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Use scRNA-Seq Profile to Define Immunologic Response after Intra-tumor Hapen Enhanced Chemotherapy on a Case of Intracranial Anaplastic Hemangiopericytoma Liver Metastasis: A Case Report

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Abstract

Purpose: AHPC (Anaplastic Intracranial Hemangiopericytoma) is a rare and malignant subset of solitary fibrous tumor/hemangiopericytoma (SFT/HPC) and foreshadow poor prognosis with a higher rate of recurrence/metastasis, it not is no effective treatment. **Methods**: We performed a novel immunotherapy, namely ultra-minimum incision personalized intra-tumor chemo-immunotherapy (UMIPIC) for treating liver metastasis of AHPC. **Findings**: We explored and compared the changes in myeloid cells, stromal cells, B cells, T cells, plasma cells, platelets, erythrocytes and epithelial cells in tumor tissues by scRNA-seq before and after UMIPIC treatments. **Implications**: Our results indicated that UMIPIC treatment can kill tumor cells and wakes immune cells in preparation of immunotherapy and aid cancer immunotherapy by PD-1 or PD-L1 to boost immune response in entire body. This study has provided a detailed understanding of killing tumor cells as well as waking immune cells for fighting cancer cells at the molecular level. This may accelerate the development of a reliable hapten enhanced sensitization immunotherapy for treatment in SFT/HPC liver metastasis patients.

Keywords: Immunotherapy; PD1 (Programmed Death-1) blockers; scRNA-seq; MHC signaling pathway-related genes, local chemotherapy of intracranial anaplastic hemangiopericytoma (AHPC)

Introduction

Anaplastic Intracranial Hemangiopericytoma (AHPC) is a rare malignant subset of solitary fibrous tumor/hemangiopericytoma (SFT/HPC) and has poor prognosis with higher rate of recurrence/metastasis compared with SFT/HPC. [1] Multimodal treatment include gross total resection, radiosurgery, and/or chemotherapy can provide the best possible outcomes for this rare but locally aggressive tumor. Despite local recurrence occurring frequently, single surgery or stereotactic radiation surgery can sometimes provide local control. In addition, metastasis such as liver metastasis is common, and the prognosis is poor due to weak or none chemosensitivity. [2] Although local treatment and other new treatment options may be able to bring tumor under control, [3] targeted therapy, such as pazopanib, emerges as a promising option. Immunotherapy is also a novel treatment strategy for multifocal tumor of hepatocellular carcinoma (HCC), i.e., Programmed Death-1 (PD-1) or Programmed Death-Ligand 1 (PD-L1) blockers, have shown also some limited success. [2, 4]

There is very little research on how to test and validate some of the new options PD-1/PD-L1 blockers for treating liver metastasis of SFT/HPC. UMIPIC (Ultra-minimum incision personalized intra-tumor chemoimmunotherapy) may provide a novel and effective option. [5] Recent progress of scRNA-seq technology (single-cell RNA sequencing) allows scientist to explore the genetic and functional heterogeneity of cellular complexes at single cell level. [6] scRNA-seq provides a suitable alternative m directly compares same cell type can before and after treatment. It can discover changes induced by UMIPIC. However, the current scRNA-seq studies of HCC mostly focus on the tumor microenvironment (TME). [7] There is still needs to investigate immune reaction differences at the single-cell level for SFT/HPC liver metastasis before and after hapten-enhanced UMIPIC.

In this study, we used scRNA-seq technology to compare changes in stroma such as myeloid cells, T and B lymphocytes, plasma cells, platelets, erythrocytes and epithelial cells in tumors before and after UMIPIC treatment. It showed that the immune cells has been awakened and enhanced cancer PD-1 or PD-L1 immunotherapy and to achieved some degree of immune response. [8] This study provides clues to understand of waking immune cell at the molecular base. These findings may accelerate development of a reliable hapten enhanced immunotherapy for treatment liver metastasis of SFT/HPC patients.

