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Mechanistic analysis of NK cells in pan-cancer tumor immunity

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Abstract. Chimeric antigen receptor (CAR) NK cell therapy has been hampered in solid tumors by an incomplete understanding of tumor-infiltrating NK cells. Based on the development of single-cell sequencing methods, this paper used single-cell sequencing data of NK cells from the GEO database to cluster them, focused on the $CD56^{bright}CD16^{lo}$-CREM subpopulation, which was statistically screened for differential genes and investigated its gene pathways. The results showed that this subpopulation can provide energy for tumor proliferation by promoting ribosomal autophagy, and at the same time express cell adhesion molecules, cytokines and related proteins to interact with monocytes and macrophages, hindering the function of NK cells and promoting tumor progression. This provides new ideas for in-depth analysis of the immunological role of NK cells in pan-cancer tumors.

Keywords: pan-cancer tumors, NK cell therapy, single-cell sequencing method, bioinformatics analysis.

1. Introduction

Immune cell-based therapies, such as natural killer (NK) cells, represent a promising strategy for treating malignant tumors without optimistic effects for traditional chemotherapy or radiotherapy. NK cells, originating from common lymphoid precursors in the bone marrow, play important roles in innate immune response against cancer. The features of NK cells are both express activating and inhibitory receptors on their surfaces, which allow them to distinguish between normal cells from cancerous or infected cells. In the tumor microenvironment, NK cells can be activated through various mechanisms, including recognizing host cells through losing their own MHC class I molecules or tumor cells through inducing certain signals. Once activated, NK cells employ several mechanisms to eliminate tumor cells, including releasing cytotoxic molecules such as perforin, granzyme, and TNF, as well as expressing Fas ligand (FasL) to induce apoptosis of tumor cells. Importantly, NK cells do not rely on specific matching of human leukocyte antigen (HLA) to exert their anti-tumor effects, making them potential candidates for allogeneic therapy, thus simplifies the treated process without HLA matching. Therefore, the NK cell-based therapies became an attractive option for patients with refractory tumors due to their convenience and efficiency [1].

Since 20 years ago, the field of NK cell-based cancer therapies has grown up, expanding into a major area in nowadays [2]. The recent research further highlights the promising advancements in chimeric antigen receptor (CAR) NK cell therapies because of the optimistic effects of the anti-CD19 CAR NK cell therapeutic B-cell cancers [3]. However, NK cell therapy faces challenges in solid tumors, partly

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due to insufficient understanding of tumor infiltrating NK cells and their disorders in the tumor microenvironment (TME) [4].

In recent years, single-cell sequencing technology in combination with different algorithms have been developing rapidly, facilitating the fine-grained subpopulation of cells due to sequencing of total RNA at the level of single cell, thus to study their evolution during the course of the disease and help to locate new subpopulations of cells. Based on the development of this technology and the optimistic prospect of NK cells in cancer therapy, this paper used single-cell sequencing data of NK cells from the GEO database to cluster them using unsupervised dimensionality-reducing clustering, and explored the genetic changes of $CD56^{bright}CD16^{lo}$-CREM subpopulation in NK cells and the related functional pathways, contributing to the understanding of the dynamics of biosignatures at the location of cancers. These analysis provides an idea for the in-depth analysis of the immunological roles of NK cells in tumors, such as pancreatic cancer.

2. Material and method

2.1. Data source

All datasets were downloaded from the NCBI GEO. Both normal and cancer samples were obtained from GSE212890. The data covered 24 kinds of tumors with 716 patients. Inclusion criteria for the data in patients of hepatocellular carcinoma and intrahepatic cholangiocarcinoma were determined based on pathologic diagnosis. Clinical staging was categorized according to AJCC guidelines and included stage I, II and III. Patient samples were obtained from East Asia, aged 49 ~ 72 years with a mean age of 60 years. The samples of adjacent tissue paired with tumors were used for NK cell isolation for single-cell sequencing with the GPL20795 sequencing platform.

