Primary Melanoma tumor immune contexture analysis: T regulatory cell to T effector cell ratio as related to MHC class II and GILT expression

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Abstract

Recent studies establish a correlation between tumor immune evasion with an increased T regulatory (Treg) cell to T effector (Teff) cell ratio in various types of primary malignant tumors. Polarization of naïve helper T cells into either Treg or Teff cells is dependent on antigenic presentation by major histocompatibility complex (MHC) class II. Furthermore, gamma-interferon-inducible lysosomal thiol reductase (GILT) plays an upstream role in antigenic processing. Our study aim was to elucidate a possible association between GILT expression in antigen presenting cells (APC's) and Treg:Teff ratio. We hypothesized GILT expression in melanoma cells would result in a decreased Treg:Teff ratio or enhanced T cell-mediated response. Primary melanoma specimens were stained and scored for Treg, Teff, CD8, MHC class II and GILT. Statistical significance was not reached relative to our hypothesized relationship of a decreased Treg:Teff ratio in the presence of GILT+ MHC class II. Similarly, we did not reach statistical significance when comparing individual cell types to GILT, MHC class II and GILT+ MHC class II. Further investigation on a larger scale is warranted given our small sample size.

Introduction

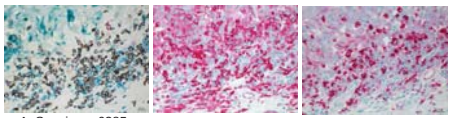
Histopathologic examination of the tumor micro-environment demonstrates the presence of a vast repertoire of infiltrating lymphocytes and APC's. Analyzing the density, location, and functionality of the immune cell populations defines the tumor immune contexture and may confer prognostic value. Treg cells are immunosuppressive lymphocytes playing a key role in prevention of autoimmunity via downregulation of Teff cells. Similarly, in the tumor microenvironment, Treg cells inhibit the anti-tumor activity of Teff cells. Previous studies specifically highlight the role of Treg:Teff cell ratio in the immunocontexture as a particularly strong prognostic factor. In the primary melanoma tumor, an increased Treg:Teff ratio has been correlated with tumor aggressiveness and decreased overall survival. This relationship led to our investigation of the factors contributing to T cell development. Polarization of helper T cells into Treg and Teff requires antigenic peptide presentation by APC's. MHC class II molecule, expressed on the surface of APC's, facilitates this presentation along with an upstream component involved in processing and presentation referred to as GILT. GILT reduces protein disulfide bonds allowing for exposure of previously buried epitopes, enhancing presentation by MHC class II. Our study, therefore, focuses on evaluating the influence of GILT expression by MHC class II (GILT+ MHC class II) on the Treg:Teff ratio within the melanoma tumor microenvironment.

Methods

Immunohistochemical staining: 17 de-identified primary melanoma specimens previously stained for FOXP3, CD8, CD3, S100, CD20 with five color immunohistochemistry and individually stained for GILT and MHC class II were scored. Markers correlating with cell type are as follows: CD3+CD8-FOXP3+ = Treg, CD3+CD8-FOXP3- = Teff, CD8+ = Cytotoxic T cell, CD20 = B lymphocyte surface antigen, S100 = tumor cell marker.

Statistical analysis: Scoring was performed by a medical student and board-certified dermatologist. Using the markers indicated above, four areas with highest observed Treg and Teff density were scored and averaged ±SD for total Treg, Teff, Treg:Teff ratio, and CD8. Using landmark association, the same four areas were identified and scored for MHC class II and GILT in APC's and tumor cells using intensity (faint, intermediate or intense) and frequency of the staining (0-100% in increments of 10%). Analysis compared average Treg, Teff, CD8, and Treg:Teff ratio to the presence/absence, intensity, and frequency of GILT. MHC class II comparisons were performed similarly. Finally, specimens demonstrating GILT+ MHC class II tumor cell staining were compared to Treg, Teff, CD8 and Treg:Teff ratio. Graphical representation of the results were constructed for all comparisons. Statistical analysis was performed using the independent *t* test (or Mann-Whitney test) for cell comparisons to the presence/absence of GILT and MHC class II, where as trend analysis or linear regression after log transformation of cell count values was used for comparison with intensity and frequency of GILT and MHC class II. Statistical significance was set at $p \leq 0.05$.

