OmniSeq®
700 Ellicott Street | Buffalo NY, 14203

Chemokine expression signatures in infiltrated vs non-infiltrated tumors

The right drug or right trial...
For Every Patient

Pabla S[1,*], Dressman D[1], Conroy JM[1,2], Nesline MK[1], Glenn ST[1,2], Burgher B[1], Qin M[1], Andreas J[1], Giamo V[1], Lenzo FL[1], Omilian A[2], Bshara W[2], Papanicolau-Sengos A[1], Wang Y[1], Ernstoff M[2], Gardner M[1], Pawel Kalinski[2], Morrison CD[1,2,**]

[1]OmniSeq Inc., Buffalo, NY; [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY

*Sarabjot.Pabla@OmniSeq.com; **Carl.Morrison@OmniSeq.com

Introduction

Chemokines are a group of small molecular weight cytokine proteins that bind to G-protein coupled chemokine receptors on the surface of leukocytes. They are crucial in regulating cell migration, immune surveillance, and inflammation via leukocyte trafficking in various diseases, including cancer. Previous studies in cancer cell lines and animal models have shown that expression of specific chemokines determines immune cell infiltration in the tumor microenvironment. More specifically, low expression of CXCL9/10/11, CXCR3, and CCL5, coupled with high expression of CCL2, CCR2, CCR4, CCR5, CCL22, CXCL12, and CXCR4, leads to an exclusion of effector T-cells in the tumor microenvironment while allowing the entry of T_{REG} and MDSC(s). In this study of 300 metastatic melanomas, we assessed the chemokine gene expression signature of tumors with apparent CD8⁺ T-cell infiltration *versus* non-infiltrated tumors with minimum to no T-cell infiltration detected by immunohistochemistry (IHC).

Methods

300 formalin-fixed, paraffin-embedded (FFPE) metastatic cutaneous melanoma samples were evaluated by the RNA-seq component of a comprehensive immune profile panel to measure transcript levels of 395 genes including 10 chemokine genes. Resultant data was QC filtered, normalized and ranked based on an assorted reference population of various tumor types.

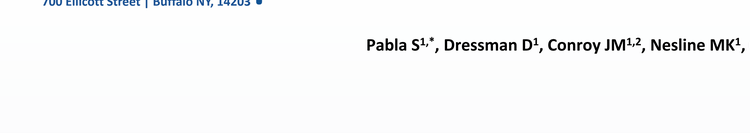

Figure 1: NGS workflow

CD8 gene expression rank was used to categorize tumors as inflamed (≥75), borderline (≥25 and <75), and immune deserts (<25). T-cell infiltration (Figure 2) is defined by CD8 immunohistochemistry with following definitions:

- Non-infiltrated referred to a sparse number of CD8⁺ T-cells that infiltrate nests of neoplastic cells and with less than 5% of the tumor showing an infiltrating pattern.
- Infiltrated represents frequent CD8⁺ T-cells that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and in more than 5% of the tumor.
- Excluded represents restriction of more than 95% of all CD8⁺ T-cells in a tumor to the periphery or interstitial stromal areas and not actively invading nest or groups of neoplastic cells.

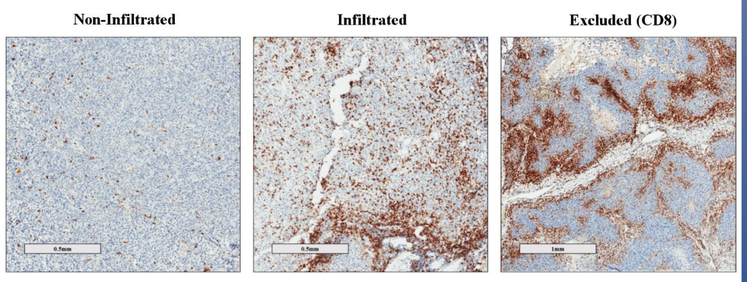

Figure 2: CD8⁺ T-cell infiltration pattern was assessed by a trained pathologist using immunohistochemistry with a CD8-specific antibody. Representative images are depicted (scale bar = 500µm or 1mm).