Materials and Methods

The patient received UMIPIC treatment at Taimei Baofa Cancer Hospital. Prior to treatment, patient had confirmed clinical diagnosis and met the criteria for local chemotherapy of UMIPIC. Informed consent was signed by patient. The project was approved by the hospital ethics committee of Taimei Baofa Cancer Hospital (TMBF 0010, 2015). Treatment procedures and study protocol were performed in accordance to relevant guidelines and regulations. [5, 9]

Clinical Parameters and Sample Collection

A 31 years male with history of brain tumor and received surgical treatment in 2015 was admitted to our hospital for 9 months due to complaints of lower limb and hip pain. Pathology on biopsies confirmed tumor was SFT/HPC, a metastasis from the brain primary. Most recent CT examination revealed space-occupying lesion in the sacrococcygeal region and in liver. Based on patient's history of brain tumors 8 years ago, patient was treated on liver metastasis, bone metastasis and left sacrococcygeal with I131 radiation particle implantation therapy (154 particles). After treatment, there was no improvement for liver metastasis. UMIPIC therapy was performed for liver metastasis only with the intra-tumor injection with combination of Adriamycin (Adr), cytarabine (Ara-C), H2O2 and penicillin as hapten into the main tumor mass in liver. Fresh tumor samples were biopsied prior injection and after injection one and two weeks twice to collect tumor tissue for this study. [5, 9]

Tissue disassociation

The fresh samples of tumor tissue stored in SCelLiVeR tissue preservation solution in GEXSCOPER (Singleron) till molecular testing. Biopsy tissue samples were washed with Hanks Balanced Salt Solution (HBSS) and then dissected into 1~2 mm pieces. [27]

Sequencing data processing and quality control

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. Briefly, after using fastqc (version 0.11.7) and cutadapt (version 1.17) for quality control and filter the data. [10] Reads were compared with the reference genome GRCh38 with ensemble version 93. Gene annotation were used STAR (version 2.6.1b). [11, 12]

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 in both of the compared groups of cells and with an average log (Fold Change) value greater than one as DEGs. [27]

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). [27]

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 were used with the "ClusterProfiler" R package 3.16.1. [11] Gene set variation analysis (GSVA) pathway enrichment analysis, the average gene expression of each cell type was used as input data using the GSVA package. [27]

UCell gene set scoring

Gene set scoring was performed using the R package UCell v 1.1.0. [12] 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) [13] to carry out single-cell trajectory analysis, and the dimensionality reduction method was DDRTree, a new manifold learning algorithm. [27]

Transcription factor regulatory network analysis (pySCENIC)

The transcription factor network was constructed by pySCENIC (v0.11.0) [14] using the scRNA expression matrix and transcription factors in AnimalTFDB. AUCell was used for regulon activity quantification for every cell. [27]

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 [15] 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. [27]

Results

Clinical benefit characteristics

The pathological diagnosis of liver biopsy confirmed the same tumor as in brain. After three treatments to liver metastasis, patient condition improved and can walk as normal.

Changes in cancer cells before and after treatment

A total of 37297 cells of three fresh samples were filtered by quality control. The cells were divided into 18 clusters after unsupervised clustering and dimensionality reduction. The seven cell types were identified based on marker gene expression and inferCNV analysis results, including cancer cells (CLU), stromal cells (COL1A1), B cells (MS4A1), TandNK cells (CD3D), neutrophils (FCGR3B), mast cell (TPSAB1) Mononuclear cells (CD14) (Figure 1 a, c, d, e, f).