2.2. Cell cluster annotation

Firstly, the dimensionality reduction clustering were performed on the single-cell RNA sequencing data with a resolution value of 0.5, and then the cell clusters were annotated. In order to study the cell subpopulations of interest in depth, the second dimensionality reduction clustering analysis was performed on specific cell subpopulations with a resolution value of 0.5.

2.3. Differential gene analysis

The differential expression (DE) analysis was performed on $CD56^{bright}CD16^{lo}$-CREM cells to identify the marker gene set according to the gene expression profiles of normal sample in paracancerous tissue. Since the expected marker gene expression variation for this data is relatively large, the limma package in the R program was used to calculate the statistical characteristics of each gene in the $CD56^{bright}CD16^{lo}$-CREM subgroup. Then, the differentially expressed genes were identified with avg_log2FC $\geqslant 1$ and corrected P-value < 0.01. Differential gene expression was visualized using the ggplot2 package.

2.4. Pathway enrichment analysis

The packages of clusterProfiler, org.Hs.eg.db, and DOSE from R program were used to perform Gene Ontology (GO) analysis to obtain important gene pathways. The STRING and cytoscape were used for protein-protein interaction (PPI) analysis using the screened 5 genes with the most up-regulation and down-regulation, separately.

3. Results and discussion

3.1. Selection of NK cell subpopulations

There were 13 cell subtypes identified in NK cells using the UMAP visual Louvain algorithm, then annotating them (Figure 1).

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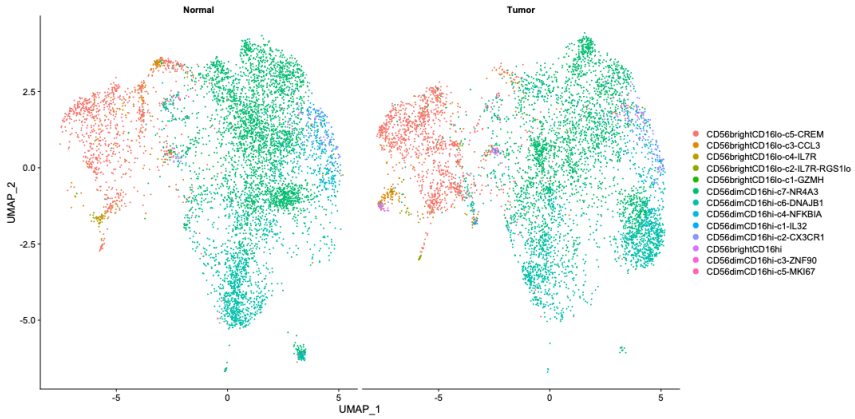

Figure 1. Labeling NK cell subpopulations.

3.2. Secondary dimensionality reduction cluster analysis

Previous data demonstrated that the immune mechanisms of CD56^{bright}CD16^{lo}-CREM in the pan-cancer were still unclear [5]. Therefore, in this paper, the CD56^{bright}CD16^{lo}-CREM NK cell subpopulations was analyzed by secondary dimensionality reduction clustering to obtain another 13 clusters. Due to the lack of relevant references, the author annotated the cell cluster using the highest expressed marker genes, and obtained the sub-clusters and their gene expression profiles (Figure 2).

