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IFN-γ Secreted by NK Cells Effectively Suppresses Metastasis of Hepatocellular Carcinoma through Inhibiting Glycolysis

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Abstract

Background: Metastasis in Hepatocellular Carcinoma (HCC) is one of the main reasons for its poor prognosis. Natural killer cells produce killing effects on tumor cells by secreting Interferon-γ (IFN-γ), the mechanism of IFN-γ for hepatocellular carcinoma metastasis remain unclear. Here we aimed to reveal the mechanism of interferon-γ secreted by natural killer cells in regulating hepatocellular carcinoma metastasis.

Methods: Natural killer cells were isolated from blood samples of healthy volunteers. Wound healing assay, transwell migration and invasion assays were applied to assess the ability of natural killer cells and IFN-γ to inhibit hepatocellular carcinoma metastasis. Glucose uptake in hepatocellular carcinoma cells measured with the Glucose Uptake Colorimetric Kit. Lactate levels and ATP production in hepatocellular carcinoma cells measured with the L-lactate colorimetric kit and the EnzyLight™ ADP/ATP colorimetric kit, respectively. Interferon-γ mRNA levels were measured by real-time quantitative Polymerase Chain Reaction (q-PCR), and interferon-γ protein levels were analyzed by Enzyme-Linked Immunosorbent Serological Assay (ELISA). Western Blot (WB) was used to detect the protein expression of Hexokinase 2 (HK2), Phosphoglycerate Kinase 1 (PCK1), and Signal Transducer and Activator of Transcription 1 (STAT1). All experiments included treatment and control groups, and at least three replicates. Results expressed as mean ± SD, and statistical analysis performed using SPSS statistical software.

Results: We found that interferon-γ produced by natural killer cells inhibited the migration and invasion of HCC by decreasing aerobic glycolysis. Furthermore, aerobic glycolysis of HCC in turn inhibited interferon-γ secretion by natural killer cells through down-regulation of Signal Transducer and Activator of Transcription 1 (STAT1) phosphorylation.

Conclusion: We reveal a unique mechanism that interferon-γ produced by natural killer cells suppress the metastasis of hepatocellular carcinoma through inhibiting the glycolysis.

Keywords: Natural killer cells; Hepatocellular carcinoma; Interferon-γ; Signal transducer and activator of transcription 1; Glycolysis

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Abbreviations

HCC: Hepatocellular Carcinoma; NK: Natural Killer; IFN: Interferon; STAT1: Signal Transducer and Activator of Transcription 1; p-STAT1: Phosphorylated STAT1; WB: Western Blot; HK2: Hexokinase 2; PGK1: Phosphoglycerate Kinase 1; 2-DG: 2-deoxy-D-glucose

Introduction

Hepatocellular Carcinoma (HCC) has a high incidence and mortality rate worldwide [1,2]. Primary liver cancer is the sixth most commonly diagnosed cancer and third leading cause of cancer death worldwide in 2020 [3]. At the same time, some HCC patients are prone to metastasis [4]. Patients with HCC metastasis suffer great pain, and at the same time, HCC patients face difficulties

in treatment. It is significant to reduce the risk of HCC metastasis and improve the efficacy of treating HCC. Therefore, the mechanism of HCC metastasis needs to be clarified urgently to facilitate the treatment of HCC.

Natural Killer (NK) cells have many functions, including immunomodulation and antitumor [5,6]. NK cell-based antitumor therapy has been a central research question. In recent years, NK cells have been found to inhibit tumor metastasis. A previous study showed that enhancing the immune function of NK cells could prevent postoperative metastasis of breast cancer and improve survival rates [7]. Cord blood-derived NK cells effectively inhibit metastasis in epidermal growth factor receptor-positive colon cancer [8]. We also found in our previous study that interleukin-2 can activate NK cells and thus inhibit lung metastasis of HCC in nude mice [9]. However, it has been shown that the immune function of NK cells was impaired in the microenvironment of HCC [10]. In addition, there is a lack of activated receptors on the surface of NK cells and a decrease in cytokine secretion by NK cells. These allow HCC to escape from the natural immune barrier. Therefore, it is imperative to determine how NK cells exert their antitumor effects and the mechanisms.

