Journal of Surgery
Yu B, et al. J Surg 9: 11057
www.doi.org/10.29011/2575-9760.011057
www.gavinpublishers.com
Research Article

Using Scrna-Seq to Define the Immune Response after Hapten Enhanced Intratumoral Chemotherapy on Cases of Rectal Cancer:Exploring Immune Surgery

Baofa Yu[1-5*], Jian Zhang[2], Feng Gao[1], Peng Jing[1], Peicheng Zhang[1], Guoqin Zheng[1]

[1]TaiMeiBaofa Cancer hospital, Dongping, Shandong Province, China

[2]Jinan Baofa Cancer hospital, Jinan, Shandong Province, China

[3]Beijing Baofa Cancer Hospital, Beijing, China

[4]Immune Oncology Systems, Inc, San Diego, CA, USA

[5]South China Hospital of Shenzhen University, Shenzhen, China

Corresponding author: Baofa Yu, TaiMeiBaofa Cancer hospital, Dongping, Shandong Province, China

Citation: Yu B, Zhang J, Gao F, Jing P, Zhang P, et al. (2024) Using Scrna-Seq to Define the Immune Response after Hapten Enhanced Intratumoral Chemotherapy on Cases of Rectal Cancer:Exploring Immune Surgery. J Surg 9: 11057 DOI: 10.29011/2575-9760.11057

Received Date: 17 May 2024; **Accepted Date:** 23 May 2024; **Published Date:** 25 May 2024

Abstract

Objective: Surgery is an major methods for rectal cancer and is significantly improved thorough not only combine with modern medical equipment also combine with immune therapy. Awaking the immune cells can be effectively eliminated residual tumor cells following the surgical of tumor. Hapten Enhanced Intratumoral Chemotherapy (HEIC) has successful applied into cancer treatment. HEIC integrated into surgery is a new way to improve outcome of surgery while the immune response is elicited during of rectal surgery.

Method: Single-Cell RNA Sequencing (scRNA-seq) was used to investigate changes of immune cells at the molecular and cellular identities in tumors before and after surgery with HEIC.

Result: A total of 43,406 cells, 10 cell types, Epithelial Cells(EpithelialCells), Endothelial Cells(ECs), Fibroblasts, B Cells(BCells), Plasma Cells(PlasmaCells), TandNK Cells(TandNK), Neutrophils, Mast Cells(MastCells), Mononuclear Phagocytes(MPs), Platelets were induced by HEIC treatment 1 hour later. Proportion of plasma cells decreased, especially the proportion of T&NK cells increased after treatment, and the number of Tumor Infiltrating Lymphocytes (TILs) increased. Activate of immune response in tumor almost immediate impact the peripheral blood samples for whole body immune response with a large proportion up of T&NK cells. The gene set was enriched into tumor-related signaling pathways such as apoptosis, IL-7 and TNF. IL-7 is an important growth factor for T cell development and survival.

Conclusion: Immune rectal cancer surgery can be performed by HEIC, it resulted in the tumor is cut out of body, then the immune response is set up and restore in the patient's own body for prevent the tumor metastasis after the removal of tumors.

Keywords: Cancer Immunotherapy; Drug Delivery; Intratumoral Injection; Intracellular Drug Delivery For Surgery; Immune Rectal Cancer Surgery, Immune Rectotomy

Introduction

The turn of the 20th century marked the beginning of the development of cancer surgery techniques, with the first abdominoperineal resection performed in 1908 by Miles, the first lobectomy being performed in 1912 and the first radical hysterectomy performed by Wertheim in 1906, all carried out under oncological criteria [1]. For more than 100 years since the advent of cancer treatment, surgery has played an important role in cancer treatment [2]. Historically, the mantra of surgeons has been that increasingly aggressive surgery will improve cure rates. Despite the development of new technologies we have witnessed over the past 50 years, surgery remains the textbook approach to cancer treatment and remains the dominant approach in oncology, with no signs of change [2]. Surgery is the cornerstone of current rectal cancer treatment, tumor cure and overall survival remain the primary goals, it requires multidisciplinary cooperation, and preoperative staging is crucial in the treatment plan of these patients [3]. Chemotherapy and radiotherapy combined with surgery have been major breakthroughs in the treatment of cancer in the past. Adjuvant chemotherapy and radiotherapy have been used to shrink tumors before surgery [4,5].

Intraoperative Radiation Therapy (IORT) is also used for surgery. Although IORT does not extend the overall survival of patients with gastric and rectal cancer, it shows good local-regional control in patients with specific stages without increasing the risk of complications [5-8]. However, metastasis or retention of tumor cells during surgery is still a clinical problem due to easy surgical compression of tumor residue. Therefore, in many clinical cases, successful surgery does not necessarily guarantee successful treatment. To improve the long term survival rate of surgical treatments and at the same time to prevent the local residual tumor and extrusion residual metastasis caused by surgery, there have been a lot of tools and surgical robots that have improved surgery to perfect and make it more successful [9]. The postoperative inflammatory response in colorectal cancer surgery was observed and it was discussed the proliferation, differentiation and secretion of immune cells, within the intestinal region following remodeling of the microenvironment, also intestinal tract is the largest digestive organ in the human body [10,11]. These studies call into question how we can use the surgery inflammatory response from the surgery to control tumor cell regeneration and tumor recovery, there is a good example for immune reaction induced by hapten enhanced intratumoral chemotherapy during esophagectomy for explore possible immunosurgery [12]. It is desirable to stimulate the body's specific immunity against residual cancer cells to suppress potential tumor metastasis at same time of surgical process. Hapten Enhanced Intratumoral Chemotherapy (HEIC) can elicit immune response to kill tumors by stimulating the release of hapten-modified tumor-associated antigens to facilitate neoantigen presentation[13,14]. HEIC also enhances the functions of CD4, CD8, DC and B cells [15-18]. In this study, we aimed to determine whether Hapten-Enhanced Intratumoral Chemotherapy (HEIC) integrate into surgery for inducing an acute immune response to prevent tumor metastasis and tumor recurrence. The drug is injected into the tumor at site of opening the abdomen and before remove the tumor and the rectal cancer tissue is completely removed 1 hour later after injection. Through comparative analysis of different samples of tumor and blood samples by scRNA-Seq, we comprehensively described the expression characteristics of malignant epithelial cells and immune cells, including bone marrow cells, stromal cells, T cells, plasma cells, B cells, platelets, epithelial cells, as well as the dynamic changes in cell percentage and cell subtype heterogeneity.

Materials and Methods

Ethical Statement

All procedures and protocols in the study has been reviewed and approved by the Ethical Committee of the Beijing Baofa Cancer Hospital (TMBF 0010, 2015). All informed consent forms form patients have been signed prior to the start of the study.

Clinical Specimens

A 83 old year male patient with rectal adenocarcinoma, with a pathological diagnosis with history of hypertension, history of coronary heart disease and he was determined as clinical stage of IIa (T1-2N0Mo) tumor (5cm x 3.2cm x1.5cm) at middle section of rectal, old age is not suitable for surgical treatment. No any other therapy before receiving treatment at Beijing Baofa Cancer Hospital, his physical condition was consistent with surgical correction and met the indications for HEIC. This was approved by the hospital ethics committee (TMBF 0010, 2015) in accordance with relevant guidelines and regulations. After prepared for operation, his skin was sterilized again under general anesthesia and a disinfecting towel is laid. When the tumor tissue was seen, a small piece of tumor tissue (2mm×1mm×1mm) was taken as an untreated sample; immediately perform the intratumoral injection of a total of 10 ml that contained 1.00 mg/ml doxorubicin hydrochloride (Dox), 0.80 mg/ml of cytarabine (Ara-C), 20.0 mg/ml of H_2O_2 and 144 mg/ml of penicillin. 1.5 hours post injection, rectal cancer tumor was totally removed, another small piece (2mm×2mm×3mm) of tumor tissue was taken as the treated samples, both sample send to analysis of scRNA-Seq. There were several lymph nodes with metastasis. A total of eight blood samples from the patient was taken as well as before and 24 hours after injection, one and two weeks after surgery for analyzed by scRNA-Seq.