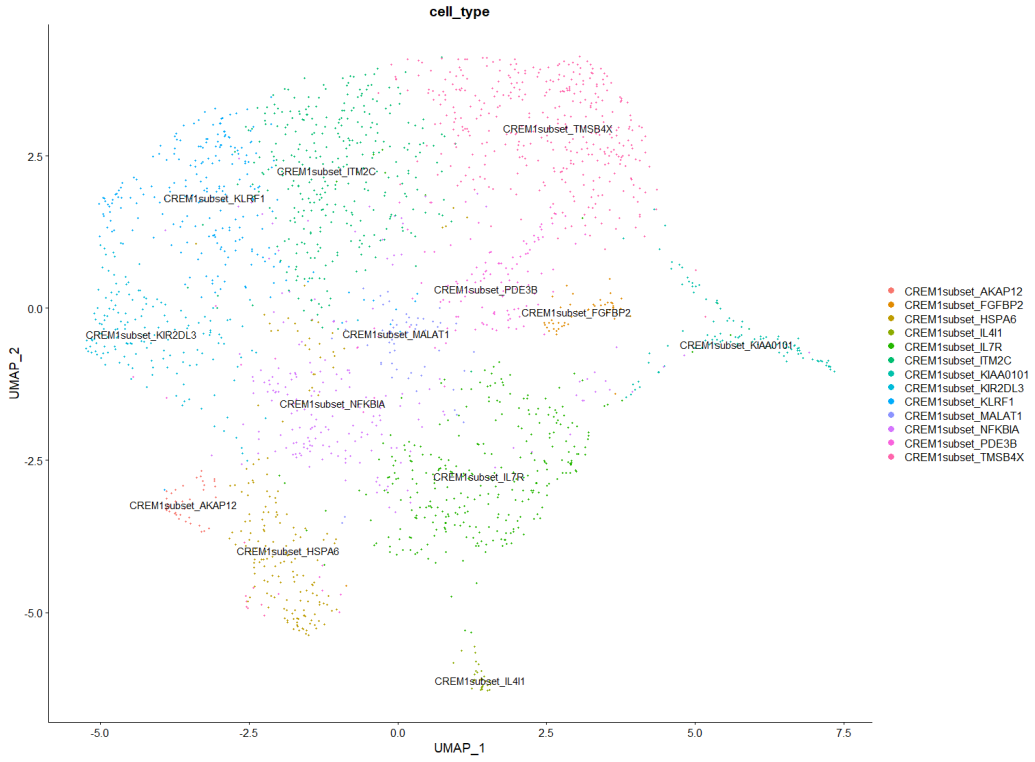

Figure 2. Dimensionality reduction clustering of CD56^{bright}CD16^{lo}-CREM cell subpopulations.

3.3. Differential gene analysis

The advantage of single cells is that they can be assessed for cellular heterogeneity based on the gene expression characteristics of the cell clusters. Compared to controls, 695 differential genes were screened in the NK cell subpopulation of CD56^{bright}CD16^{lo}-CREM in patients (Figure 3).

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Figure 3. The volcano plot of differential expressed genes. The blue dots are differential expressed genes with upregulation in the right and downregulation in the left, and red dots are unimportant genes.

Subsequently, the top five up- and down-regulated genes were selected for subsequent functional enrichment and protein interaction analysis, respectively (Table 1).

Table 1. Differential gene names, Log2FC values, corrected P values and their associated functions.

	Avg_log2FC	P_val_adj	Related functions
HSPA1B.5	4.266189771	5.97E-109	Stress response, protein folding, protein transportation, apoptosis regulation, immune regulation
HSPA6	3.764764094	8.72E-111	Stress response, protein folding, protein transportation, apoptosis regulation, immune regulation
HSPA1A.3	3.621812399	4.58E-97	Stress response, protein folding, protein transportation, apoptosis regulation, immune regulation
CCL4.7	-4.774639113	2.56E-07	Chemotactic activity, immunomodulation, inflammation regulation, tissue reparation
NKG7.6	-4.163386614	1.04E-13	Chemotactic activity, immunomodulation, inflammation regulation, tissue reparation
CCL5.10	-3.930642371	1.61E-07	Chemotactic activity, immunomodulation, inflammation regulation, tissue repair

3.4. Bioenrichment analysis on the CD56^{bright}CD16^{lo}-CREM subpopulation

The author subsequently analyzed the selected differential genes for the same functional nodes and compared their differences to controls. GO analysis serves to extend the simple annotation of individual genes to the group-wise analysis of multiple genes with better interpretation of the obtained data. For example, pericyte subpopulation 2, susceptible to retinal capillary dysfunction, discovered by GO enrichment analysis, thus enhancing the understanding of retinal cellular complexity and heterogeneity and implications for the future therapeutic candidates [6]. Here, the author hypothesized that differential

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genes in CD56^{bright}CD16^{lo}-CREM influenced tumorigenesis by regulating certain immune functions. The GO enrichment analysis showed that this subpopulation of NK cells is involved in biological processes mainly related to monocyte differentiation, regulation of cell-cell adhesion and leukocyte-cell adhesion (Figure 4).