Fig. 1

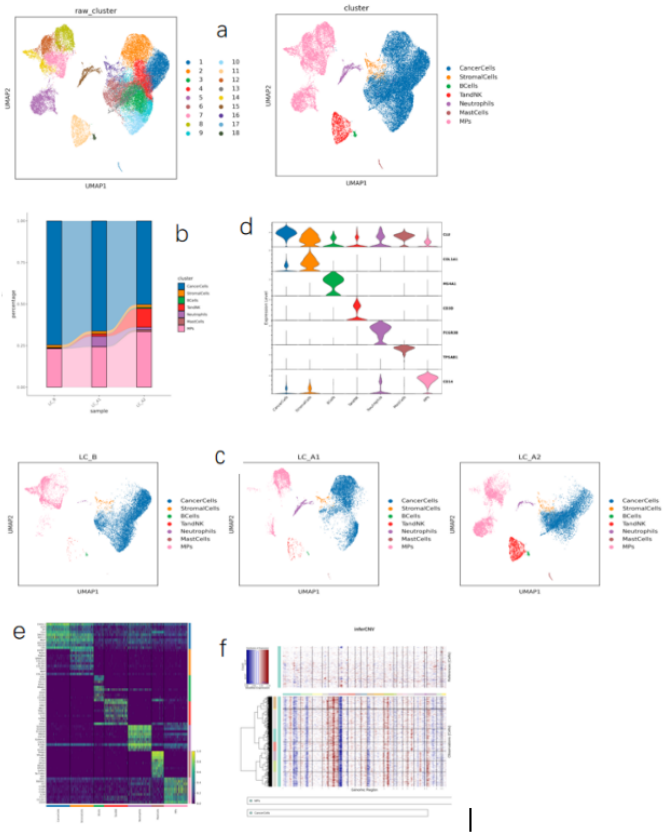

Figure 1: Seven cell types were identified based on marker gene expression and inferCNV analysis results.

Transcriptome characteristics of metastasis before and after drug administration

To investigate impact of drug therapy on tumor cells, collected 23447 Cancer Cells were divided, annotated into 8 different subtypes CancerCells1-8 (Figure 2 a) by cell proportion and heterogeneity analysis. We found that cancer cells at different stages of treatment exhibited significant characteristics heterogeneity (Figure 2 b, c). Based on GSVA analysis we identified the functions of each cluster (hallmark database): cluster 1 enriches G2M

checkpoints and KRAS signaling pathways; Cluster 2 enriched oxidative phosphorylation signaling pathway; Cluster 3 enriches P53 and Notch signaling pathways; Cluster 4 enriches fatty acid metabolism pathways; Cluster 5 enriches the WINT signaling pathway; Cluster 6 enriches the Hedgehog signaling pathway; Group 7 enriches epithelial mesenchymal transformation EMT-TGF-β signaling pathway; Cluster 8 enriched IFN- α and IFN- γ signaling pathway (Fig. 2 d). The significant active regulators in the TF regulatory network of these 8 subtypes were anal sized using Psychic analysis. Results showed cluster 8 upregulated interferon regulatory factor (IRF2), which corresponds to the results of gene enrichment analysis. Cluster 4 upregulates the tumor suppressor gene RUNX3, while cluster 4 is mainly enriched in LC_ A2 sample (Figure 2 e).

Fig. 2

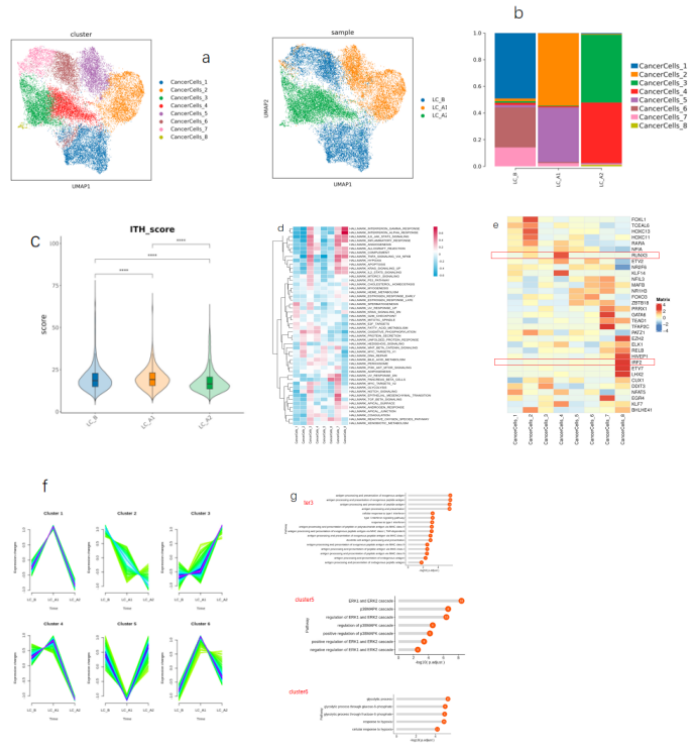

Figure 2: Transcriptome characteristics of metastasis.

