OmniSeq
700 Ellicott Street | Buffalo NY, 14203

Distinct pathways of DC-induced CD8⁺ T cell differentiation revealed by single-cell mRNA sequencing analysis

PRESENTER: **Sarabjot Pabla**

Dong B[1], Pabla S[2#], Giamo V[2], Jiang W[1], Conroy JM[1,2], Taniguchi K[3], Glenn ST[1,2], Shirai M[3], Kalinski P[1]

[1] Roswell Park Comprehensive Cancer Center, Buffalo, NY
[2] OmniSeq, Inc., Buffalo, NY
[3] Hitachi, Ltd., Research & Development, Tokyo Japan

INTRODUCTION

Dendritic Cells (DC) maturing in the conditions of acute/early versus chronic/late inflammation (such as alpha-type-1-polarized DCs [αDC1s] and "standard" nonpolarized DCs; sDCs) deliver differential "signal 3" to resting T cells, resulting in their different induction of effector functions in tumor-specific CD8⁺ T cells. Here, using single-cell mRNAseq (scRNAseq), a unique technology allowing for unbiased analysis of gene expression (GEX), we evaluated the GEX patterns and cellular heterogeneity of CD8⁺ T cells expanded by cancer-cell loaded αDC1s or sDCs.

METHODS

αDC1s and sDCs were generated from human monocytes and maturated in conditions mimicking, respectively, acute and chronic inflammation as described [1]. Freshly isolated autologous (to DCs) CD8⁺ T cells were in vitro sensitized for 1 week, using either αDC1s loaded with UV/gamma-radiation-killed ovarian cancer (OvCa) cell lines OVCAR3 or SKOV3, or sDCs loaded with the same antigens. αDC1s and sDCs not loaded with exogenous antigen were used as controls (Figure 1). The expanded CD8+ T cells were harvested, captured at the single-cell level and immobilized in a vertical flow array chip (VFAC), which contain 100-microchambers packed with 105 beads immobilizing 1010 oligo(dT) probes with unique cell-ID, UMI, and PCR-tags. Each cell was lysed and its mRNA was individually tagged with unique cell ID sequences prior to library preparation and targeted scRNAseq using a custom 94-gene inflammation RNA-seq panel was performed. Post-clustering (Scanpy), unsupervised cell-type identification (louvain), and downstream analyses were performed on GEX data to determine impact of αDC1s versus sDCs (loaded with OVCAR or SKOV3 versus not loaded with antigens) on the effector and memory pathways of differentiation of the expanded T cells and their unique functional status (Figures 2-6).

AACR 2020 – Abstract #6078 – Poster #968

Study Design

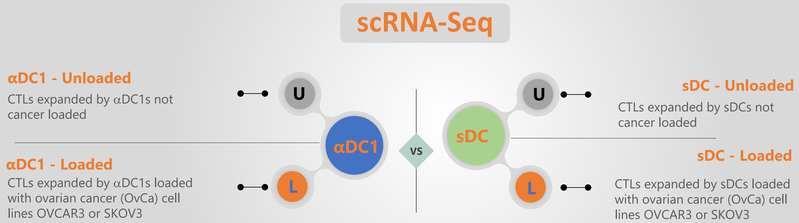


Figure 1: Study design presenting various conditions for CD8⁺ T cells in vitro sensitization (IVS).

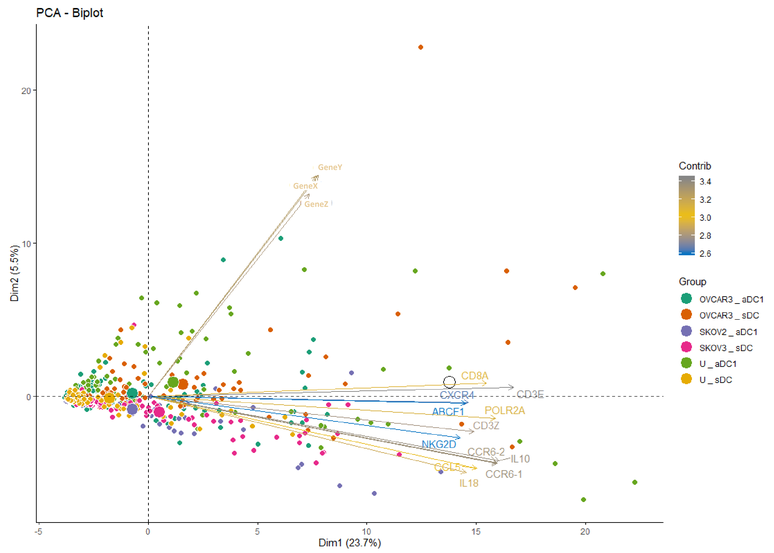


Figure 2: Principal component analysis plot showing first and second principal component of all gene features for CD8⁺ T cells primed by both types of DCs under loaded and unloaded conditions. 15 genes most contributing to the separation of the groups are labeled.