Scrna-Seq Data Processing and Quality Control: The fresh samples of blood stored in SCelLiVeR tissue preservation solution in GEXSCOPER (Singleron) till molecular testing [19]. Original gene expression matrix data were generated using the CeleScopeR (https://github.com/singleron-RD/CeleScope) software. CeleScopeR is a single-cell data processing software developed by Singleron.quality control and filter the data was carried. [19] Reads were compared with the reference genome GRCh38 with ensemble version 93. Gene annotation were used STAR (version 2.6.1b) [19,20].

Differentially Expressed Genes (Degs) Analysis (Scanpy): Identify differentially expressed genes (DEGs) was studied by using the scanpy.tl.rank_genes_groups function based on the Wilcoxon rank sum test with default parameters and selected the genes expressed in more than 10% of the cells with an average log (Fold Change) value greater than one as DEGs [20].

Cell Type Annotation: Cell type identity in each cluster was determined by the expression of canonical markers found in the DEGs using the SynEcSys database (Singleron Biotechnologies) [20].

Subtyping of Major Cell Types, CNV Detection Based on Scrna-Seq and Pathway Enrichment Analysis: To investigate the potential functions of DEGs between clusters, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes And Genomes (KEGG) analysis with the "ClusterProfiler" R package 3.16.1. [19] Gene Set Variation Analysis (GSVA) pathway enrichment analysis [20].

UCell Gene Set Scoring: Gene set scoring was performed using the R package UCell v 1.1.0 [18]. UCell scores based on the Mann-Whitney U statistic by ranking query genes in the order of their expression levels in individual cells.

Trajectory Analysis: We used the R package monocle (version 2.18.0) [20] to carry out single-cell trajectory analysis, and the dimensionality reduction method was DDRTree [21].

Transcription Factor Regulatory Network Analysis (pySCENIC): The transcription factor network was constructed by pySCENIC (v0.11.0) using the scRNA expression matrix and transcription factors in AnimalTFDB. AUCell [22].

Cell-Cell Interaction Analysis: Cell-cell interactions (CCI) between different cell types were predicted based on known ligand-receptor pairs by Cellphone DB v2.1.0. Predicted interaction pairs with p-value < 0.05 and average log expression > 0.1 were considered significant. Differentially activated ligand-receptor pairs between groups were visualized by dot plot in ggplot2 [20-22].

Result

Clinical Benefit Characteristics

After rectal surgery, result of pathology diagnosis confirmed rectal adenocarcinoma and metastasis was found in lymph nodes, which is not found before surgery. Follow-up examination every four weeks after the treatment for a total of six month period, physical checkup and CT of the patient showed no signs of tumor mass was found after treatment and the patient was in good health living a normal life 3 years now.

Landscape of Single Cell Transcriptome Sequencing Before and After Cancer Surgery

It was found a total 43,406 cells, 10 cell types, Epithelial Cells(EpithelialCells), Endothelial Cells(ECs), Fibroblasts, B Cells(BCells), Plasma Cells(PlasmaCells), TandNK Cells(TandNK), Neutrophils, Mast cells(MastCells), Mononuclear phagocytes(MPs), Platelets. The especially the proportion of T&NK cells increased, and the number of Tumor Infiltrating Lymphocytes (TILs) increased in rectal adenocarcinoma, while the proportion of epithelial cells in tumor decreased and the proportion of plasma cells decreased (Figure 1 a,b). It indicated patient's immune system is being activate and remobilized to attack the tumor (Figure 1 c,d).

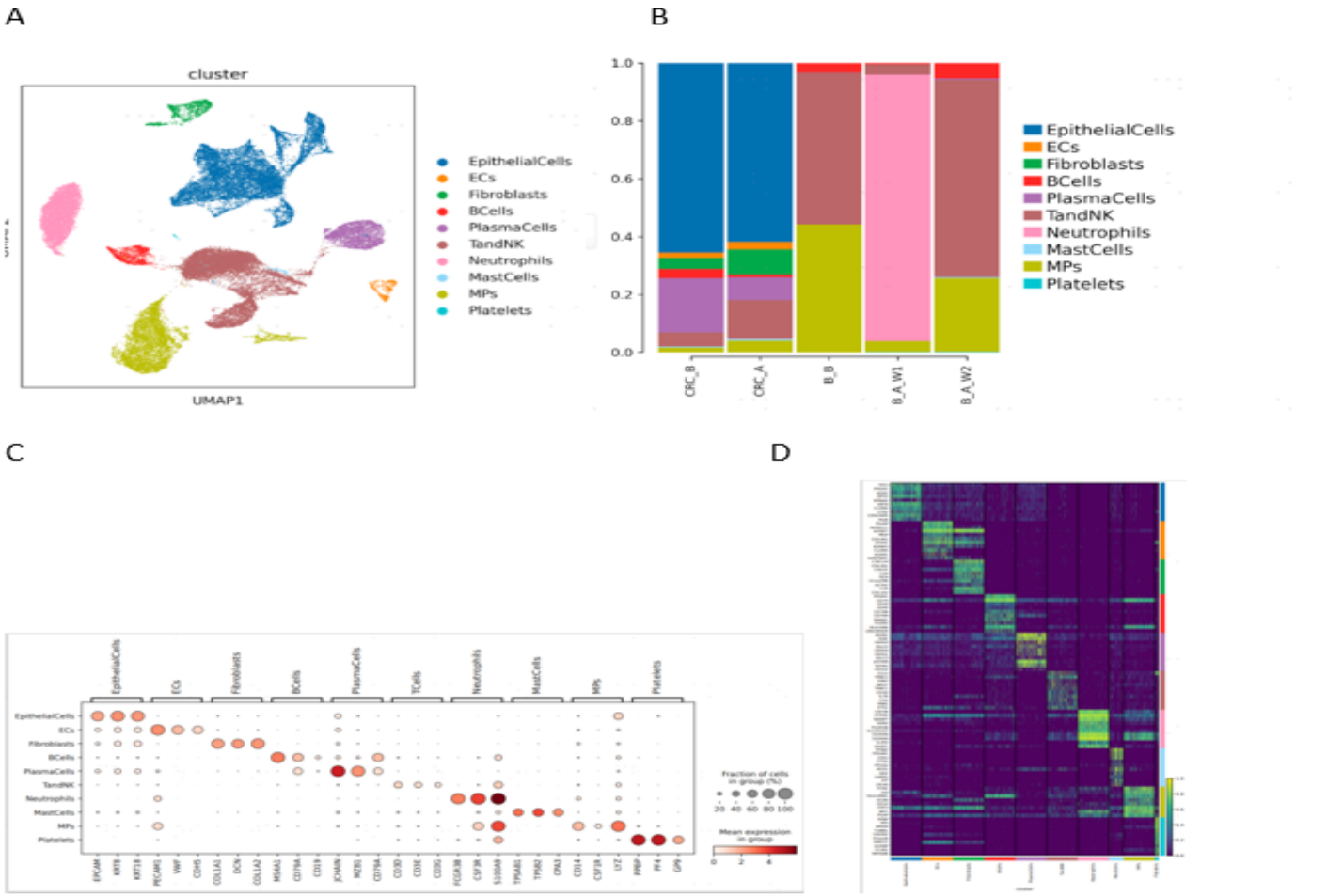


Figure 1: a. Cell type dimension reduction display; b. Histogram of the proportion of cell types from different sample sources; c. marker gene bubble map display of different cell types; d. Heat map display of Top10 differential genes in different cell types

