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Basic Study

Glutamine Deprivation Decreases the Function of Infiltrating CD8$^+$T Cells in Hepatocellular Carcinoma by Inducing Mitochondrial Damage and Apoptosis

Wang W *et al*. Glutamine deprivation induced CD8$^+$T cells dysfunction

Abstract

BACKGROUND

Functions of infiltrating CD8$^+$T cells are often impaired due to tumor cells causing nutrient deprivation in the tumor microenvironment. Thus, the mechanisms of CD8$^+$T cell dysfunction have become a research hotspot, which has increased interest in how changes in metabolomics correlate with CD8$^+$T cells dysfunctions.

AIM

To investigated whether and how glutamine metabolism affects the function of infiltrating CD8$^+$T cells in hepatocellular carcinoma .

METHODS

Immunohistochemical and immunofluorescence staining was performed on surgically resected liver tissues from patients. Differentially expressed genes of hepatocarcinoma-infiltrating CD8$^+$T cells were detected using RNA sequencing. Activated CD8$^+$T cells were co-cultured with Huh-7 cells for 3 d. The function and mitochondrial status of CD8$^+$T cells were analyzed by flow cytometry, qRT-PCR and transmission electron microscopy. Next, CD8$^+$T cells were treated with the mitochondrial protective and damaging agents. Functional alterations in CD8$^+$T cells were detected by flow

cytometry. Then, complete medium without glutamine was used to culture cells and detect their functional changes and mitochondrial status.

RESULTS

A large number of infiltrating PD-1^{+}CD8^{+}T cells in liver cancer tissues.Next, we co-cultured CD8^{+}T cells and Huh-7 cells to explore the regulation of CD8^{+}T cells by hepatoma cells. Flow cytometry results revealed increased PD-1 expression and decreased secretion of perforin (PRF1) and granzyme (GZMB) by CD8^{+}T cells in the co-culture group. Meanwhile, JC-1 staining was decreased and levels of reactive oxygen species (ROS) and apoptosis were increased in CD8^{+}T cells of the co-culture group; additionally, the mitochondria of these cells were swollen. When CD8^{+}T cells were treated with the mitochondrial protective and damaging agents, their function was restored and inhibited, respectively, through the mitochondrial damage and apoptotic pathways. Subsequently, complete medium without glutamine was used to culture cells. As expected, CD8^{+}T cells showed functional downregulation, mitochondrial damage, and apoptosis.

CONCLUSION

Glutamine deprivation decreases the function of hepatocarcinoma-infiltrating CD8^{+}T cells through the mitochondrial damage and apoptotic pathways.

Key Words: Glutamine; Mitochondrial damage; CD8^{+}T cells; T cell function

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Core Tip: In this study, we discovered that glutamine deprivation decreases the function of hepatocarcinoma-infiltrating CD8+T cells through the mitochondrial damage and apoptotic pathways.

INTRODUCTION

CD8+T cells are important effector immune cells in the tumor microenvironment that primarily kill tumor cells by secreting granzymes (GZMB) and perforin (PRF1)[1]. Owing to factors that include chronic stimulation by tumor antigens, the physicochemical state of the tumor microenvironment is imbalanced including low pH, hypoxia, and low nutrition availability[2-3], which trigger T cell dysfunction and ultimately cell depletion[4-5]. Exhausted T (Tex) cells are characterized by loss of effector functions, elevated and sustained expression of inhibitory receptors (IRs), and a distinct metabolic profile[6-7]. Recently, the mechanisms of CD8+T cell exhaustion have become a research hotspot, which has increased interest in how changes in metabolomics correlate with changes in immune cell functions.

Tumor cells consume high levels of energy sources such as glucose[8], resulting in nutrient depravation in the tumor microenvironment. This deprives immune cells of the energy sources they require, altering the phenotype and function of affected immune cells[9]. Glutamine (Gln) is the most abundant free amino acid in serum[10]. Gln is not only involved in the occurrence, development, and metastasis of tumor cells[11-12], but also regulates the growth and function of immune cells[13]. Gln has been reported to regulate the phenotype of CD4 cells, and increasing Gln levels can skew regulatory CD4+T cells toward more inflammatory subtypes[14-15]. However, whether Gln also regulates CD8+T cells and the mechanism of this regulation have not been reported.