Gene expression pattern analysis revealed six gene modules that varied with the treatment process (Figure 2 f, g).

Changes in T and NK cells before and after drug administration

Total of 1576 T and NK cells were identified into 6 different subtypes by detailed annotation: ProliferatingT, CD8Teff, NK, NaiveT, Th17, and Treg (Figure 3 a, b, c, d, e, f).

Fig. 3

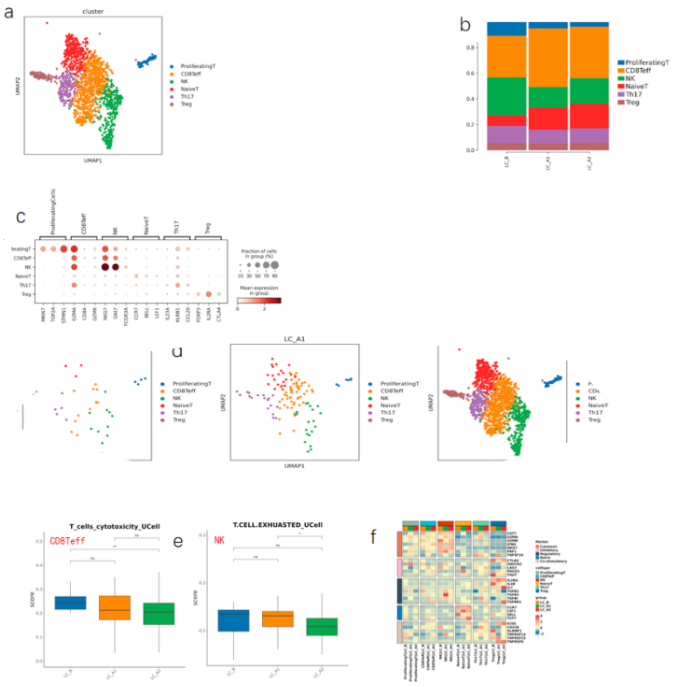

Figure 3: Changes in T and NK cells.

Changes in MPs before and after treatment

We collected total of 6929 macrophages. Using unsupervised clustering results further divided into 4 heterogeneous subgroups (Figure 4 a, b, c, d, e, f). Cluster 1 highly expresses the source characteristics of embryonic precursors (FOLR2), those are tissue anchored macrophages (Figure 4 g, h). We found that the proportion of cluster 1 increased after treatment (Figure 4 d). Cluster 2 cells was highly expressing angiogenic features or proangiogenic factors (VEGFA, CXCL8), these are TAMs that promote angiogenesis (Figure 4 g, h). This cluster initially decreases and then increases during treatment (Figure 4 d).