Changes of Epithelial Cells Before and After Treatment

It was found a total of 12,953 EpithelialCells were epithelialcells, and four different subtypes were obtained from epithelialcells including Cancer Cells(CancerCells), Goblet Cells(GobletCells), Tuft Cells(TuftCells), ProgenitorCells (Figure 2a). The proportion of cancer cells and the proportion of Tuft cells in CRC_A group increased (Figure 2b). The inferCNV and CNVscore analysis indicated that epithelial subtype Tuft cells were also malignant cells, indicating a high degree of malignancy in the whole epithelial cells (Figure 2d). Cancer cells were significantly enriched in hypoxia and tumor dryness gene sets, GobletCells were significantly enriched in antigen presentation, and TuftCells were significantly enriched in cell proliferation gene sets. ProgenitorCells were significantly enriched in the interstitial transformation gene set (Figure 2f). The proportion of epithelial at the end of differentiation and development increased (Figure 3a,b). The treatment mainly affected the state3 stage of epithelial differentiation, in which the cell proportion of CRC_A group was significantly reduced (Figure 3c). KEGG enrichment analysis suggested that State3 up-regulated genes in group CRC_A were mainly enriched in allograft rejection, antigen processing and presentation, cell adhesion molecules and other pathways (Figure 3 d).

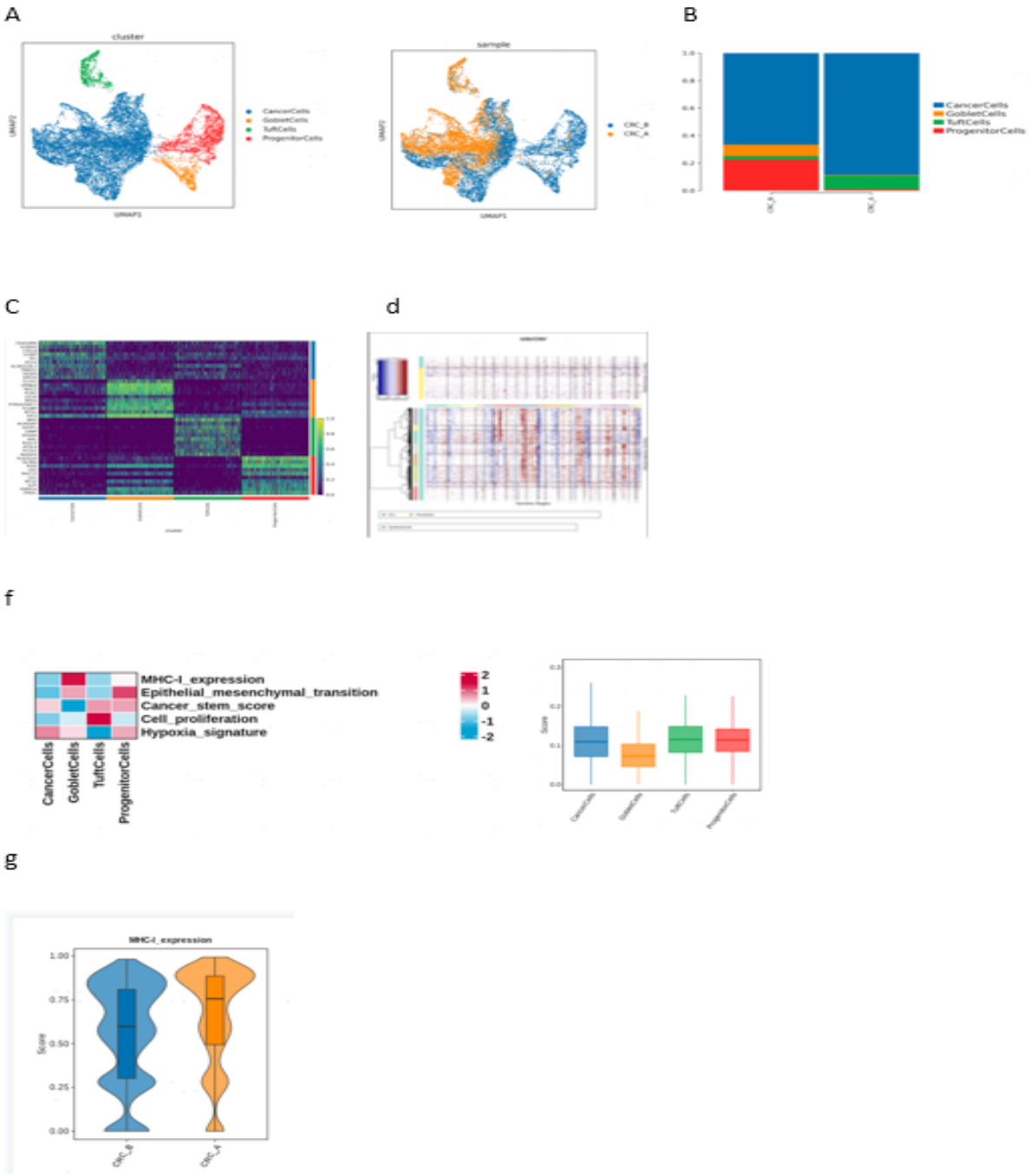


Figure 2: a. epithelial cell subtype vitreogram; b. Histogram of proportion of epithelial cell subtypes; c. Heat maps of marker genes in each cell type; d. Chromosome Copy Number Variation (CNV), where genes are arranged from left to right across the entire chromosome. Chromosome amplification is shown as a red block, while chromosome deletion is shown as a blue block. e.CNV Score box diagram, the horizontal coordinate is different modules, and the vertical coordinate is the quantized CNV score value. The larger the CNV score value, the greater the CNV variation degree of the cell type. f. Gene set scores of each subgroup of epithelial cells; g. MHC-I molecular score of epithelial cells