Measuring CD8⁺ T-cell Infiltration

We assessed the correlation between the semiquantitative measurement of CD8 gene expression by RNA-seq and the qualitative measurement of CD8 infiltration pattern by immunohistochemistry (IHC). CD8 infiltration by IHC showed high correlation with CD8 gene expression by RNA-seq with infiltrated tumors showing significantly higher expression of CD8 than non-infiltrated tumors (one way ANOVA *p*-value <2e-16). Post hoc Tukey HSD confirmed significantly different expression of CD8 for all infiltration patterns (Figure3).

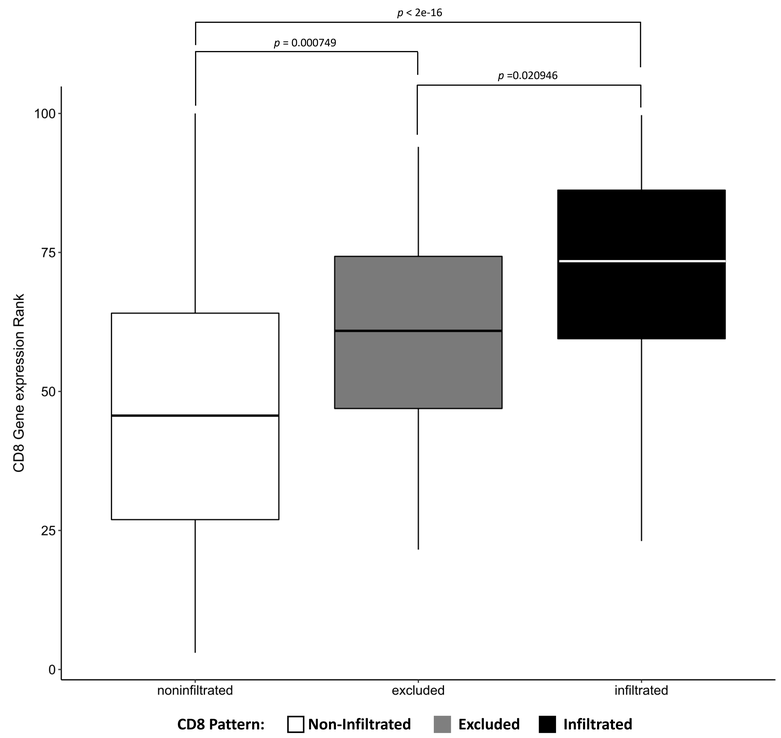

Figure 3: CD8 gene expression rank versus CD8 infiltration pattern by IHC. Tukey HSD pairwise comparison *p* values indicated.

Next, we investigated the CD8 gene expression rank categories and its relationship to infiltration patterns detected by RNA-seq. Within the CD8 gene expression rank groups of inflamed, borderline, and immune deserts, the vast majority of inflamed tumors (Figure 4A) were classified as infiltrated (73%) and almost all immune desert tumors (Figure 4B) were classified as non-infiltrated (93%).

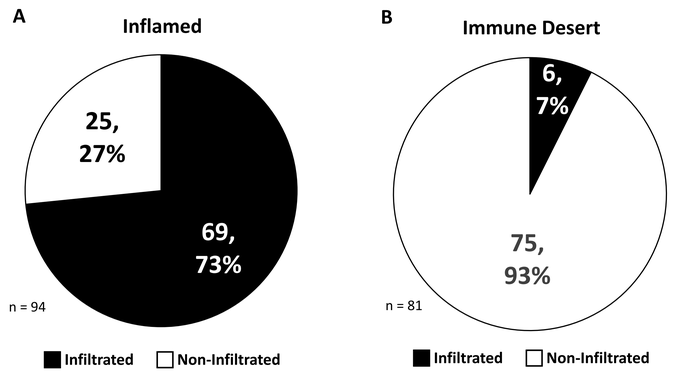

Figure 4: Proportion of infiltrated and non-infiltrated tumors in CD8 gene expression rank groups of inflamed and immune desert tumors.