Mitochondria are important intracellular organelles that provide energy and biosynthetic substrates for cell survival through oxidative phosphorylation[16]. Mitochondria are also highly nutrient-sensitive. When cells are deficient in nutrients, their mitochondria undergo depolarization and appear damaged. Damaged mitochondria can release pro-apoptotic proteins such as cytochrome c and other

substances to induce the formation of apoptotic complexes that activate caspase 9, which causes apoptosis. Recent research has demonstrated abnormal mitochondrial damage in tumor tissues that is closely related to remodeling of the tumor microenvironment. It has also been shown in the literature that after glucose deprivation, $CD8^+$T cells in the tumor microenvironment produce reactive oxygen species (ROS), which subsequently triggers mitochondrial damage[17]. Whether Gln similarly regulates the function of mitochondria in $CD8^+$T cells has not been reported.

Therefore, this study investigated whether Gln regulated $CD8^+$T cell function through the mitochondrial damage and apoptotic pathways to clarify the relationship between Gln metabolism and $CD8^+$T cell depletion, laying a foundation for new anti-tumor treatments.

MATERIALS AND METHODS

Immunohistochemical analysis

Immunohistochemical staining was performed on surgically resected tissues from patients with clinicopathologically confirmed hepatocellular carcinoma (HCC) and adjacent non-cancerous tissues. The study was approved by the Medical Ethics Committee of North China University of Science and Technology (No.2018109).

Immunofluorescence chemical staining analysis

Immunofluorescence staining of paraffin-embedded human liver tissue sections was performed using anti-PD-1/CD279 (66220-1-Ig, Proteintech, Rosemont, IL, USA) and anti-CD8α (PB9249, Boster Bio, Pleasanton, CA, USA) antibodies. Antigen retrieval was performed with EDTA buffer (AR0023, Boster Bio). Tissue sections were blocked with 10% goat serum (AR1009, Boster Bio), and then incubated with rabbit anti-CD8α antibody (1:200 dilution) and mouse anti-PD-1 antibody (1:4000 dilution) at 4°C overnight. Secondary antibodies including DyLight594 fluorescein goat anti-mouse (BA1141, Boster Bio) and fluorescein DyLight488 goat anti-rabbit (BA1127, Boster Bio) IgGs were mixed and incubated for 45 min at 37°C. DAPI staining solution (AR1176,

Boster Bio) was used for counterstaining, and the stain was incubated at room temperature for 3 min, and then washed with PBS (pH 7.2–7.6) (AR0030, Boster Bio). Slides were mounted using anti-fluorescent quench mounting medium (AR1109, Boster Bio). Finally, whole-slide scanning (APERIOVERSA8, Leica, Wetzlar, Germany) and image acquisition (BX51, Olympus, Tokyo, Japan) were performed.

Cell sorting

First, 100 mL of peripheral blood was collected from healthy volunteers, from which peripheral blood mononuclear cells (PBMCs) were obtained by diluting peripheral blood with an equal volume of PBS followed by centrifugation (800 ×g, 20 min). The pellet was then washed with a 5×volume of PBS and centrifuged (200 ×g, 5 min). Cells were resuspended in x-vivo15 (SH30809.01B, Hyclone, Logan, UT, USA). PBMCs were stained with anti-human CD8 antibody (Biolegend, San Diego, CA, USA). Cell sorting was performed using a MofloXDP sorting flow cytometer (Beckman Coulter, Brea, CA, USA). The post-classification purity of all included samples was >85%.

Cell culture and activation

The hepatoma cell line HuH-7 (RRID: CVCL_0336) was confirmed *via* short tandem repeat markers by Procell Life Science & Technology Co., Ltd. (Hyderabad, India). The results showed that the DNA typing of this cell line matched 100% with other typing in the CRC cell bank, and no human cell cross contamination was found. HuH-7 cells were cultured in Dulbecco's modified eagle medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Waltham, MA, USA) and 1% penicillin/streptomycin (Hyclone). $CD8^+$T cells were cultured *in vitro* using Roswell Park Memorial Institute (RPMI) 1640 medium (SH30809.01B; Hyclone), supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Hyclone). $CD8^+$T cells were stimulated with 10 μg/mL anti-human CD3 antibody (kx10-3A; Beijing Kexin Biological, Beijing, China) and 2.5 μg/mL anti-human CD28 antibody (kx10-28A; Beijing Kexin Biological) in medium containing 100 U/mL IL-2 (PeproTech, Rocky Hill,

NJ, USA) for 2 d. Both cell types were maintained at 37°C in an atmosphere of 50 mL/L CO_2.