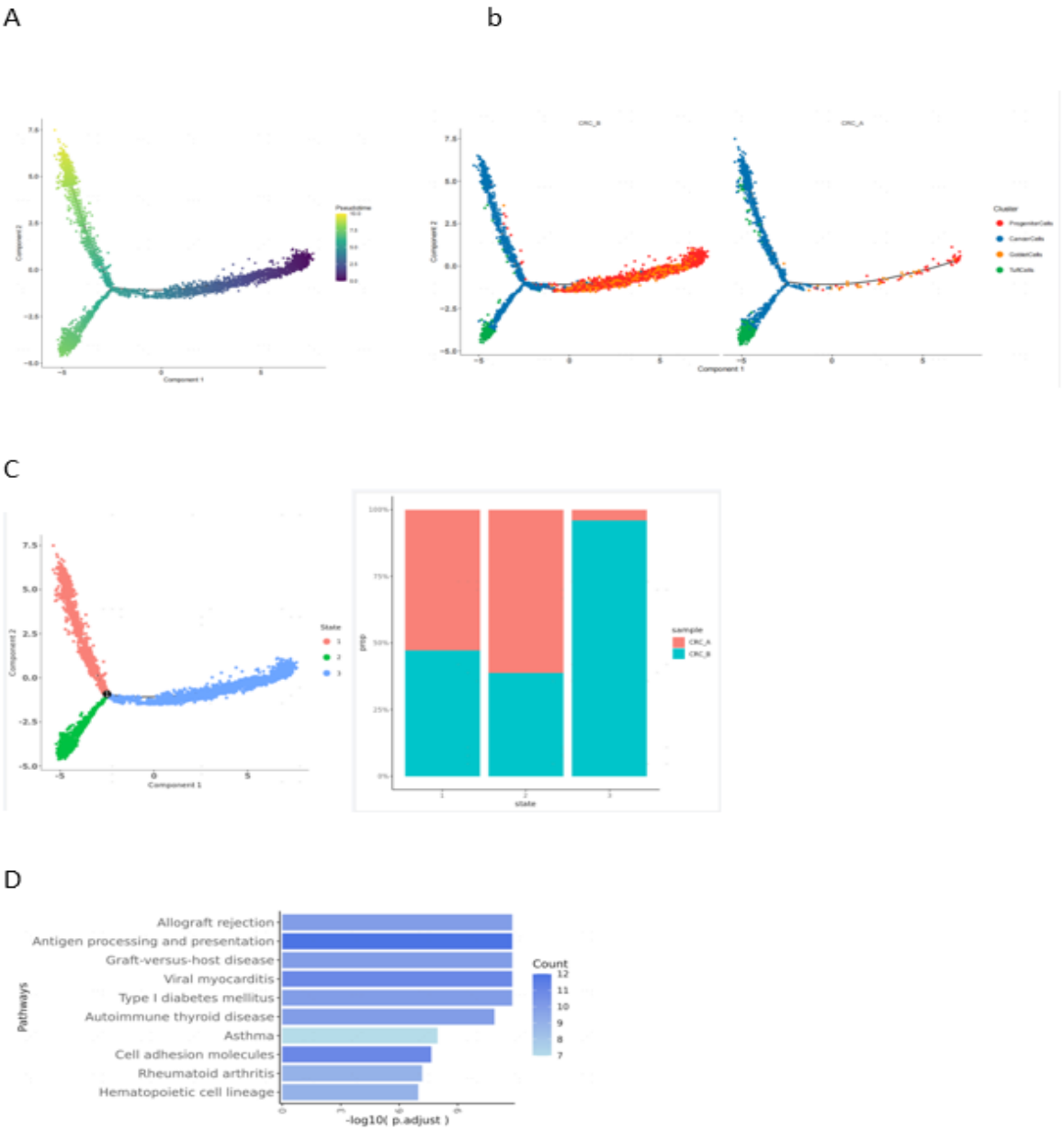
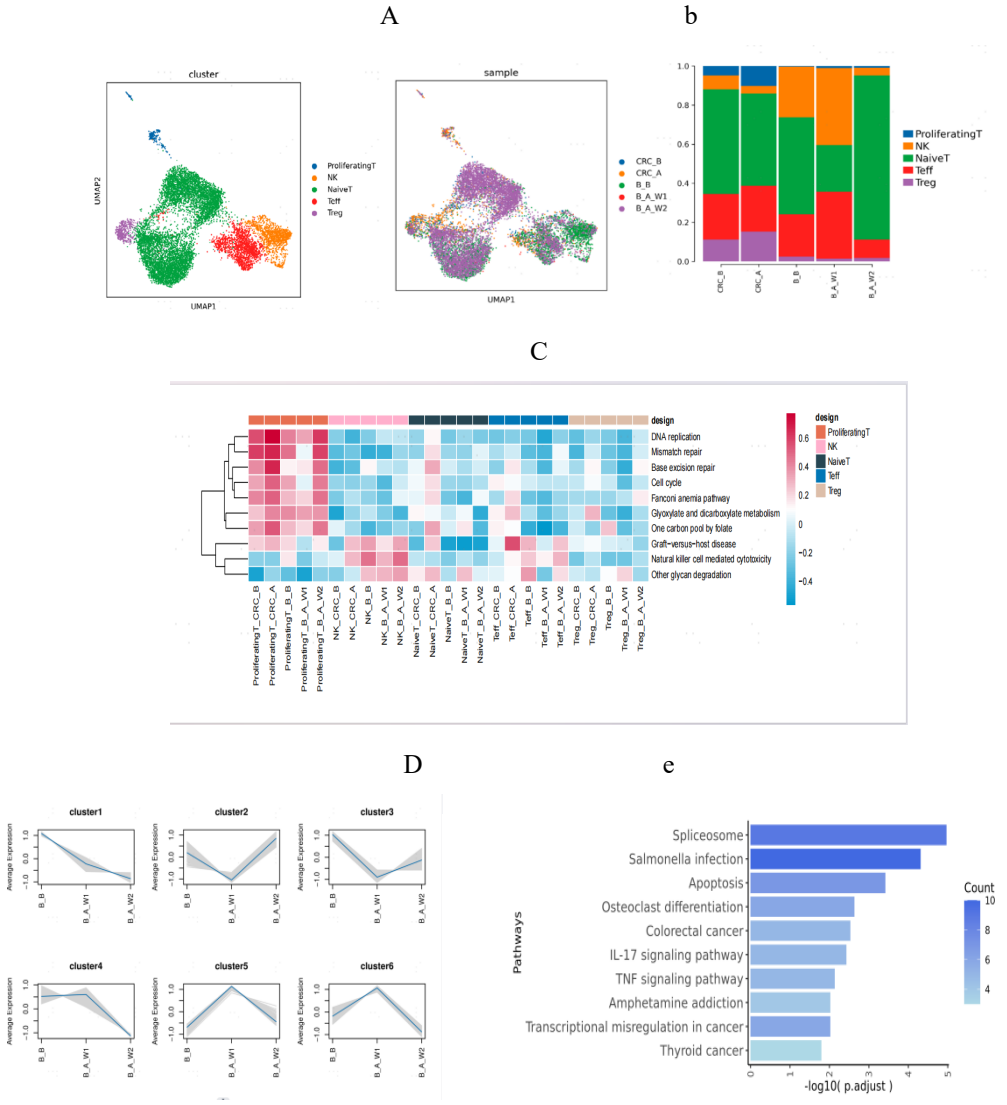


Figure 3: a. Diagram of all cells of epithelial cell subtypes in quasi-sequential order; b. Grouping display of subtypes of epithelium by pseudo time series analysis -monocle2; C. Changes in the proportion of each subtype of epithelium in different state stages of pseudo time series before and after treatment; d. Bar chart for KEGG enrichment analysis of state_3 differential gene

Citation: Yu B, Zhang J, Gao F, Jing P, Zhang P, et al. (2024) Using Scrna-Seq to Define the Immune Response after Hapten Enhanced Intratumoral Chemotherapy on Cases of Rectal Cancer:Exploring Immune Surgery. J Surg 9: 11057 DOI: 10.29011/2575-9760.11057

Changes in Immune Cell T&NK Before and After Administration

It was found a total of 12025 T and NK cells were subdivided into 5 different subtypes including ProliferatingT cells(ProliferatingT), Natural killer cells(NK), NaiveT cells(NaiveT), Effector T cells(Teff), Regulatory T cells(Treg) (Figure 4 a,b). Proliferating T pathways in tissue, such as DNA repair, mismatch repair, base excise repair, etc., are up-regulated after treatment, indicating that the drug may promote DNA damage repair of cells, and may promote the proliferation of T cells through this pathway (Figure 4 c). The graft-anti-host pathway was up-regulated in Effector T cells, which related to dicarboxylic acid metabolism is up-regulated in Treg (Figure 4 d,e). In peripheral blood samples, T&NK cells accounted for a large proportion. According to the gene expression pattern cluster analysis (Figure 4 f,g), the gene set of expression pattern 2 was significantly up-regulated after treatment, and gradually decreased in the later stage of treatment. TEFF cytotoxicity scores in tissues and blood samples were significantly improved after treatment. TEFF killed tumor cells by releasing cytotoxic substances such as perforin and cytokines (IFN-γ, TNF-α), and cytotoxic T cells would release effector molecules such as perforin and granase.