Distinct Chemokine Signature

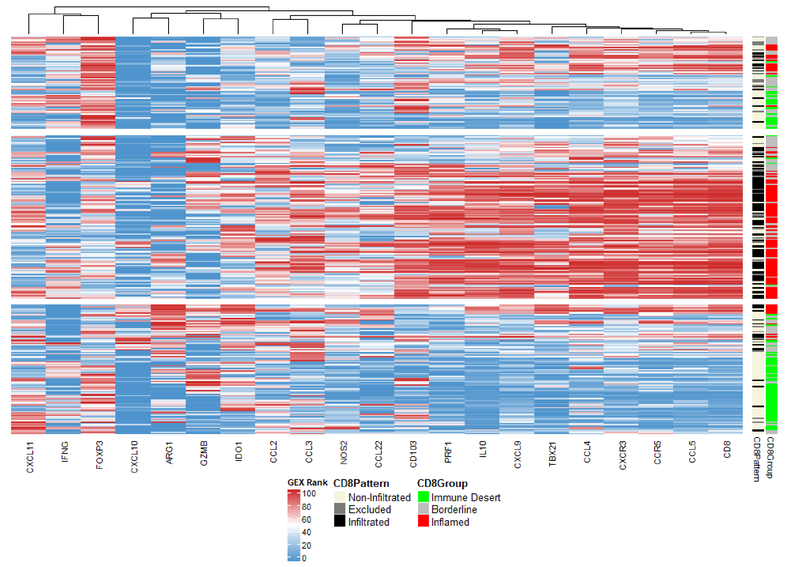

Figure 5: Distinct chemokine signatures revealed by unsupervised clustering of gene expression ranks of 300 melanoma cases annotated by CD8 infiltration pattern by IHC and CD8 inflammation group by gene expression.

The following genes related to leukocyte infiltration were found to be differentially expressed (Wilcoxon test *p*-value < 0.05) between infiltrated and non-infiltrated tumors: CD8, CCL5, CCR5, CCL4, IL10, CXCR3, CXCL9, TBX21, PRF1, CD103, NOS2, CCL2, CCL22, CCL3, CXCL10 and IDO1.

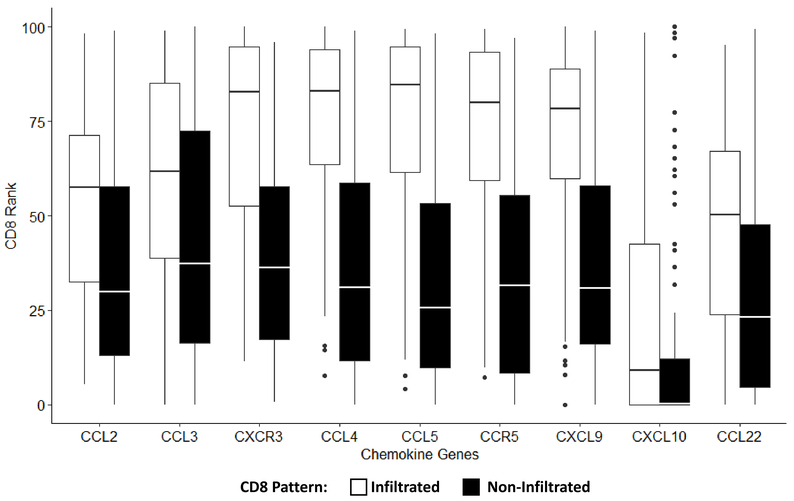

Figure 6: Chemokine genes significantly differentially expressed (Wilcoxon rank sum test *p*-values < 0.05) between infiltrated vs non-infiltrated tumors.

Conclusions

In 300 metastatic cutaneous melanoma cases, we demonstrated that tumor inflammation status by CD8 expression by RNA-seq correlated with CD8 infiltration pattern by IHC. Moreover, expression of infiltrated and non-infiltrated tumors shows distinct chemokine signatures where higher CD8 T-cell infiltration correlates with higher expression of studied chemokines in the tumor microenvironment. These results make biological sense with the signaling of leukocytes by cytokines leading to infiltration of these cells. However, it requires further investigation to better understand the interplay of specific chemokines and cytokines in the tumor microenvironments that drive the immune cycle in melanoma.