Cell co-culture

HuH-7 and $CD8^+$T cells were co-cultured in transwell inserts for 3 d, with consistent initial numbers of both cells used.

RNA sequencing of $CD8^+$T cells

Total RNA was extracted from $CD8^+$T cells, and total RNA-Seq libraries were obtained by a three-step method: 1) RNA library construction and on-board sequencing, sequencing data were aligned to the reference genome of the project species to obtain comprehensive transcript information and to perform gene expression quantification; 2) the transcriptome sequencing project was completed on the Illumina sequencing platform (San Diego, CA, USA), the IlluminaPE library (approximately 300 bp) was constructed for sequencing; and 3) the obtained sequencing data were analyzed by bioinformatics after performing quality control.

Flow cytometry analysis

$CD8^+$T cells isolated from peripheral blood were cultured in groups and stained for intracellular markers. Analytical flow cytometry (NovoCyte, ACEA Biosciences Inc., San Diego, CA, USA) and a flow analyzer (CytoFLEXS, Beckman Coulter) were used for the analysis. $CD8^+$T cells were stained with an Annexin V-FITC Apoptosis Detection Kit (A211-02; Vazyme, Nanjing, China) to detect apoptosis. $CD8^+$T cells were stained with JC-1 fluorescent dye (J8030; Solarbio, Beijing, China) and a ROS Detection Kit (CA1410; Solarbio) to detect mitochondrial damage and ROS levels, respectively. Cells were fixed and permeabilized with Fixation/Permeabilization solution (554714; BD Biosciences, Franklin Lakes, NJ, USA), and then $CD8^+$T cells were stained with anti-GZMB (515403; Biolegend) and anti-PRF1 (154305 ; Biolegend) antibodies to detect activation status.

Finally, $CD8^+$T cells were stained with PE-conjugated anti-human CD279 (PD-1) antibody (329905; Biolegend) to detect functional changes to $CD8^+$T cells.

Quantitative Real-time PCR (qRT-PCR)

First, mRNA was extracted from sorted $CD8^+$T cells using the MiniBEST Universal RNA Extraction Kit (Cat.#9767; TaKaRa, Dalian, China) according to the manufacturer's protocols. Extracted mRNAs were reverse-transcribed into cDNA after mixing mRNA template with the Primescript RT Reagent Kit with gDNA Eraser (AK3920; TaKaRa) for qPCR. The qPCR reactions were run on a Light Cycler 480SYBRGreenIMaster (04887352001; Roche, Basel, Switzerland). The primer sequences were as follows: GZMB (forward: 5′-GGTGCGGTGGCTTCCTGAT-3′, reverse: 5′-ACTGCTGGGTCGGCTCCTGT-3′), PRF1 (forward: 5′-TGCCGCTTCTACAGTTTCCA-3′, reverse: 5′-CCACCTCGTTGTCCGTGAG-3′), and GAPDH (forward: 5′-TCAAGAAGGTGGTGAAGCAGG-3′, reverse: 5′-TCAAAGGTGGAGGAGTGGGT-3′). All reactions were performed for 40 cycles. The qPCR steps included activation at 95°C for 10 min, denaturing at 95°C for 10 s, annealing at 60°C for 15 s, and extension at 72°C for 20 s. All qPCR reactions were performed on a Bio-Rad real-time quantitative fluorescence PCR instrument (CFX Connect, Bio-Rad, Hercules, CA, USA). Relative gene expression was calculated with respect to the internal standard (GAPDH). Expression levels were normalized against GAPDH using the $2^{-\Delta\Delta Ct}$ method.

Transmission electron microscopy (TEM)

Cells in each group were digested to prepare 1×10^6 cells/mL solutions, centrifuged, and after discarding the supernatant, were fixed with 3% glutaraldehyde fixative for 2 h and 1% osmic acid for 1 h. After dehydration with a graded ethanol series and acetone, the cells were embedded in epoxy resin, ultrathin sectioned, and double stained with uranium and lead. The ultrastructural changes of mitochondria in each group of cells were observed by TEM.