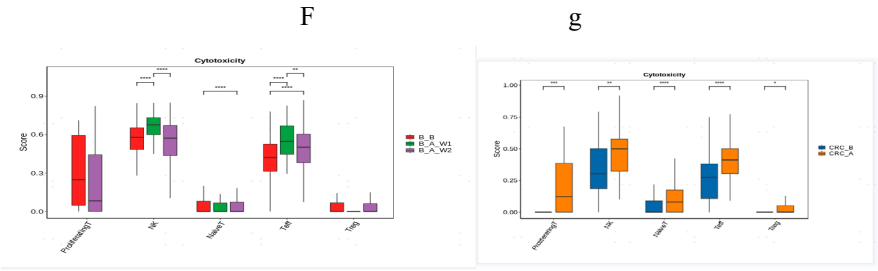


Figure 4: A. TandNK cell subsets subdivision UMAP dimension reduction display; B. TandNK subpopulation cell proportion histogram; C. TandNK cell GSVA enrichment analysis; D. Genes with the same pattern expression (for example, the expression has been down-regulated over time) cluster together, forming 6 patterns; E. GO pathway enrichment analysis was performed on the genes of cluster6 formed by gene expression pattern clustering of T&NK cells; F. Blood cytotoxicity score before and after treatment; G. Cytotoxicity scores of tissue samples before and after treatment

Changes of TCR Before and After Administration

There was no significant change in the frequency of clonotype between the two groups (Figure 5a), but the clonal diversity was significantly enhanced after treatment (Figure 5b). After treatment, the types of effector T cells with a large number of clones were significantly up-regulated (Figure 5c). Different T cell subtypes have a certain degree of clonal expansion, but cytotoxic-related effector T cells have more obvious clonal expansion than other subgroups, and the top 5 clonal expansion are mainly distributed in effector T cells (Figure 5d).

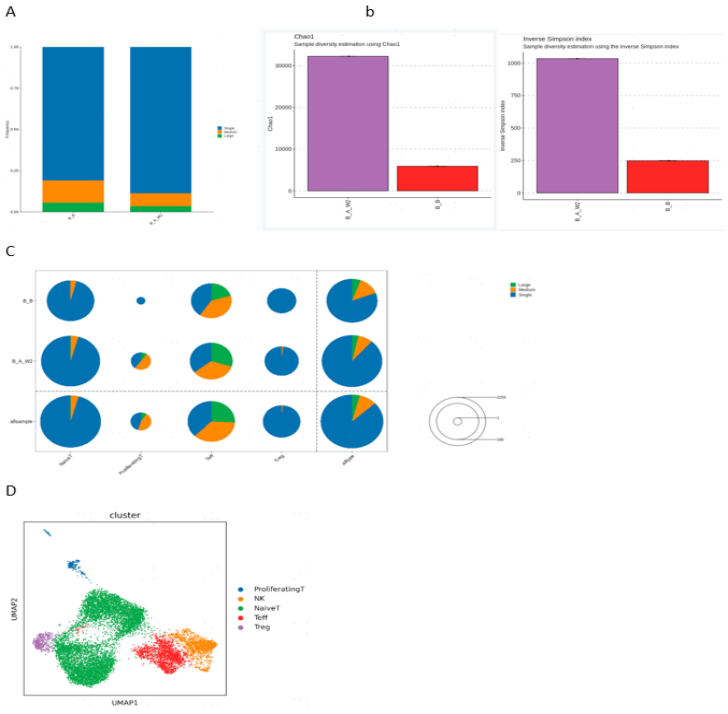


Figure 5: a. Display the proportion according to the frequency of clone type; b. Display of clonal diversity in the two groups; C. Show the frequency and proportion of grouped clone types with different frequency of clone types, and the circle size represents the total number of clone types; d. Distribution of top5 clones on the umap and whether the cells have immune receptors on the umap

Changes in Immune B Cells Before and After Administration

It was found a total of 1245 BCells with three subtypes, NaiveB cells(NaiveB), Bmem_1, and Bmem_2 (Figure 6 a,b,c). Bmem_1 is mainly present in tumor. GO enrichment analysis showed that this subgroup is highly expressed in protein folding, ubiquitin-like protein ligase binding, heat shock protein binding and other related pathways, which are related to the normal function and stress response of B cells. Immunotherapy may play a role by enhancing anti-tumor immune response, including promoting the activation and proliferation of B cells (Figure 6 d,e).

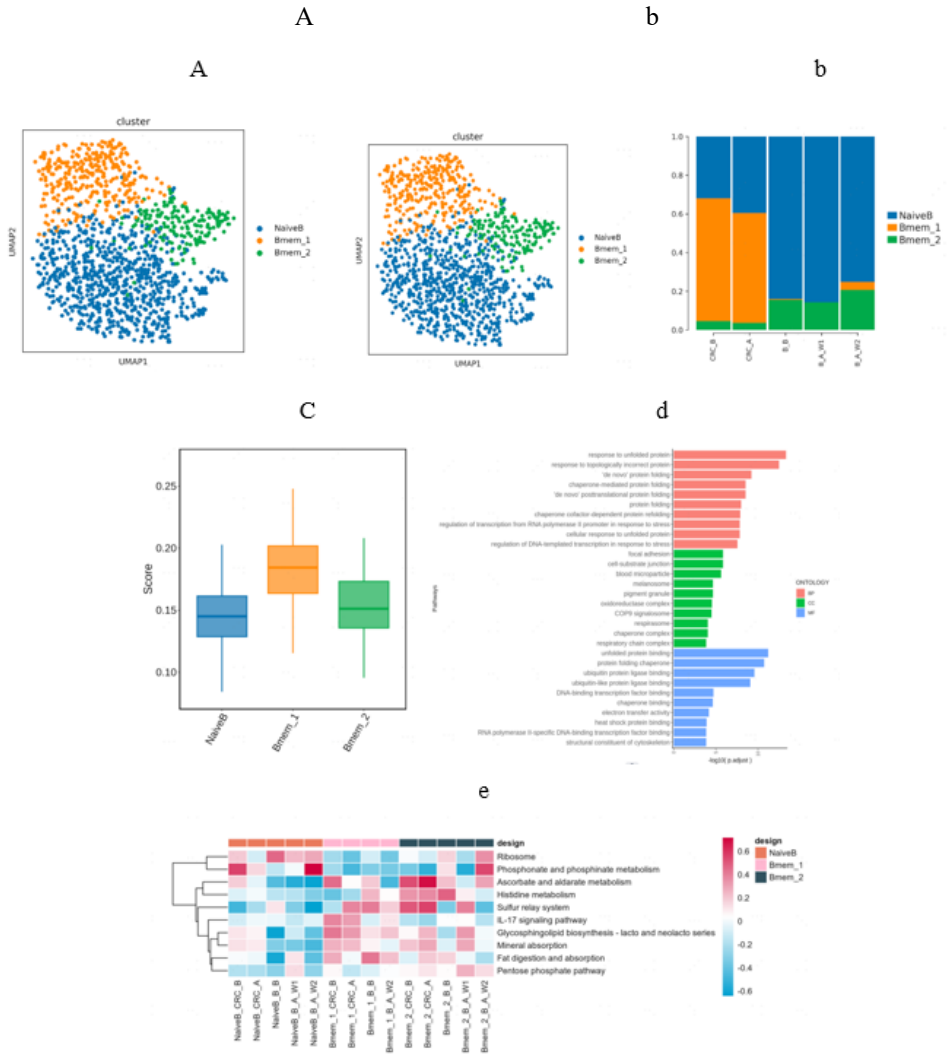


Figure 6: a. B cell subgroup subdivision UMAP dimension reduction display; b. Histogram of cell proportion of B cell subsets; c. NFKB signal channel scoring box diagram; d. GO enrichment analysis of Bmem_1 subgroup; e. Analysis of GSVA enrichment in each subgroup of e. B cells before and after treatment.

