PS3-01-04

# Single-Cell Transcriptomic and Phenotypic Profiling Reveals T Cell Dysfunction in cancer-free *BRCA1* Mutation Carriers

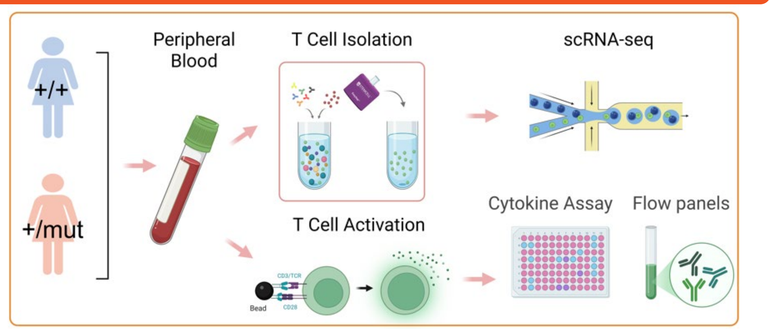
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## ABSTRACT

Women harboring germline mutations in *BRCA1* have an approximately 80% lifetime risk of developing breast cancer. Extensive research on the breast epithelium, the cell of origin for *BRCA1*-related tumors, has shed light on the molecular functions of BRCA1 in tumor suppression. However, all somatic cells in a germline *BRCA1* mutation carrier contain the same mutant allele, yet it remains unclear whether *BRCA1* heterozygosity in stromal compartments could also contributes to the increased risk of *BRCA1*-associated cancer. To determine the impact of *BRCA1* heterozygosity on T cells at the transcriptional and functional levels, we first investigated a cohort of T cells isolated from healthy individuals who are *BRCA1* mutation carriers (*BRCA1*$^{mut/+}$) and non-carriers, using single cell RNA sequencing (scRNA-seq) and high-parametric spectral flow cytometry. Single-cell transcriptomic analysis revealed a significant enrichment of a gamma-delta (γδ) T cell subpopulation in *BRCA1*$^{mut/+}$ samples. A similar trend, albeit not statistically significant, was observed for a SOX4$^{high}$ T cell subpopulation. Functionally, *BRCA1*$^{mut/+}$ T cells exhibited a delayed response to CD3/CD28 costimulation and showed an enrichment of naïve cells in the CD4 compartment. In addition, the production of cytokine IL-17 is significantly reduced in *BRCA1*$^{mut/+}$ T cells after 24 hours of activation. In summary, our data from clinical samples suggest that the heterozygous *BRCA1* mutation status leads to T cell–intrinsic functional alterations and compromised antitumor immunity. We propose that germline *BRCA1* mutations exert a "double whammy" effect—contributing to elevated cancer risk through their presence in both the cell of origin and the surrounding stromal compartments.