Results



A. Specimen 6385 B. Specimen 6385 C. Specimen 6385

Figure 1. *Serial sections of a primary melanoma biopsy were stained for, A. FOXP3 (nuclear, brown), CD3 (cell surface, blue), CD8 (cell surface, black), CD20 (cell surface, pink), & S100 (nuclear and cell surface, aqua). B. MHC class II (red). C. GILT (red)*

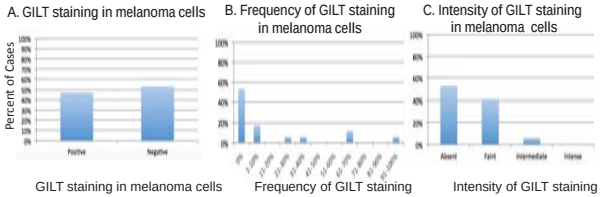


Figure 2. *Heterogeneous GILT staining in melanoma cells. A. Percent of cases with positive & negative staining. B. Percent of cases associated with frequency or estimated area with staining. C. Percent of cases associated with absent, faint, intermediate & intense staining.*

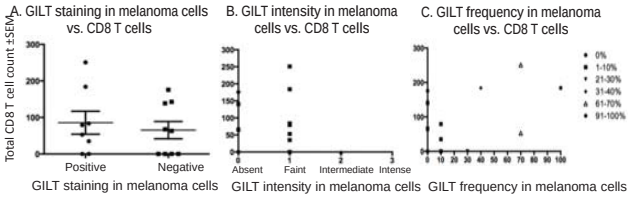


Figure 3. *Comparisons between GILT staining in melanoma cells & CD8 T cell count. A. No statistically significant association was derived between GILT presence & CD8 T cells (p = 0.59), B. GILT intensity & CD8 T cells (p = 0.66), & C. GILT frequency & CD8 T cells (p = 0.78). Analysis was performed using Mann-Whitney test for graph A & trend analysis with log transformation of cell count values for graphs B & C.*

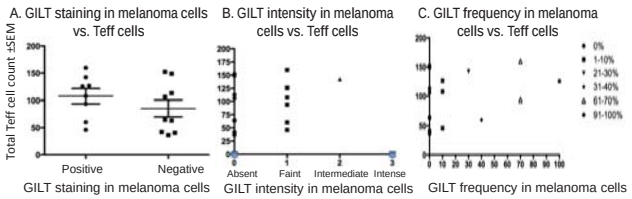


Figure 4. *Comparisons between GILT staining in melanoma cells & Teff cell count. A. No statistically significant association was derived between GILT presence & Teff cells (p = 0.32, B. GILT intensity & Teff cells (p = 0.18), & C. GILT frequency & Teff cells (p = 0.19). Analysis was performed using Mann-Whitney test for graph A & trend analysis with log transformation of cell count values for graphs B & C.*

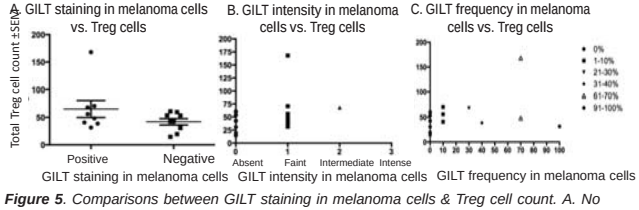


Figure 5. *Comparisons between GILT staining in melanoma cells & Treg cell count. A. No statistically significant association was derived between GILT presence & Treg cells (p = 0.28), B. GILT intensity & Treg cells (p = 0.12), & C. GILT frequency & Teff cells (p = 0.26). Analysis was performed using Mann-Whitney test for graph A & trend analysis with log transformation of cell count values for graphs B & C.*

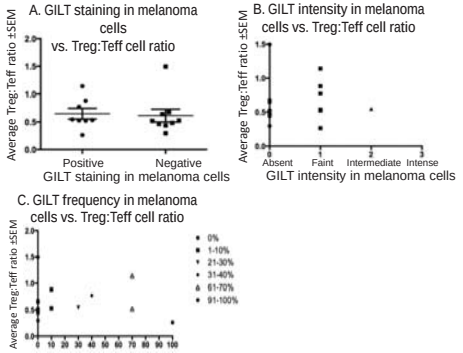


Figure 6. *Comparisons between GILT staining in melanoma cells & Treg:Teff ratio. A. No statistically significant association was derived between GILT presence & Treg:Teff cells (p = 0.32). B. Demonstrates lack of association between GILT intensity & Treg:Teff ratio & C. GILT frequency & Treg:Teff ratio. Analysis was performed using Mann-Whitney test for graph A.*

Comparisons between GILT+MHC class II and Treg, Teff, CD8, and Treg:Teff cell ratio, similarly, did not reach statistical significance when assessing for an association ($p = 0.37, 0.52, 0.22$ and 0.12, respectively; data not shown). Only 3/17 specimens demonstrated both GILT+ MHC class II staining. We ultimately were unable to reject the null hypothesis for all comparisons.

Discussion and Conclusions

Statistically significant associations between Treg, Teff, CD8 and Treg:Teff ratio and the presence of MHC class II, GILT, and GILT+ MHC class II were not reached in our study. The inability to reach statistical significance in all cases may be attributed in part or largely due to the small sample size or total number of specimens scored, leading to a type II error or inability to reject the null hypothesis. Further investigation of these factors on a larger scale as well as other factors influencing lymphocytic development is warranted in the context of the primary melanoma tumor.

Acknowledgements

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