Changes of BCR Before and After Administration

The proportion of B cell clonotype frequency greater than 1 was significantly increased (Figure 7a), with richer clonal expansion and increased clonal diversity after treatment (Figure 7b), indicating strong activation of B cell activity and humoral immune response after treatment. Compared with sample before treatment, Bmem2 cells had a higher proportion of clones and a higher proportion of

intercluster clones (Figure7 c,d), and the clonotype was much higher than before treatment.

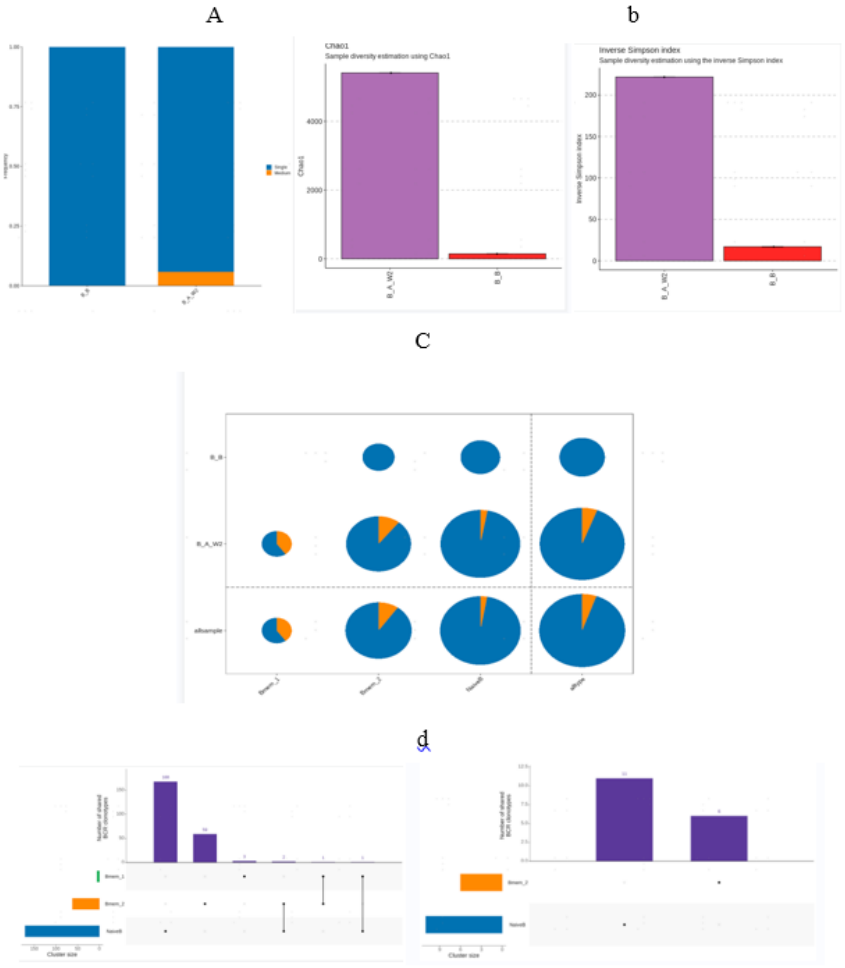


Figure 7: a. Display the proportion according to the frequency of clone type; b. Display of clonal diversity in the two groups; C. Show the frequency and proportion of grouped clone types with different frequency of clone types, and the circle size represents the total number of clone types; d. Show the clonotype data of each cell type and the intersection of clonotypes between different cell types, the top is after treatment, and the bottom is before treatment. The clonotype after treatment is much higher than before treatment, and there are certain common clones of different subgroups

Changes in Plasma Cells Before and After Administration

It was found a total of 2955 PlasmaCells with 6 different subtypes includes PlasmaCells_1, PlasmaCells_2, PlasmaCells_3, PlasmaCells_4, PlasmaCells_5, and PlasmaCells_6 (Figure 8 a,b).

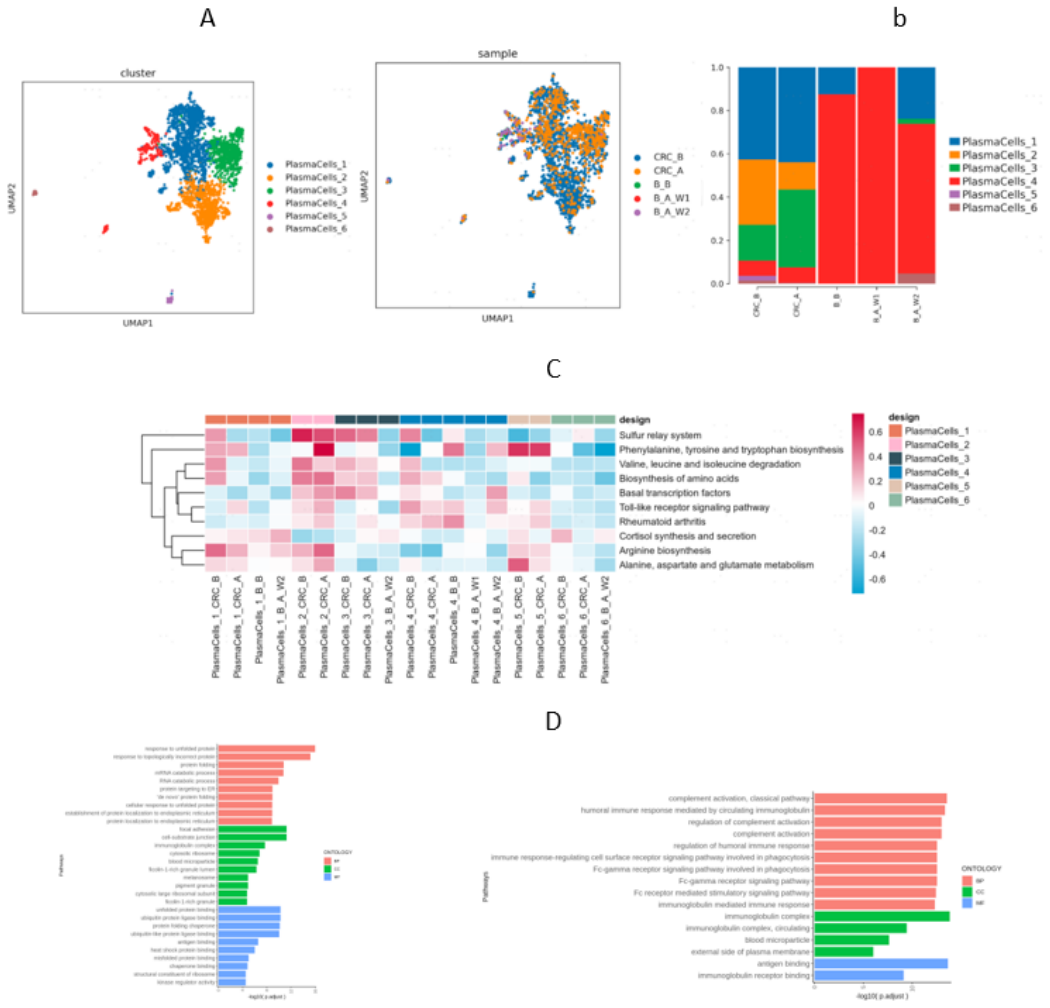


Figure 8: a. Plasma cell subpopulation subdivision UMAP dimension reduction display; b. Histogram of cell proportion of plasma cell subsets; c. GSVA enrichment analysis of plasma cell subgroups before and after treatment; d. PlasmaCells_2 and PlasmaCells_3 GO enrichment analysis

Changes of Mps Cells Before and After Treatment

It was found a total 6030 MPs cells with 6 different subtypes by subdivision annotation (Figure 9 a/b). Including Macrophages, Classical Monocytes(ClassicalMono), Non-Classical Monocytes(NonClassicalMono), Mature Dendritic Cells(MatureDCs), Conventional Type 2 Dendritic Cells(cDC2), Plasmacytoid Dendritic Cells(pDCs). MPs showed significant changes in the proportion of major categories (Figure 9 a/b). Therefore, cluster analysis of the expression pattern of MPs was conducted, and it was found that cluster4 was significantly down-regulated after treatment (Figure 9 c,d), and gradually up-regulated over time. Enrichment analysis of this gene set showed that it is related to SRP-dependent cotranslation protein, MHC functional complex, MHCll receptor activity, etc. cluster5 showed activation of T cell activation, myeloid cell differentiation, positive regulation of cytokine production and other related pathways (Figure 9 e), all of above is indicating that the body may be undergoing acute inflammatory response or tissue repair process, and promoting local and systemic immune response, affecting the functional state and proliferation and differentiation of other immune cells of T cells and B cells.