Statistical analysis

All analyses were performed with SPSS 21.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA, USA). Measurement data were first examined using the Kolmogorov–Smirnov test to check whether the measurement data of each group were positively distributed. Results are expressed as mean ± standard error of mean (mean ± SEM) for measurement data with a positive distribution. Analyses between two groups were performed using the two-tailed Student's t test. One-way analysis of variance was used for multiple group comparisons, and the LSD-t test was used for pairwise comparisons between multiple groups. Significance was accepted at $P<0.05$.

RESULTS

$CD8^+$T cell infiltration in liver tissues

To investigate T cell infiltration in the tumor microenvironment, we performed immunohistochemical staining of resected liver tissues. These results showed significantly more $CD8^+$T cells in HCC tissues than in paracancerous tissues (Figure 1). Next, we used immunofluorescence staining to detect PD-1 expression on $CD8^+$T cells. Compared with paracancerous tissues, PD-1 was more abundantly expressed on the surface of $CD8^+$T cells in HCC tissues (Figure 2).

Hepatoma cells induced decreased $CD8^+$T cell function

After observing a large number of infiltrating $PD\text{-}1^+CD8^+$T cells in HCC tissues, we next co-cultured $CD8^+$T cells with Huh-7 cells to evaluate functional alterations in $CD8^+$T cells. Flow cytometry and qRT-PCR were used to examine the function of $CD8^+$T cells. These data indicated that compared with in the control group, $CD8^+$T cells in the co-culture group expressed higher PD-1 Levels (Figure 3A) and lower levels of GZMB and PRF1 (Figure 3B-E). Increased expression of IRs and reduced ability to secrete effector molecules suggested functional inhibition of $CD8^+$T cells; thus, hepatoma cells induced a decrease in $CD8^+$T cell function.

Hepatoma cells induced differential gene expression in CD8⁺T cells

To interrogate potential mechanisms of the functional impairment of T cells, we performed RNA-seq of CD8⁺T cells co-cultured with Huh-7 cells. We found that genes related to mitochondrial damage (such as mitochondrion disassembly) and apoptosis (such as regulation of apoptotic pathways) were upregulated (Figure 4A). The expression of genes related to cell metabolism (such as multi-organism metabolic processes) and T cell function (such as T cell proliferation) were downregulated (Figure 4B). These data illustrated that CD8⁺T cells co-cultured with hepatoma cells underwent alterations associated with cellular metabolism, mitochondrial damage, and apoptosis.

Hepatoma cells induced mitochondrial damage and apoptosis in CD8⁺T cells

Mitochondria are the primary source of ROS production, and JC-1 is a dye used to reflect mitochondrial integrity. To examine whether hepatoma cells had a regulatory effect on CD8⁺T cell mitochondria, CD8⁺T cells were co-cultured with Huh-7 cells. Flow cytometry analysis revealed that CD8⁺T cells from the co-culture group had decreased JC-1 staining (Figure 5A) and increased levels of ROS (Figure 5B) and apoptosis (Figure 5C) compared with the control group. Next, mitochondrial morphology was further studied by TEM to examine the ultrastructure of mitochondria. Mitochondria of CD8⁺T cells from the control group were identified with well-defined integral double membranes and regular cristae (Figure 6A). In contrast, mitochondria of CD8⁺T cells in the co-culture group showed significant swelling (Figure 6B). In summary, co-culture with hepatoma cells induced damage to the mitochondria of CD8⁺T cells and caused apoptosis.

Mitochondrial damage induced by hepatoma cells decreased CD8⁺T cell function

Next, we explored the relationship between mitochondrial damage and T cell function in hepatocarcinoma-infiltrating CD8⁺T cells. First, we co-cultured CD8⁺T cells with Huh-7 cells, adding to the culture system mitochondrial division inhibitor 1 (Mdivi-1),