Fig. 4

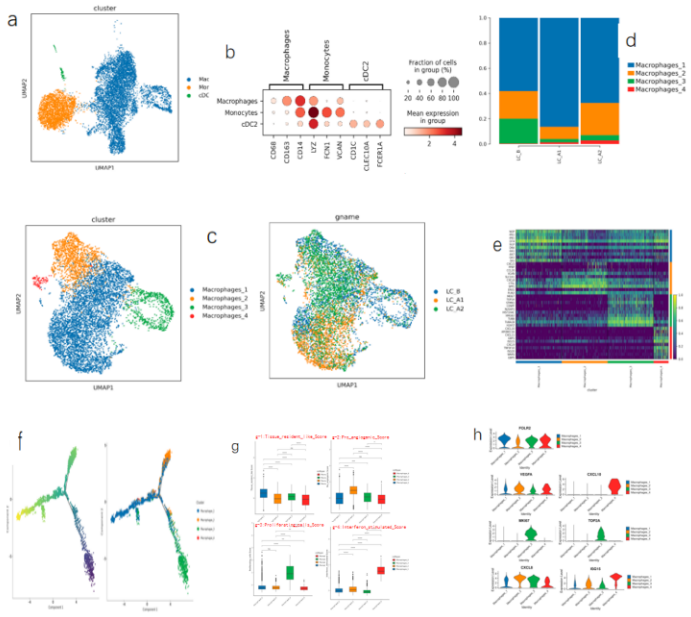

Figure 4: Changes in MPs

Changes in neutrophils

A total of 689 cells were identified as neutrophils, two different subtypes were identified through detailed annotation: neutrophils-1 and Neutrophils-2 (Figure 5 a, b, c). Monocle2 trajectory analysis shows that cluster 1 is in the early stage of development, while cluster 2 is in the late stage of development (Figure 5 d). Cluster 1 upregulates ICAM3/HCK/ANXA3, exhibiting the characteristics of polymorphonuclear leukocytes (PMNs), while cluster 2 is tumor associated neutrophils (TANs) which upregulate VEGFA/ LDHA/BHLHE40 (Figure 5 e). Functional enrichment analysis showed the innate immune response, antigen presentation and phagocytosis signals of cluster 1 were enhanced; Cluster 2 upregulates endoplasmic reticulum stress and hypoxia pathways (Figure 5 f).

Fig. 5

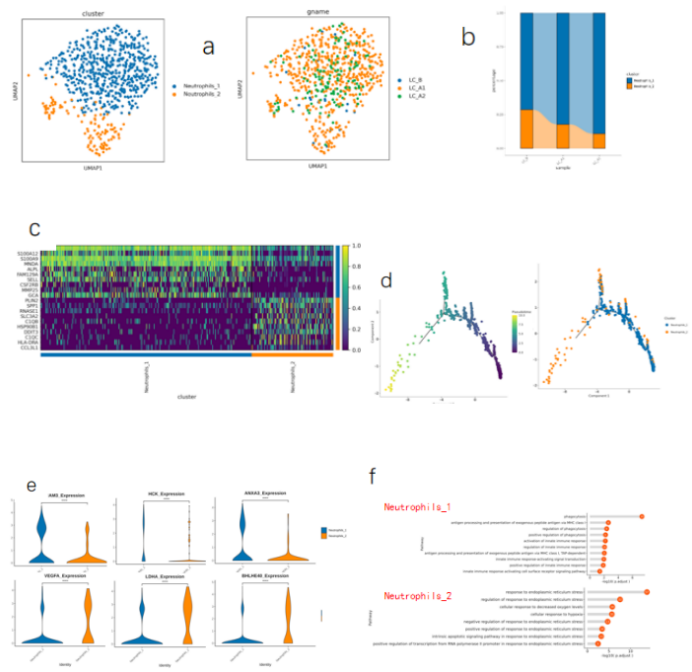

Figure 5: Changes in neutrophils.

Changes in CellphoneDB

CellPhoneDB database is based on ligand receptor interaction. It was used to analyze the interaction of each major cell cluster in three groups of samples. The results showed that the interaction relationship of cancer cells, stromal cells and mononuclear cells increased first and then decreased with the treatment process (Figure 6 a, b). Further analysis found that SPP1 between monocyte phagocyte and cancer cells, and between monocyte phagocyte and stromal cells. The CD44 signal intensity gradually decreases with the treatment process (Figure 6 c).