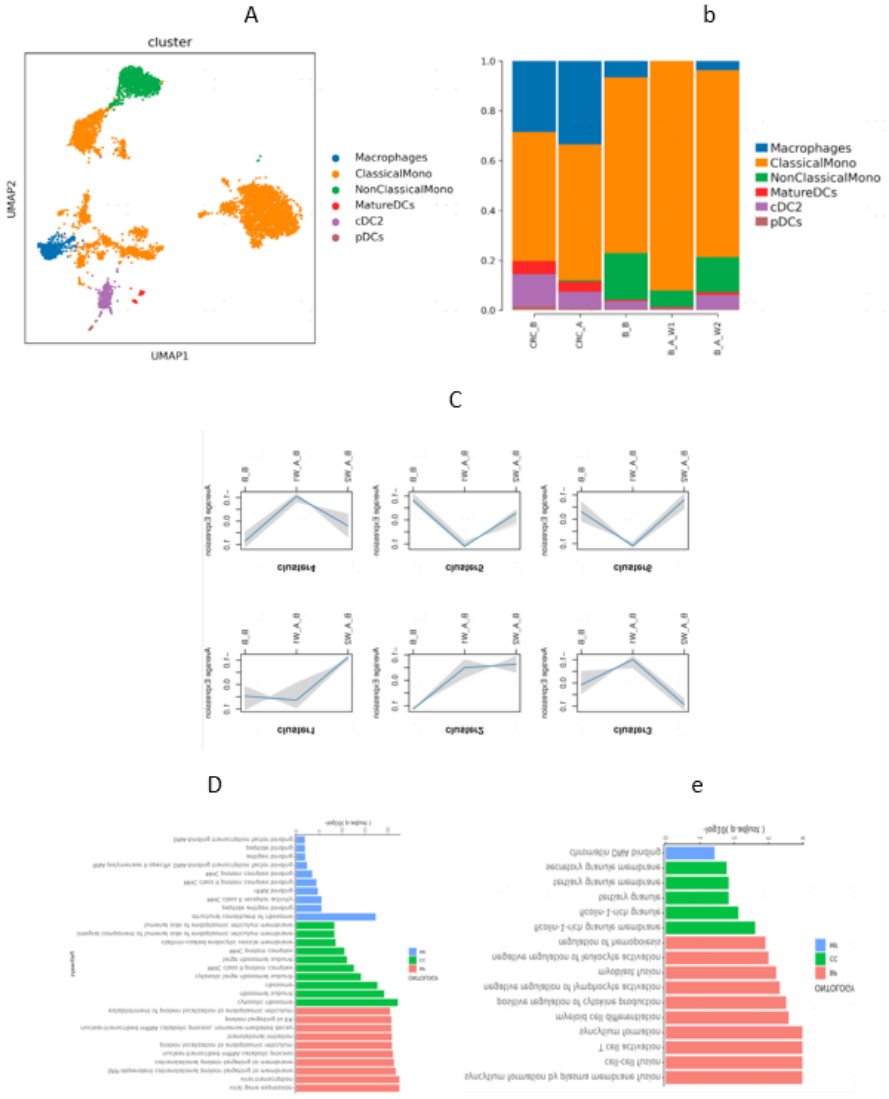


Figure 9: a. MPs subgroup subdivision UMAP dimension reduction display; b. Histogram of MPs cell proportion; c. Genes with the same pattern expression in MPs cells were grouped together to form 6 patterns; d. GO pathway enrichment analysis was performed on the genes of cluster4 formed by the clustering of gene expression patterns in MPs cells; e. Enrichment analysis of GO pathway for genes of cluster5 formed by clustering of gene expression patterns in MPs cells

Discussion

The surgical of rectal cancer has been around for more than 100 years [1]. Surgery no longer brings optimistic results for advanced stages of rectal cancer. This is most likely due to cancer cell metastasis after rectal cancer surgery. Current study suggests that HEIC in process of rectal cancer surgery is a promising strategy for preventing cancer metastasis and improving treatment outcomes. The inflammatory in cancer with the proliferation, differentiation and secretion of immune cells were observed within the intestinal region, and mucosal membranes of intestinal is a large immune organs [10,11]. How to use intraoperatively induced inflammatory responses to control tumor cells left over surgery is very important, HEIC has been successfully integrated into esophagectomy and has shown to effectively immune responses following surgery [12]. In this study, it was found a lot of 43,406 cells, 10 cell types, including

Epithelial Cells(EpithelialCells), Endothelial Cells(ECs), Fibroblasts, B Cells(BCells), Plasma Cells(PlasmaCells), TandNK Cells(TandNK), Neutrophils, Mast Cells(MastCells), Mononuclear Phagocytes(MPs), Platelets at HEIC treatment 1 hour later after injection. The proportion of T&NK cells increased and the number of tumor infiltrating lymphocytes (TILs) increased after treatment. The increase of lymphocytes in tumor and blood samples indicates the treatment may up-regulate the immune response of the body, and the patient's immune system is being activating and re-mobilizing to attack the tumor. The MHC-I molecular immune score of epithelial cells was significantly improved after treatment [19].Under normal circumstances, epithelial cells would also express appropriate MHC-I molecules, which is used to present endogenous antigens to CD8+ T cells (i.e., cytotoxic T cells) to check for the presence of viral infection or other abnormal proteins. The significant increase in the expression of MHC-I class molecules may be due to the interaction of tumor antigens and the need to enhance the immune system's recognition and clearance of these abnormal cells [23].

Immune response in tumor almost immediate impact the peripheral blood samples, T&NK cells accounted for a large proportion up. GO pathway enrichment analysis showed the gene set enriched into tumor-related signaling pathways such as apoptosis, IL-7 and TNF. IL-7 is an important growth factor for T cell development and survival. TNF has the dual function of inducing apoptosis in certain cell types (including possibly T cells) [22] and inhibiting apoptosis by activating the expression of anti-apoptotic genes such as Bcl-2 via the NF-κB pathway. TEFF cytotoxicity scores in both of tumor tissues and blood samples were significantly improved after treatment, it indicated the tumor is cut out of body, then the immune response is set up in the body. TEFF killed tumor cells by releasing cytotoxic substances such as perforin and cytokines (IFN-γ, TNF-α), and cytotoxic T cells would release effector such as perforin and granase. Perforin forms holes in the target cell membrane, resulting in ion imbalance and changes in intracellular osmotic pressure, while granulozyme enters the target cell and induces programmed cell death [23]. In this study, it showed the significant change in the frequency of clonotype between the two groups, the clonal diversity was significantly enhanced and indicated that the immune pool capacity of T cells including effector T cells was increased, it indicates the ability of effector T cells to recognize tumors was enhanced after drug administration, and patients may lack effective cloning and expansion of effector T cells before treatment [23].