which has been shown to protect from mitochondrial damage. Flow cytometry was used to examine the function of Mdivi-1-treated $CD8^+$T cells in co-culture. The results showed reduced expression of PD-1 (Figure 7A) and increased expression of PRF1 (Figure 7B) and GZMB (Figure 7C) in $CD8^+$T cells from the Mdivi-1 group compared with $CD8^+$T cells from the co-culture alone group, demonstrating that the function of hepatoma-co-cultured $CD8^+$T cells was not decreased after protecting from mitochondrial damage. Next, we cultured $CD8^+$T cells with different concentrations of the oxidative phosphorylation uncoupler carbonyl cyanide-3-chlorophenylhydrazone (CCCP) and examined their function. As shown from flow cytometry analysis, PD-1 expression gradually increased (Figure 8A), and there was a gradual decrease in the secretion of PRF1 (Figure 8B) and GZMB (Figure 8C) by $CD8^+$T cells with increasing CCCP concentrations. These results illustrated that mitochondrial damage led to the inhibition of $CD8^+$T cell function. In summary, co-culture with hepatoma cells decreased the function of $CD8^+$T cells through the mitochondrial damage pathway.

Gln deprivation induced mitochondrial damage, apoptosis, and functional impairment of $CD8^+$T cells

As a primary energy source, Gln affects both the development of tumor cells and the functions of immune cells. Therefore, we assessed whether Gln metabolism affected the function of $CD8^+$T cells. We co-cultured $CD8^+$T cells with Huh-7 cells and supplemented the co-culture system with RPMI 1640 with normal concentrations of Gln or without Gln. The findings demonstrated that $CD8^+$T cells in the co-culture system without Gln expressed lower PD-1 (Figure 9A) and higher PRF1 (Figure 9B) and GZMB (Figure 9C) levels compared with the co-culture alone group; additionally, JC-1 staining was decreased (Figure 9D), and there were increased levels of ROS (Figure 9E) and apoptosis (Figure 9F). Next, $CD8^+$T cells were cultured alone in RPMI 1640 with the normal Gln concentration or without Gln to further validate the effect of Gln on $CD8^+$T cells. The results showed that $CD8^+$T cells in the group lacking Gln had increased PD-1 expression (Figure 10A) and decreased secretion of perforin (Figure 10B) and granzyme

(Figure 10C) compared with those in the control group. Similarly, JC-1 staining was decreased (Figure 10D), and levels of ROS (Figure 10E) and apoptosis (Figure 10F) were increased. Together, these data show that hepatoma cells compete with $CD8^+$T cells for Gln. Gln deficiency inhibited the function of $CD8^+$T cells and induced mitochondrial damage and apoptosis.

DISCUSSION

In the tumor immune microenvironment, $CD8^+$T cells are the main immune effector cells and play a crucial role in the host immune environment. A large number of $CD8^+$T cells infiltrate tumor tissues in patients. However, due to chronic stimulation by tumor antigens, most of the T cells are functionally impaired and have differentiated into $CD8^+$Tex cells, which mediate tumor immune escape[18-20]. Therefore, interrogating the mechanism of decreased $CD8^+$T cell function is essential for tumor immunity. Tex cells are characterized by the loss of effector functions and elevated and sustained expression of IRs[6][21-22]. In this study, expression of the PD-1 was increased and the secretion of cellular effector molecules such as GZMB and PRF1 were decreased in $CD8^+$T cells from the co-culture group. Our observations illustrated that the function of infiltrating $CD8^+$T cells was suppressed induced by HCC cells. Therefore, we next analyzed the specific mechanisms through which $CD8^+$T cell function was inhibited.

Mitochondria are central to cellular metabolism and play key roles in the functional regulation of immune cells such as $CD8^+$T cells[23]. When mitochondria are slightly impaired, the damage is neutralized by fusion with healthy mitochondria. However, when mitochondrial damage exceeds the range buffered by fusion, the cell promotes mitochondrial division and damage[24]. Severely injured mitochondria usually show increased ROS production and decreased membrane potential[25], which decreases cellular function. As shown by our data, the mitochondria of $CD8^+$T cells co-cultured with HCC cells undergo swelling, increased ROS production, decreased membrane potential, and the cells undergo apoptosis, implying that hepatoma cells induce mitochondrial damage and apoptosis in $CD8^+$T cells. Next, we interrogated the

relationship between mitochondrial damage and CD8$^+$T cell function in using Mdivi-1 and CCCP to regulate mitochondrial status. Mdivi-1 is a quinazolinone derivative that penetrates cell membranes and attenuates mitochondrial damage by inhibiting mitochondrial division[26]. CCCP can interrupt oxidative phosphorylation, which impairs mitochondrial function and prompts mitochondria to produce high levels of ROS[27]. Together, these data show that mitochondrial damage can decrease the function of CD8$^+$T cells, *i.e.*, hepatoma cells can decrease the function of CD8$^+$T cells through the mitochondrial damage and apoptotic pathways.