## MATERIAL & METHODS




## RESULTS

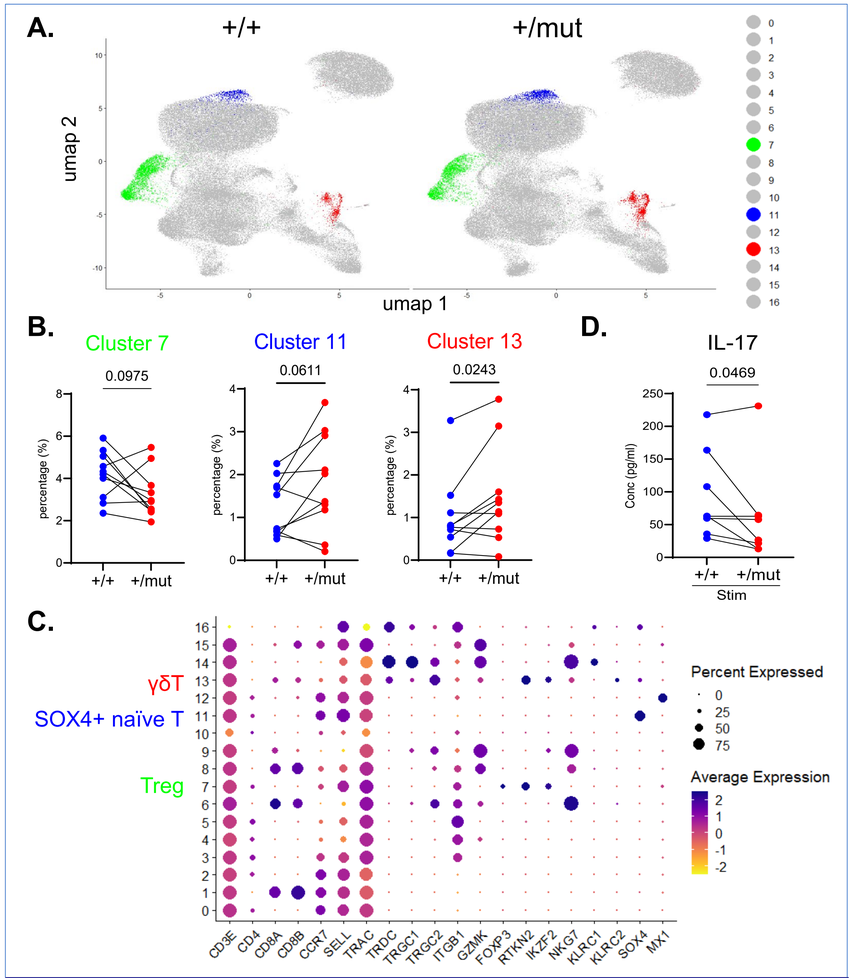



**Figure 1. Single-cell transcriptomic analysis reveals an enrichment of SOX4 high T cell population in *BRCA1* mutation carriers.** (**A**) UMAP comparison between controls and *BRCA1* mutation carriers (**B**) Proportions of each cluster in controls and *BRCA1* mutation carriers (**C**) Dot plot depicting the mean expression levels of signature genes and the percentage of cells expressing them across clusters (**D**) IL-17 production after 24 hours of stimulation.

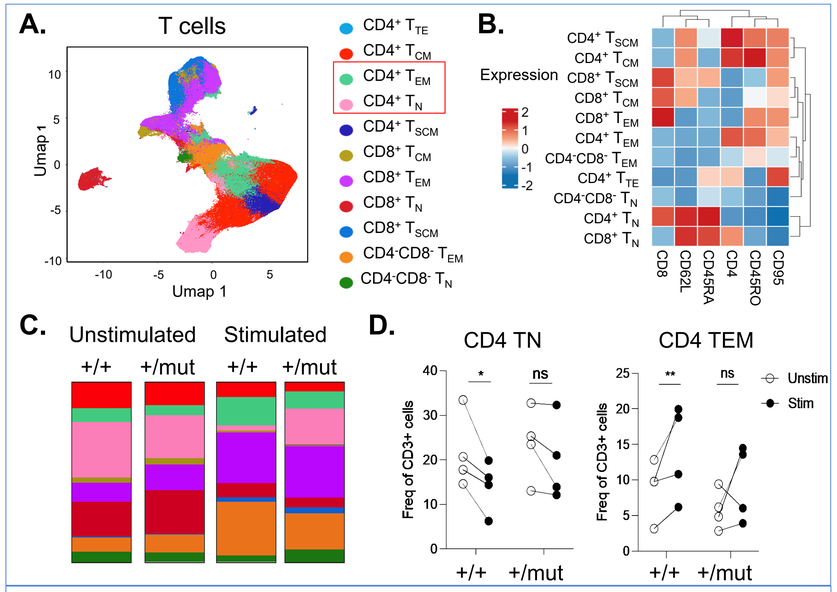
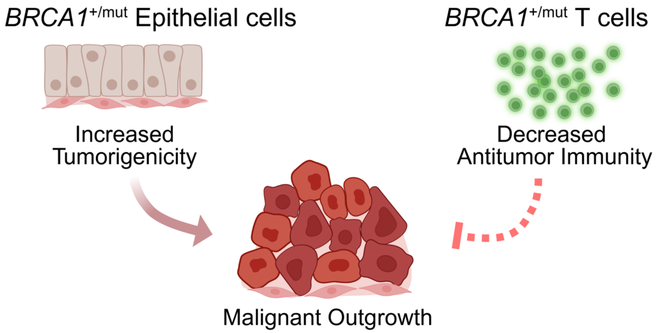



**Figure 2. Early activation of CD4 T cells is impaired in *BRCA1* mutation carriers.** (**A**) UMAP representation of CD3$^+$ T cells from four age-matched female controls and *BRCA1* mutation carriers (**B**) Heatmap depicting the expression levels of phenotypic T cell markers in the cells shown in (A) (**C**) Stacked bar plots representing the proportions of all T cell populations from (A) (**D**) Slope chart showing CD4$^+$ subpopulations before and after 24 hours of stimulation. * $p < 0.05$, ** $p < 0.01$; paired Tukey's multiple comparison test. TE: terminal effector, CM: central memory, EM: effector memory, N: naïve, SCM: stem cell memory.

## SUMMARY & FUTURE DIRECTION




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