After being stimulated by antigen, it is rapidly activated, proliferated, migrated to the site of inflammation, and releases immunoactive substances for immune response. These results suggest that the increased proportion and activity of effector T cells, as well as the efficient clonal expansion, may be the reason why immunotherapy can target tumors more effectively. In this study, it showed that a total of 1245 BCells were subdivided into three subtypes, NaiveB cells(NaiveB) in tissues. GO enrichment analysis shows that this subgroup is highly expressed in protein folding, ubiquitin-like protein ligase binding, heat shock protein binding and other related pathways, which are related to the normal function and stress response of B cells. Moreover, the existence of certain shared clones in different subpopulations indicated that effector B cells experienced dynamic state changes [22].The increased proportion of B cell clones suggests that B cell-mediated humoral immunity is effectively mobilized during treatment, contributing to the formation of an effective defense mechanism against tumor cells, suggesting that the treatment of HEIC may be inducing a beneficial immune response and may be associated with better treatment outcomes. Study has showed that increasing of autoantibodies of Tumor Associate Antigens (TAA) like P53, P16 after HEIC has confirmed B cell involved the activity of immune response [17]. In this study, it was found a 6030 MPs cells with 6 different subtypes (fig4a/b) and an significant changes in the proportion of major categories. It showed that cluster4 was significantly down-regulated after treatment and gradually up-regulated over time. Thus enhancing the coordinated response of the whole immune system [12, 23]. HEIC treatment also strengthened the interaction between Fibroblasts, B cells(BCells), Plasma cells(PlasmaCells), TandNK cells(TandNK), Neutrophils, Mast cells(MastCells), Mononuclear phagocytes(MPs) through in the expression of MHC-I class molecules. Studies have shown that these genes have a negative impact on the prognosis of patients in the tumor microenvironment, [24] while CEACAM5 the interaction strength with CEACAM6 is up-regulated. According to reports, CEACM can transmit signals that produce multiple effects, including the activation of neutrophils and lymphocytes [25]. In summary, our present study has demonstrated that the HEIC treatment induces the interaction among TandNK cells, MPs, and BCells, DC, Mast cells (MastCells) and (PlasmaCells), and immediate promotes the expression of a large number of genes contributing to the upregulation of immune response during rectal cancer surgery. Our study provides evidence that hapten-mediated intratumoral chemotherapy can induce a systemic immune response to cancer by initiating an immune response within the tumor during the process of cancer surgery, thus achieving an desirable clinical results.

References

1. Arruebo M, Vilaboa N, Berta Sáez-Gutierrez B (2011) Assessment of the evolution of cancer treatment therapies. Cancers (Basel) 3: 3279-3330.
2. Wyld L, Audisio RA, Poston GJ (2015) The evolution of cancer surgery and future perspectives. Nature Reviews Clinical Oncology 12: 115-124.

3. McCourt M, Armitage J, Monson JRT (2009) Rectal cancer. Surgeon 7: 162-169.

4. Mo DC, Liang Z, Chen L (2022) Efficacy and safety of adjuvant therapy with PD-1/PD-L1 inhibitors in cancer. Exp Ther Med 24: 749.

5. Al Qaraghuli MM, Palliyil S, Broadbent G, Cullen DC, Charlton KA et al., (2015) Defining the complementarities between antibodies and haptens to refine our understanding and aid the prediction of a successful binding interaction. BMC Biotechnology 15: 99.

6. Coccolini F, Nardi M, Montori G (2018) Neoadjuvant chemotherapy in advanced gastric and esophago-gastric cancer. Meta-analysis of randomized trials. Int J Surg 51: 120-127.

7. Gao P (2017) Intraoperative radiotherapy in gastric and rectal cancer cancer: meta-analysis of long-term outcomes and complications. Minerva Med 108: 74-83.

8. Wang Q, Lang J, Li T (2020) Postoperative adjuvant chemotherapy versus chemoradiotherapy for node-positive rectal cancer squamous cell carcinoma: a propensity score-matched analysis. Radiat Oncol 15: 119.

9. Marcos-Pablos S, José García-Peñalvo F (2022) More than surgical tools: a systematic review of robots as didactic tools for the education of professionals in health sciences. Adv Health Sci Educ Theory Pract 27: 1139-1176.

10. Kampman SL, Smalbroek BP, L M Dijksman LM (2023) Postoperative inflammatory response in colorectal cancer surgery: a meta-analysis. Int J Colorectal Dis 38: 233.

11. Zhou B, Yuan Y, Zhang S, (2020) Intestinal Flora and Disease Mutually Shape the Regional Immune System in the Intestinal Tract. Front Immunol 11: 575.

12. Yu BF, Zhang J, Gao Z (2023) Immune Reaction Induced with Hapten Enhanced Intratumoral Chemotherapy during Esophagectomy: Explore Possible Immunosurgery. Journal of Cancer Science and Clinical Therapeutics 2023: 259-270.

13. Adair K, Meng X, and Naisbitt DJ (2021) Drug hapten-specific T-cell activation: Current status and unanswered questions. PROTEOMICS 21: 2000267.

14. Yu B, Lu Y, Gao F (2015) Hapten-enhanced therapeutic effect in advanced stages of lung ancer by ultra-minimum incision personalized intratumoral chemoimmunotherapy therapy. Lung Cancer (Auckl) 6: 1-11.

15. Yu B, Fu Q, Han Y, Zhang J, Chen D (2022) An Acute Inflammation with Special Expression of CD11 & CD4 Produces Abscopal Effect by Intratumoral Injection Chemotherapy Drug with Hapten in Animal Model. J Immunological Sci 6: 1-9.

16. Yu B, Fu Q (2020) Drug Mixed by H2O2 Injection Intratumoral to turning an Extracellular Matrix into Autologous Coagulum as Drug Depot. Novel Research in Sciences 4.

17. Yu B, Han Y, Fu Q (2023) Awaken Immune Cells by Hapten Enhanced Intratumoral Chemotherapy with Penicillin Prolong Pancreatic Cancer Survival. Journal of Cancer 14: 1282-1292.

18. Yu B, Fu Q, Han Y, Zhang J, Chen D (2022) An Acute Inflammation with Special Expression of CD11 & CD4 Produces Abscopal Effect by Intratumoral Injection Chemotherapy Drug with Hapten in Animal Model. J Immunological Sci 6: 1-9.

19. Kechin A, Boyarskikh U, Kel A (2017) cutPrimers: A New Tool for Accurate Cutting of Primers from Reads of Targeted Next Generation Sequencing. J Comput Biol 24: 1138-1143.

20. Yu B, Gao F, Jing P (2023) Cancer Immunotherapy Preparation and Immune Cells Activation through Hapten-Enhanced Chemotherapy in Primary Lung Cancer. J Basic Clin Pharma 14: 10-16.

21. Dobin A, Davis CA, Schlesinger F (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29: 15-21.

22. Guo W, Zhang C, Xia Wang X (2022) Resolving the difference between left-sided and right-sided colorectal cancer by single-cell sequencing. JCI Insight 7: e152616.

23. Wang S, Qu Y, Xia P (2020) Transdifferentiation of tumor infiltrating innate lymphoid cells during progression of colorectal cancer. Cell Res 30: 610-622.

24. Li E, Yang X, Du Y, (2021) CXCL8 Associated Dendritic Cell Activation Marker Expression and Recruitment as Indicators of Favorable Outcomes in Colorectal Cancer. Front Immunol 12: 667177.

25. Schrand B, Clark E, Levay A (2018) Hapten-mediated recruitment of polyclonal antibodies to tumors engenders antitumor immunity. Nature Communications 9: 3348.