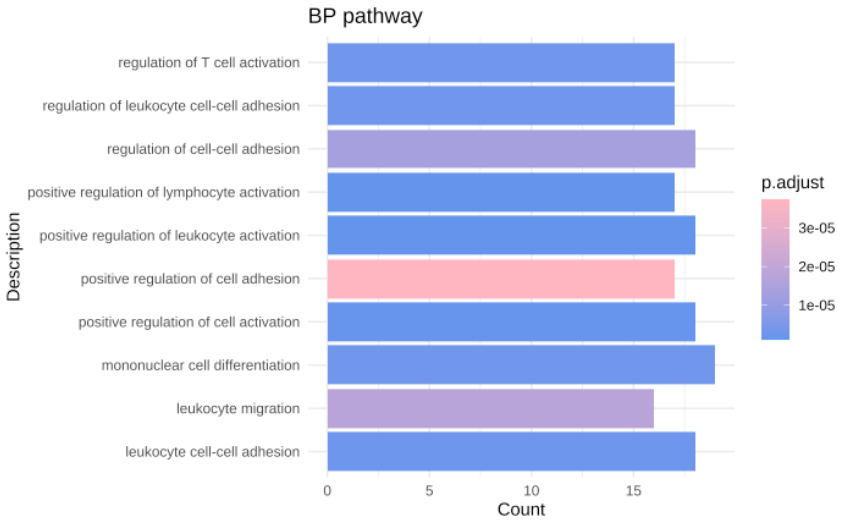

Figure 4. Biological processes of GO analysis.

NK cells can express receptors for several monocyte-derived cytokines, such as IL-1, IL-10, and IL-18, suggesting that the interaction of NK cells with the mono-macrophages in the inflammatory microenvironment of tumors. Studies have also shown that tumor-associated monocytes impair NK cell function by producing cytokines. For instance, the concentration of TGFβ1, a cytokine that inhibits NK cell function, was found to be significantly higher in monocytes of gastric cancer tissues compared to non-tumor tissues, suggesting that monocytes/macrophages may secrete TGFβ1 to damage NK cell functions [7]. Combined with the results of this paper, the release of cytotoxic and perforin and other killing substances in this subsets of NK cells declined due to affecting the differentiation of monocytes, thus inhibiting their tumor-killing function.

CD56bright NK cells exhibit elevated expression levels of functional L-selectin (CD62L) as well as a repertoire of adhesion molecules including CD2, CD44, and intracellular adhesion molecule 1 (ICAM-1 or CD54). Leukocyte adhesion molecules (LAMs) are crucial for tumor cell metastasis through binding to adhesion molecules on the surface of vascular endothelial cells, allowing tumor cells to adhere to vascular endothelial cells and then metastasize to other sites through blood circulation. This suggests that the CD56^{bright} subpopulation of NK cells influences tumor metastasis by regulating intercellular adhesion.

In addition, the cellular components involved in the functional changes of this subpopulation of NK cells were mainly reflected in the enrichment of ribosomal and cytoplasmic ribosomal components (Figure 5). Moreover, the pathways involved in the enriched molecular functions were also shown to be associated with ribosome composition (Figure 6).

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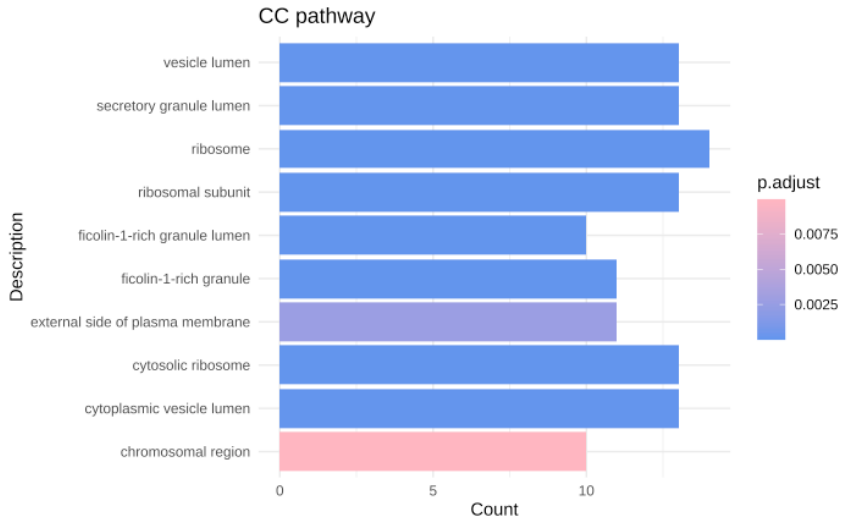