It is well known that NK cells exert anti-tumor functions in various ways. In addition to antibody-dependent cell-mediated cytotoxicity, cytokines secreted by NK cells, such as Interferon (IFN) and tumor necrosis factor, are becoming increasingly important. These modalities are not independent but cooperate. Previous studies have shown that IFN-γ was first identified in lymphocyte culture supernatant by Younger and Salvin in 1973. Since then, IFN-γ has been found to have antiviral, anti-tumor and immunomodulatory functions. It regulates the expression of 30 genes and produces a variety of cellular responses, including promotion of antigen presentation and improvement of lysosomal activity in macrophages. The causes of HCC metastasis are complex, and the exact mechanisms are unclear. IFN-γ has attracted great interest in our quest. Recently, a growing body of evidence also suggests that metabolic reprogramming may contribute to tumor development [11]. In the 1920s, Otto Warburg first discovered that, unlike normal cells, which break down glucose through oxidative phosphorylation in mitochondria, tumor cells tend to convert glucose to lactic acid for energy supply even under conditions of sufficient oxygen [12]. In addition, extensive studies have shown that aerobic glycolysis produces up to 50% to 70% of the ATP supply in different tumors. In addition, lactate produced by aerobic glycolysis provides an acidic environment that contributes to cancer invasion and metastasis. Whether IFN-γ is associated with aerobic glycolysis requires further study.

Signal Transducer and Activator of Transcription (STAT) is a unique family of proteins activated by cytokines, such as interleukin-6, IFN-γ, epidermal growth factor, and platelet-derived growth factor. It is a nuclear transcription factor involved in the regulation of gene expression, particularly in cells with IFN as a signaling molecule. Several studies have shown that STAT1 is closely related to the function of NK cells. Ho-Bum Kang has reported that Genkwadaphnin can induce IFN-γ secretion from NK-92 cells *via* STAT1 [13]. It reported that miR-146a negatively regulated the function of NK cells through STAT1 signaling. Defective STAT1 activation is closely associated with impaired secretion of IFN-γ in NK cells [14,15]. In the tumor microenvironment, many immune cells, including NK cells, are recruited around the tumor by chemokines for tumor cell destruction, but the tumor cells still avoid immune killing, which ultimately leads to treatment failure. The most likely reason is that the function of NK cells is directly or indirectly inhibited by tumor cells. We will also perform further validation in this study [9]. In this study, we further investigated the mechanism of which NK cells inhibit HCC metastasis. We found that NK cells inhibit the metastasis of HCC by secreting the cytokine IFN-γ to inhibit aerobic glycolysis of HCC cells. This study provides experimental evidence for the clinical treatment of HCC.

Methods and Materials

Cells isolation and culture

The study protocol complied with the ethical guidelines of the Declaration of Helsinki and approved by the Research Ethics Committee of the First Hospital Research Institute of Xiamen University (Xiamen, China). First, we isolated peripheral blood mononuclear cells from blood samples of healthy volunteers. Then, NK cells were isolated from peripheral blood mononuclear cells using a human NK cell isolation kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Finally, we characterized NK cells according to the methods of a previous study [9]. NK cell was cultured in RPMI-1640 medium (HyClone; GE Healthcare Life Sciences, Logan, Utah, USA) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc). MHCC-97H cells, human highly metastatic hepatocellular carcinoma cells, were purchased from Shanghai Liver Cancer Institute and cultured in RPMI-1640 medium containing 10% FBS and 1% antibiotics. The study designed according to the principle of randomized controlled trials, experiment included treatment group and control group, and every experiment repeated at least three times.

Wound-healing assay

Cell migration and invasion were measured using BD BioCoat Transwell chambers without or with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). In the vehicle group, the normal medium containing 5×10^4 MHCC-97H cells were added to the upper compartment of the Transwell chamber. In the NK group, 200 μl of NK cell culture supernatant containing 5×10^4 MHCC-97H cells were added to the upper compartment of the Transwell. In the IFN-γ group, 200 μl of 0.2% FBS medium containing 150 U/ml IFN-γ and 5×10^4 MHCC-97H cells were added to the upper part of the Transwell compartment. Then 600 μl of medium containing 10% FBS was added to the lower chamber. After 48 h of incubation in a CO2 humidified incubator at 37°C, non-invasive cells in the upper chamber were removed with a cotton swab, and invasive cells were stained and counted with a microscope (x200) along the longitudinal or transverse axis, including five fields, without any overlap in the fields evaluated. The mean value of cells in each field was calculated.