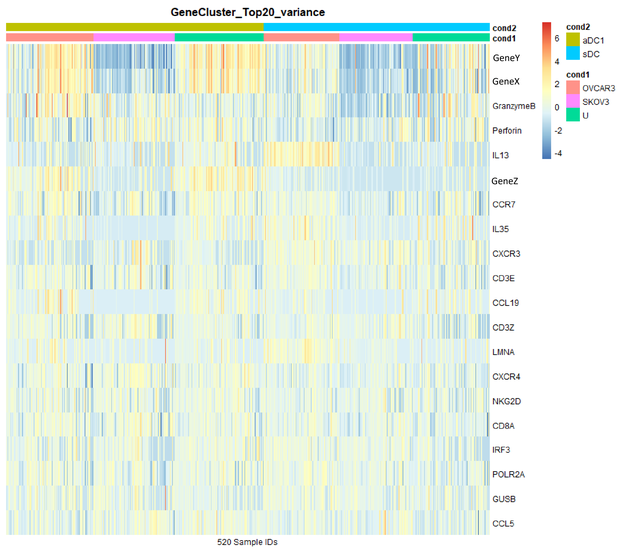


Figure 3: Heatmap of gene expression showing top20 most variable genes between CD8⁺ T cells primed by two DC types under loaded and unloaded conditions. For instance, αDC1-primed T cells show higher expression of Gene X, Gene Y and GranzymeB in unloaded and OVCAR3 loaded conditions compared to sDC-primed T cells under all conditions.

Figure 4: Fold change of differentially expressed inflammation genes (p<0.05) for CD8⁺ T cells primed by: a) loaded αDC1 compared to loaded sDC and b) unloaded αDC1 compared to unloaded sDC.

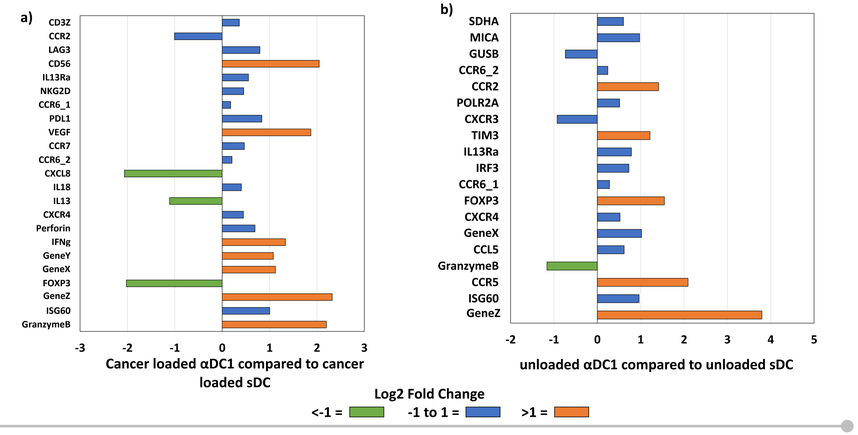


Figure 5: Fold change of differentially expressed inflammation genes (p<0.05) for CD8⁺ T cells primed by: a) OVCAR3 loaded αDC1 compared to unloaded αDC1 and b) SKOV3 loaded αDC1 compared to unloaded αDC1.

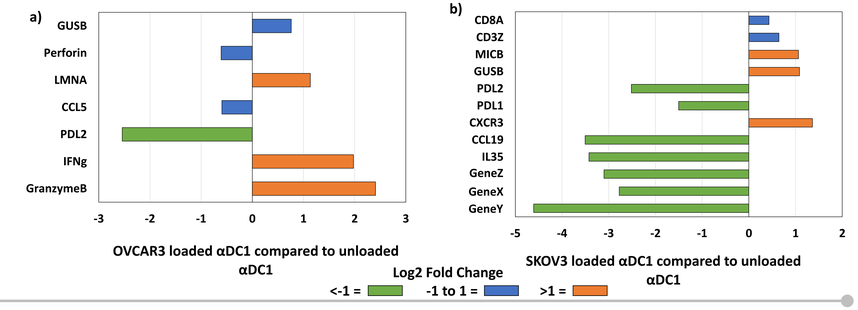
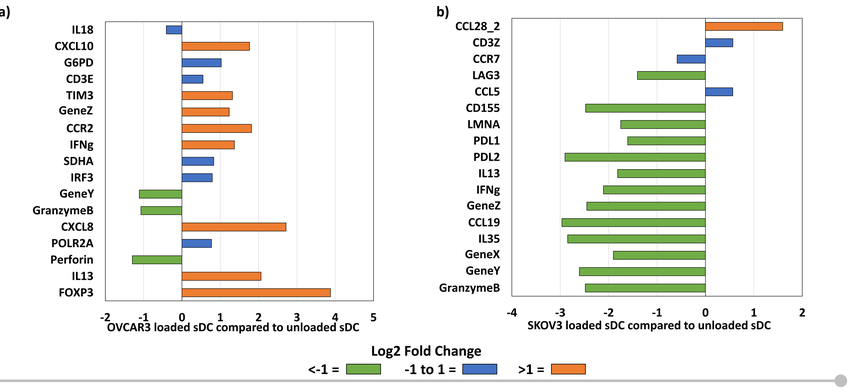


Figure 6: Fold change of differentially expressed inflammation genes (p<0.05) for CD8⁺ T cells primed by: a) OVCAR3 loaded sDC compared to unloaded sDC and b) SKOV3 loaded sDC compared to unloaded sDC.



CONCLUSION

scRNAseq was able to identify distinct single-cell T-cell phenotypes caused by induction of αDC1s or sDCs (loaded with OVCAR or SKOV3), consistent with the differential ability of these two DC types to induce effector, effector memory and central memory CD8+ T cells in response to different tumor targets and with their differential ability to modulate peripheral tissue homing patterns.

REFERENCES

1. Mailliard, R.B., Wankowicz-Kalinska, A., Cai, Q., Wesa, A., Hilkens, C.M., Kapsenberg, M.L., Kirkwood, J.M., Storkus, W.J., and Kalinski, P., alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. *Cancer Res*. **64**(17): 5934-7 (2004).