Figure 5. Cellular components (CC) of GO analysis.

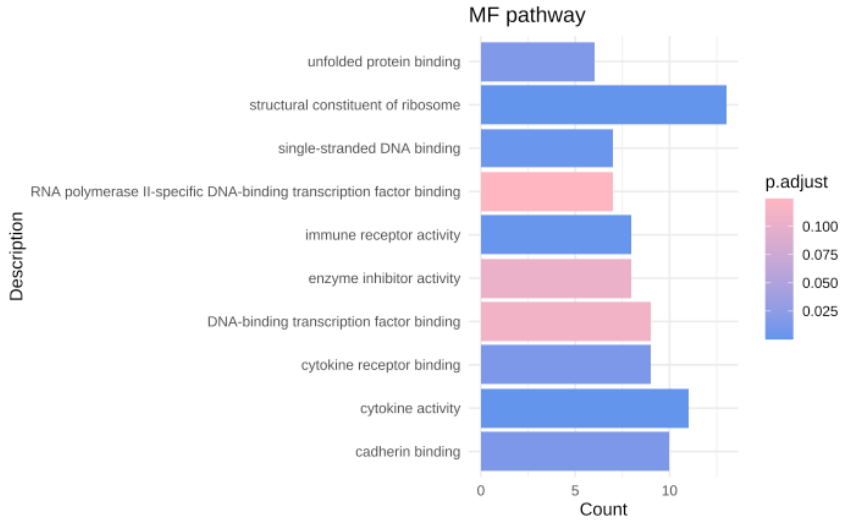

Figure 6. Molecular function (MF) of GO analysis.

Overactive ribosomes are a hallmark of tumor proliferation, which are targets of autophagy-mediated degradation, providing a source of energy for the constant and abnormal proliferation of tumor cells *in vivo* under the situation of nutrient starvation [8]. Similar to previous research data, this subset of NK cells promotes autophagy to supply the energy required for tumor cell proliferation by up-regulating *HSF1* of ribosome biogenesis-related genes [9].

3.5. PPI network of CD56^{bright}CD16^{lo}-CREM in pan-cancer tumors

Next, the authors conducted a PPI network of CD56^{bright}CD16^{lo}-CREM subpopulations using the GeneMANIA online program to investigate possible processes by which CD56^{bright}CD16^{lo}-CREM plays a role in cancer carcinogenesis. As shown in Figure 7, CD56^{bright}CD16^{lo}-CREM showed a significant physical interactions. Combined with the results of differential gene expression analysis (Figure 3, Table 1), it is suggested that this subpopulation of NK cells participates in tumor killing mainly by up-regulating the expression of *HSP* family genes and activating related signaling pathways.

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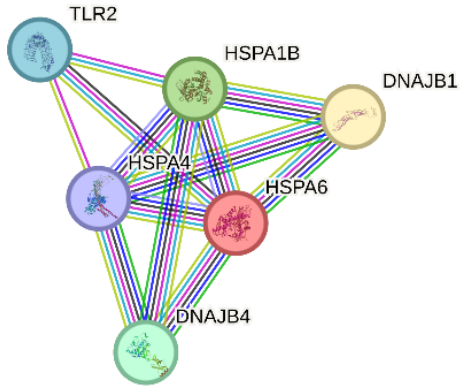

Figure 7. PPI network of the CD56^{bright}CD16^{lo}-CREM.