Glucose uptake and lactate release and ATP assay

Glucose uptake of MHCC-97H cells was measured by the glucose uptake colorimetric assay kit (Biovision, CA, USA). Briefly, MHCC-97H cells were seeded at 1×10^4 cells/well in 96-well plates and reached a density of 70% to 80% after 48 h. At this point, 2-Deoxy-2-[(7-Nitro-2,1,3-Benzoxadiazol-4-yl) amino]-D-Glucose (2-NBDG) was added to a glucose-free medium at a final concentration of 100 μg/mL and incubated for 2 h. Cells were harvested in a cell-based analysis buffer and suspended in 1 μg/mL of the PI solution to exclude dead cells. Glucose uptake was determined by measuring the fluorescence intensity of 2-NBDG in PI-negative cells. lactate levels and ATP production in MHCC-97-H cell lysates were determined

using the colorimetric L-lactate assay kit (Abcam, Cambridge, UK) and the EnzyLight™ ADP/ATP ratio assay kit (BioAssay Systems, Hayward, CA, USA) were measured according to the manufacturer's instructions. Total cell counts were used for normalization.

RNA extraction and qRT-PCR

Total RNA was extracted using Trizol reagent (TaKaRa, Dalian, China) and then reverse transcribed into cDNA using the PrimeScript™ RT kit (TaKaRa, Dalian, China), according to the manufacturer's instructions. Real-time quantitative Polymerase Chain Reaction (qPCR) performed by SYBR Green I fluorescent dye (TaKaRa, Dalian, China) and Light Cycler 96 real-time PCR system (Roche, Switzerland) for real-time quantitative Polymerase Chain Reaction (qPCR). GAPDH as an internal control. The following primer sequences were used: hIFN-γ, primer pair 5′GGTTCTCTTGGCTGTTACTG3′ and 5′GACAGTTCAGCCATCACTTG3′. hGAPDH, primer pair 5′-TGCACCACCAACTGCTTAGC-3′ and 5′-GGCATGGACTGTGGTCAT GAG-3′. mRNA level quantified by the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked-immunosorbent serologic assay

Cells supernatant was collected from the Vehicle group (1×10^6 NK cells cultured in normal medium), S group (1×10^6 NK cells cultured in MHCC-97H medium supernatant), 2-DG group [1×10^6 NK cells cultured in MHCC-97H with 10 mM 2-DG (MedChemExpress, Shanghai, China) medium supernatant], G1 group (1×10^6 NK cells cultured in MHCC-97H with 5 mM glucose medium supernatant), and G2 group (1×10^6 NK cells cultured in MHCC-97H with 10 mM glucose medium supernatant). The concentration of IFN-γ was measured by ELISA (R&D Systems) according to the manufacturer's instructions.

Western blot

Collect the cell lysate using the Whole Protein Extraction Kit (Keygen, Jiangsu, China). Centrifuge at 13,000 rpm for 15 min at 4°C and recover the supernatant. The concentration of protein measured by the BCA method equals the amounts of total protein separated by SDS-PAGE. Proteins were transferred to PVDF membranes (Merck Millipore, New York, USA) and the membranes were blocked with 5% skim milk for 1 h at room temperature. Then the membranes were incubated at 4°C with primary antibodies [HK-2 (Abcam, Cambridge, UK), PGK-1 (Santa Cruz Biotechnology, CA, USA), STAT1 (Bioworld Technology Inc., St. Louis Park, MN, USA), p- STAT1 (Bioworld Technology Inc., St. Louis Park, MN, USA)] incubated overnight. After washing with TBST, the membranes were incubated with the corresponding peroxidase-conjugated secondary antibodies for 1 h at room temperature. Specific bands were detected on autoradiographic film using enhanced chemiluminescence reagents (ECL; Perkin Elmer Life Sciences, Massachusetts, USA).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, United States). Data from IHC was analyzed by Pearson's χ^2 test, and overall survival was assessed by