Fig. 6

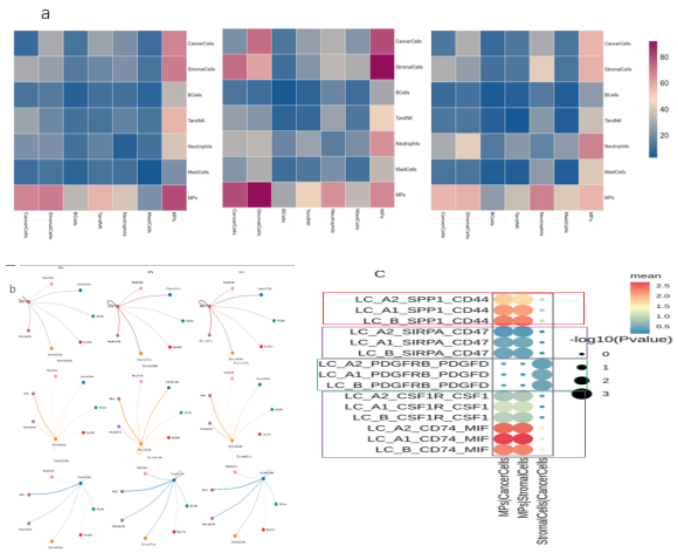

Figure 6: Changes in CellphoneDB.

Discussion

AHPC is a rare disease. Three is very limited research on basic molecular and treatment response. In this study, we used scRNA-seq and analysis of single cell level and gen express from tumor tissue before and after treatment with hapten enhanced intra-tumor chemotherapy (HEIC). UMIPIC with different drugs, provides evidence that HEIC treatment of liver metastasis of AHPC can induce acute immune response. As a result, it can help to control the spreading of tumor cells locally.

Clinical benefit showed this patient felt better and walked normally after three HEIC treatments.

Our study has demonstrated that immune cells can be wakened by HEIC, such as B cells (MS4A1), T and NK cells (CD3D), neutrophils (FCGR3B), mast cell (TPSAB1) and mononuclear phagocytic cells (CD14). As the treatment progressed, the proportion of cancer cells gradually decreased, while the proportion of mononuclear phagocytic cells and T and NK cells gradually increased. IFN- α and IFN- γ signal pathway are active regulators in the TF regulatory network. These results indicate that drug therapy induces the remodeling of tumor microenvironment and gathering of immune cells.

Our study has demonstrated by using gene expression pattern analysis. Data showed six gene modules that varied with the treatment process and in the course of processing and presentation of cancer cell antigens, as well as the response function of type I interferon. It was continuously upregulated with the treatment process. The effect was inhibition of immune-escape of tumor cells. [16,17] Our result also showed that p38MAPK cascade signaling pathway and ERK1 and ERK2 cascade connectivity first decreased and then increased following the treatment. Excessive activation of the p38MAPK cascade signaling pathway, ERK1, and ERK2 cascade pathways promotes cancer occurrence, metastasis, and drug resistance. [18, 19] The hypoxia and glycolysis pathways in cancer cells showed initial increase and then decrease. Previous studies hda showed these are related to tumor cell proliferation. [18, 19] The results indicate that treatment induces activation of related pathways and suppresses tumor immune escape and also induces pathways for drug resistance at different treatment stages.

Our study has further revealed changes in T and NK cells before and after drug administration. T and NK included total of 1576 cells categorized with 6 different subtypes. These 6 types include: Proliferating T, CD8Teff, NK, Naïve T, Th17, and Treg. The expression of costimulatory genes ICOS, CD226, TNFRSF14, and TNFRSF25 in Naive T cells gradually upregulate the followed treatment. [22] The CD8Teff toxicity gradually decreased following the treatment process, while the NK cell depletion gradually decreased.

Result showed changes in MPs before and after treatment. Total of 6929 macrophages were divided into 4 heterogeneous subgroups. Cluster 1 highly expresses the source characteristics of embryonic precursors (FOLR2), these are tissue resident macrophages. Some studies had shown that staining density of FOLR2+macrophages in breast cancer were positively correlated with better patient survival. Our results showed that treatment process induced more transformation of cluster 3 to cluster 1 (FOLR2+macrophages). [23]

We also found changes in Neutrophils cells of Neutrophils_ 1 and Neutrophils_ 2 before and after drug treatment. Monocle2 trajectory analysis shows that cluster 1 is in the early stage of development while cluster 2 is in late stage of development. Some studies had shown that tumor related neutrophils TAN-1 infiltrated in invasive pancreatic ductal adenocarcinoma are enriched to hypoxia, endoplasmic reticulum stress, IL-1 and TNF signaling pathways and glycolysis. These cells are in terminal differentiation stage which can be significantly related to the poor prognosis of PDAC. [24]

