell type specific expression of TIGIT and PD-1



The high variability of TIGIT and PD-1 expression was also independent from the T-cell type. However, HL with high TIGIT or PD-1 expression on CD8 positive cells sometimes showed low level expression on CD4 or FOXP3 positive T cells and vice versa.

Relative expression of TIGIT an PD-1 on CD3⁺ T-cells is independent from the CD3⁺ cell density



Expression of TIGIT and PD-1 was unrelated to the amount of the lymphocytic infiltrate, i.e., the density of CD3 positive T-cells as indicated by the size of the circles.

Conclusions

- TIGIT expression is a frequent feature of the lymphocytic background in Hodgkin's lymphoma.
- TIGIT and PD-1 are typically co-expressed on T-cells, but their relative importance varies between patients.
- TIGIT and PD-1 are expressed typically at variable levels in individual patients irrespective of the HL subtype or T-cell subtype.
- Refractory or relapsed HL may benefit from future therapeutic strategies aiming in co-targeting of TIGIT and PD-1.

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