4. Conclusion

In this paper, the author explored the role of the CD56^{bright}CD16^{lo}-CREM subpopulation in tumors using the single-cell sequencing data of NK cells. The results showed that this population of cells promotes ribosomal autophagy to provide energy for the continuous proliferation of tumors mainly through the upregulation of the *HSF1* gene, and also promotes tumor progression through the expression of cell adhesion molecules, cytokines, and related proteins, etc., which interact with the monocyte macrophages, inhibit their function, and reduce the killing of tumor target cells.

References

[1] Li Yujuan, He Mansen, Li Xiaoming, Wu Xiaoming, Wu Xiaoming, Wu Xiaoming. Chimeric antigen receptor modified human ipsc natural killer cells enhance anti-tumor activity. Cell Stem Cells, 23(2), 181-192. https://doi.org/10.1016/j.stem.2018.06.002

[2] Don, F. , J. , qi, L, Liu, D, Bo, Y. , Qin, era, Miao, Y. K, Hou, W, Lee, J. Peng, J. , Z, Zhu, L, Peng, H, Wang, & Zhang Z(2023). Pancarcinoma single-cell panorama of human natural killer cells. Cell, 186(19), 4235-4251.e20. https://doi.org/10.1016/j.cell.2023.07.034

[3] Liu, E., Marin, D, Banerjee, P., Macapinlac, H. A., Thompson, P., Basar, R., Nassif Kerbauy, L. , Overman, B., Thall, P., Kaplan, M., Nandivada, V., Kaur, I., Nunez Cortes, A., Cao, K., Daher, M., Hosing, C., Cohen, E. N., Kebriaei, P., Mehta, R., Neelapu, S., Rezvani, K.(2020). Application of CAR-transduced natural killer cells in CD19-positive lymphoid tumors New England Journal of Medicine, 382(6), 545-553. https://doi.org/10.1056/NEJMoa1910607

[4] Habif, G Crinier, A Andr é, P Vivier, E. and Narni Mancinelli, E (2019). Targeting natural killer cells in solid tumors. Cellular and Molecular Immunology, 16 (5), 415-422. https://doi.org/10.1038/s41423-019-0224-2

[5] Bagaev, A Kotlov, N Nomie, K Svekolkin, V Gafurov, O Isaeva, O Kozlov, I Frenkel, F Gancharova, O, Almog, N Tsiper, M Ataullakhanov, R. and Fowler, N (2021). Conservative pan cancer microenvironment subtypes predict response to immunotherapy. Cancer Journal, 39 (6), 845-856. https://doi.org/10.1016/j.ccell.2021.04.014

[6] Xia, M., Jiao, L., Wang, X. H., Tong, M., Yao, M. D., Li, X. M., Yao, J., Li, D., Zhao, P. Q., & Yan, B. (2023). Single-cell RNA sequencing reveals a unique pericyte type associated with capillary dysfunction. Theranostics, 13(8), 2515–2530. https://doi.org/10.7150/thno.83532

[7] L.S., j Y. Teng, Y. Zhao, Y L.T. T. T. Lv Yongping, Cheng, Li. Chen, N, Duan, M, Chen, W, Guo, G. Zou, Question: M, Zhuang, Y (2017). Tumor associated monocytes/macrophages transform growth factors- β 1. Impairment of nk cell function in human cancer, Tumor Immunology, 5 (3), 248-256. https://doi.org/10.1158/2326-6066.CIR-16-0152

[8] Smith, S L. Kennedy, P R. Stacey, K B. Worboys, J D. Yarwood, A SEO, S Solloa, E H. Mistreta, B Chatterjee, S Gunarane, P Allette, K Wang, Y C. Smith, M L. Sebra, R Mace, E M. Horowitz, A Thomson, W Martin, P Eyre, S. and Davis, D M. (2020). Identification of diversity of peripheral blood NK cells through single-cell RNA sequencing. The progression of blood, 4 (7), 1388-1406. https://doi.org/10.1182/bloodadvances.2019000699

[9] Pelletier, J., Thomas, G., & Volarević, S. (2018). Ribosome biogenesis in cancer: new rules and therapeutic pathways. Nature Review. cancer, 18(1), 51–63. https://doi.org/10.1038/nrc.2017.104