Further analysis we found some interesting changes in SPP1 between monocyte phagocyte and cancer cells, monocyte phagocyte and stromal cells. CD44 signal intensity gradually decreases with the treatment process. Previous studies have shown that intrinsic SPP1 in tumor cells can promote the transformation of macrophages into M2-like tumor associated macrophages (TAM) through mediating crosstalk between HCC malignant cells and macrophages. By coupling the expression with survival data,

researchers found that the high expression of SPP1 significantly correlate with poor prognosis in HCC; [25] Targeting CSF1 (R) can reduce the number of TAMs or alter their phenotype in inhibiting tumor promotion characteristics. [26]

In short, we have demonstrated that immune response was induced by UMIPIC. It involves not only B cell activity but also involves T cell activity with multiple genes participating at the molecular level. This may play an important role in the modification of associate tumor antigens (ATAs) to improve neu ATAs. This may also provide an opportunity for production of immune cells due to immune cells activation becomes sensitive to tumor antigens, in turn, it may turn microenvironment ready for immunotherapy like PD-1 or PD-L1. [27] Further study with more patients of AHPC for hapten-enhanced intra-tumor injections are needed for comprehensive understanding of the full mechanism and therapeutic potential.

Competing Interests: No competing financial interests.

Statement on Data Availability: Data from this study will be made available but some restrictions apply. Data may be requested with reasonable written cause from Dr. Baofa Yu.