Studies have shown that tumor cells inhibit anti-tumor immunity by competing for essential nutrients and reducing the metabolic adaptability of tumor-infiltrating immune cells, which in turn decreases the function of immune cells[28]. The nutrients required for cellular metabolism include glucose, amino acids, and fatty acids. Both tumor cells and activated T cells show a significant Gln requirement[29]. CD4$^+$T cells lacking Gln have diminished proliferative capacity and decreased cytokine secretion. We found that CD8$^+$T cells lacking Gln have a reduced ability to secrete effector molecules and increased expression of inhibitory receptors such as PD-1, suggesting that Gln deprivation inhibits the function of CD8$^+$T cells. In states of energy deficiency, mitochondrial function is abnormal, which further activates pro-apoptotic downstream regulators that induce apoptosis[30]. The literature suggests that upon glucose deprivation, cells largely mobilize oxidative phosphorylation to maintain energy homeostasis, causing mitochondria to produce high levels of ATP and ROS[31]. Our data showed that in the absence of Gln, the mitochondria of CD8$^+$T cells undergo morphological changes with reduced mitochondrial membrane potential and generated high levels of ROS, and induced apoptosis. However, the above results are only from in-vitro experiments, more details need to be further studied.

CONCLUSION

We found that Gln deprivation decreased the function of CD8$^+$T cells through the mitochondrial damage and apoptotic pathways.

ARTICLE HIGHLIGHTS

Research background

Functions of infiltrating $CD8^+$T cells are often impaired due to tumor cells causing nutrient deprivation in the tumor microenvironment. Thus, the mechanisms of $CD8^+$T cell dysfunction have become a research hotspot, which has increased interest in how changes in metabolomics correlate with $CD8^+$T cells dysfunction.

Research motivation

To explore the effect of glutamine metabolism on the function of tissue-infiltrating $CD8^+$T cells, so as to provide a new strategy for reversing the exhausted $CD8^+$T cells in hepatocellular carcinoma.

Research objectives

This study aims investigated whether and how glutamine metabolism affects the function of infiltrating $CD8^+$T cells in hepatocellular carcinoma .

Research methods

Immunohistochemical and immunofluorescence staining was performed on surgically resected liver tissues from patients. Differentially expressed genes of hepatocarcinoma-infiltrating $CD8^+$T cells were detected using RNA sequencing. Activated $CD8^+$T cells were co-cultured with Huh-7 cells for 3 d. The function and mitochondrial status of $CD8^+$T cells were analyzed by flow cytometry, qPCR and transmission electron microscopy. Next, $CD8^+$T cells were treated with the mitochondrial protective and damaging agents. Functional alterations in $CD8^+$T cells were detected by flow cytometry. Then, complete medium without glutamine was used to culture cells and detect their functional changes and mitochondrial status.

Research results

A large number of infiltrating PD-1^{+}CD8^{+}T cells in liver cancer tissues. Next, we co-cultured CD8^{+}T cells and Huh-7 cells to explore the regulation of CD8^{+}T cells by hepatoma cells. Flow cytometry results revealed increased PD-1 expression and decreased secretion of perforin (PRF1) and granzyme (GZMB) by CD8^{+}T cells in the co-culture group. Meanwhile, JC-1 staining was decreased and levels of reactive oxygen species (ROS) and apoptosis were increased in CD8^{+}T cells of the co-culture group; additionally, the mitochondria of these cells were swollen. When CD8^{+}T cells were treated with the mitochondrial protective and damaging agents, their function was restored and inhibited, respectively, through the mitochondrial damage and apoptotic pathways. Subsequently, complete medium without glutamine was used to culture cells. As expected, CD8^{+}T cells showed functional downregulation, mitochondrial damage, and apoptosis.

Research conclusions

Glutamine deprivation decreases the function of hepatocarcinoma-infiltrating CD8^{+}T cells through the mitochondrial damage and apoptotic pathways.

Research perspectives

From this study, We confirmed the potential mechanisms of CD8^{+}T cells dysfunction induced by glutamine deprivation, which would provide a novel strategy for reversing the exhaustion of CD8^{+}T cells in hepatocellular carcinoma.

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