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References

1. Yamashita D, Suehiro S, Kohno S, Ohue S, Nakamura Y, et al. (2020) Intracranial Anaplastic Solitary Fibrous Tumor/Hemangiopericytoma: Immunohistochemical Markers for Definitive Diagnosis. Neurosurg Rev. 44(3):1591-1600.
2. Lottin M, Escande A, Peyre M, Sevestre H, Maurage CA (2020) What's New In The Management Of Meningeal Solitary Fibrous Tumor/Hemangiopericytoma? Bull Cancer 107(12):1260-1273.
3. Liu CY, Chen KF, Chen PJ (2015) Treatment of Liver Cancer. Cold Spring Harb Perspect Med 5: A021535.
4. Xu F, Jin T, Zhu Y, Dai C (2018) Immune Checkpoint Therapy In Liver Cancer. J Exp Clin Cancer Res 37: 110.
5. Gao F, Jing P, Liu J, Lu Y, Zhang P, et al. (2015) Hapten-Enhanced Overall Survival Time In Advanced Hepatocellular Carcinoma By Ultro-Minimum Incision Personalized Intratumoral Chemoimmunotherapy. J Hepatocell Carcinoma 2: 57-68.
6. Ziegenhain C, Vieth B, Parekh S, Reinius B, Guillaumet-Adkins A, et al. (2017). Comparative Analysis Of Single-Cell RNA Sequencing Methods. Mol Cell 65: 631-643.E634.
7. Ma L, Hernandez MO, Zhao Y, Mehta M, Tran B, Kelly, et al. (2019) Tumor Cell Biodiversity Drives Microenvironmental Reprogramming In Liver Cancer. Cancer Cell 36: 418-430.E416.
8. Yu BF, 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.
9. Yu BF, Han Y, Fu Q, Gao F, Jing P, et al. (2023) Awaken Immune Cells by Hapten Enhanced Intratumoral Chemotherapy With Penicillin Prolong Pancreatic Cancer Survival. Journal of Cancer. 14(8): 1282-1292.
10. Kechin A, Boyarskikh U, Kel A, Et Al. (2017) Cutprimers: A New Tool For Accurate Cutting Of Primers From Reads Of Targeted Next Generation Sequencing. J Comput Biol. 24(11):1138-1143.
11. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Et Al. (2013) STAR: Ultrafast Universal RNA-Seq Aligner. Bioinformatics 29(1):15-21.
12. Yu G, Wang L, Han Y, He Q (2012) Cluster Profiler: An R Package For Comparing Biological Themes Among Gene Clusters. OMICS: A Journal of Integrative Biology 16(5):284-287.
13. Qiu XJ, Hill A, Packer J, Lin D, Yi-An M, Et Al. (2017) Single-Cell Mrna Quantification And Differential Analysis With Census. Nat Method. 14(3):309-315.
14. Efremova M, Vento-Tormo M, Teichmann SA, Vento-Tormo R (2020) "Cellphonedb: Inferring Cell-Cell Communication From Combined Expression Of Multi-Subunit Ligand-Receptor Complexes." Nature Protocols 15.4 (2020): 1484-1506.
15. Sun X, Niu X, Chen R, He W, Chen D, et al. (2016). Metallothionein-1G Facilitates Sorafenib Resistance Through Inhibition Of Ferroptosis. Hepatology 64: 488-500.
16. Dhatchinamoorthy K, Colbert JD, Rock KL (2021) Cancer Immune Evasion Through Loss Of MHC Class I Antigen Presentation. Front Immunol. 12:636568.
17. Favuzzi E, Huang S, Saldi GA, Binan L, Ibrahim LA, et al. (2021) GABA-Receptive Microglia Selectively Sculpt Developing Inhibitory Circuits. Cell. 184(15):4048-4063.E32.
18. Xu Y, Sun Q, Yuan F, Dong H, Zhang H, et al. (2020) RND2 Attenuates Apoptosis and Autophagy In Glioblastoma Cells By Targeting The P38 MAPK Signalling Pathway. J Exp Clin Cancer Res. 39(1):174.
19. Zhu G, Herlyn M, Yang X (2021) TRIM15 And CYLD Regulate ERK Activation Via Lysine-63-Linked Polyubiquitination. Nat Cell Biol. 23(9):978-991.
20. Yao B, Li Y, Chen T, Niu Y, Wang Y, et al. (2021) Hypoxia-Induced Cofilin 1 Promotes Hepatocellular Carcinoma Progression By Regulating The PLD1/AKT Pathway. Clin Transl Med. 11(3): E366.
21. Liu Y, Liang G, Xu H, Dong W, Dong Z, et al. (2021) Tumors Exploit FTO-Mediated Regulation Of Glycolytic Metabolism To Evade Immune Surveillance. Cell Metab. 33(6):1221-1233.E11.
22. Zhou W, Yu M, Mao X, Pan H, Tang X, et al. (2022) Landscape of The Peripheral Immune Response Induced By Local Microwave Ablation In Patients With Breast Cancer. Adv Sci (Weinh). 9(17):E2200033.
23. Ramos RN, Missolo-Koussou Y, Gerber-Ferder Y, Bromley CP, Bugatti M, et al. (2022) Tissue-Resident FOLR2+ Macrophages Associate With CD8+ T Cell Infiltration In Human Breast Cancer. Cell. 185(7):1189-1207.E25.
24. Wang L, Liu Y, Dai Y (2022) Single-Cell RNA-Seq Analysis Reveals BHLHE40-Driven Pro-Tumour Neutrophils with Hyperactivated Glycolysis In Pancreatic Tumour Microenvironment. Gut. 72(5):958-971.

25. Zhu L, Oh JM, Gangadaran P, Kalimuthu S, Baek SH, Jeong SY, Et Al. (2018) Targeting and Therapy Of Glioblastoma In A Mouse Model Using Exosomes Derived From Natural Killer Cells. Front Immunol. 9:824.

26. Pittet MJ, Michielin O, Migliorini D (2022) Clinical Relevance Of Tumour-Associated Macrophages. Nat Rev Clin Oncol. 19(6):402-421.

27. Yu B, Gao F, Jing P, et al. (2023) Cancer Immunotherapy Preparation and Immune Cells Activation Through Hapten-Enhanced Chemotherapy In Primary Lung Cancer. J Basic Clin Pharma 14(S1):10-16.