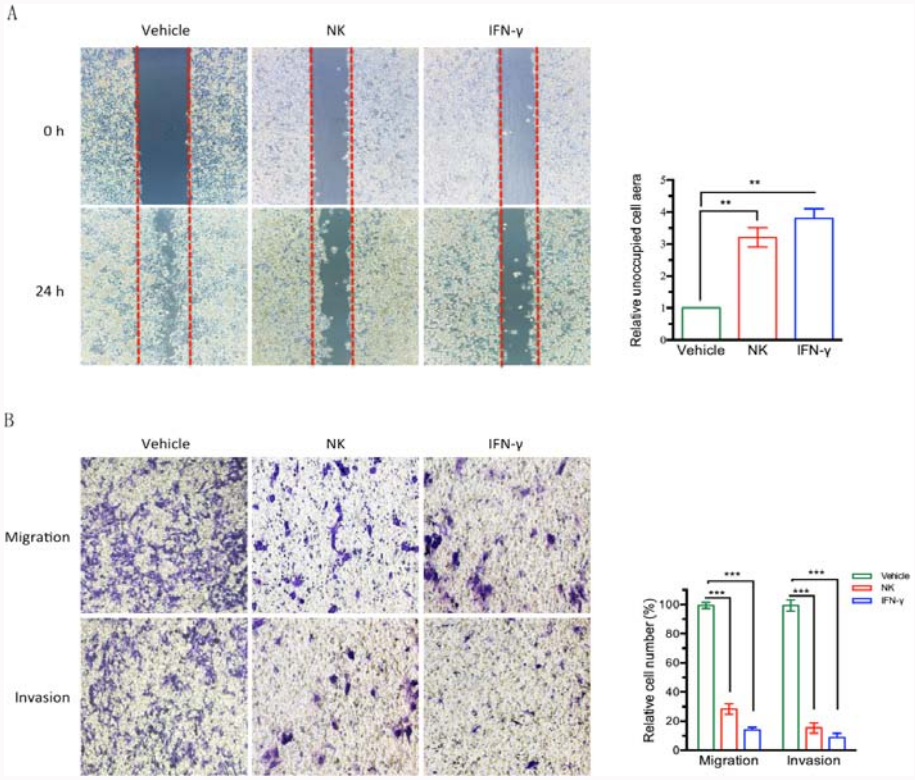

Figure 1: Inhibition of migration and invasion of MHCC97-H cells by NK cells and IFN-γ.
MHCC97-H cells were cultured with complete medium (vehicle), NK cell culture supernatant (NK), and complete medium containing IFN-γ (IFN-γ), respectively. NK cell culture supernatant was obtained from NK cell culture medium for 24 h. (A) Wound healing was observed microscopically (magnification, x100) and assessed as [(24 h cell-free area)/(0hr cell-free area)] × 100%. (B) MHCC-97H cell migration and invasion analysis (staining, crystal violet; magnification, x200).
Relative number = [(number of migrating cells in the vehicle or NK or IFN-γ groups)/(number of migrating cells in the vehicle group)]. The invasion was evaluated in the same way as migration. Data are expressed as mean ± standard deviation (n=3).
* p<0.05; ** p<0.01; *** p<0.001

the Kaplan-Meier method. All quantitative results are expressed as mean ± SD. Statistically significant differences were obtained using Student's t-test or one-way ANOVA. Values represent the results of three independent experiments. Values of P<0.05 were considered statistically significant.

Results

IFN-γ from NK cells inhibited the migration and invasion of MHCC-97H cells

To investigate the effect of IFN-γ on inhibition of HCC *in vitro*, HCC cells were cultured with a normal medium, NK cell culture supernatant, and the normal medium supplemented with IFN-γ for 24 h. Wound healing assay and Transwell assay were performed to evaluate the migration and invasion of MHCC-97H cells cultured with the above three media. Our results showed that the healing distance of MHCC-97H cells was significantly decreased in the NK and IFN-γ groups compared with the vehicle group, but there was no significant difference between the NK and IFN-γ groups (Figure 1A). Meanwhile, the Transwell migration assay showed that the NK and IFN-γ groups had significantly lower cell numbers compared with the vehicle group, and the inhibition of these two groups had the same results in the Transwell invasion assay (Figure 1B). Thus, the results indicate that IFN-γ alone or IFN-γ from NK cells can inhibit the migration and invasion of MHCC-97H cells.

Translated with www.DeepL.com/Translator (free version) IFN-γ from NK cells inhibited aerobic glycolysis of HCC

Abnormal glucose metabolism can promote the proliferation of tumor cells [16], and is an important hallmark of tumors. Based on the above results, we further explored the effect of IFN-γ on aerobic glycolysis in HCC cells. After culturing MHCC-97H cells with normal medium, NK cell supernatant, and normal medium-plus IFN-γ, glucose uptake, lactate production, and cellular ATP levels were detected. The results showed that the glucose uptake in the NK and IFN-γ groups was significantly lower than that in the vehicle group (Figure 2A). In addition, lactate release in the NK and IFN-γ groups was also significantly lower than that in the vehicle group (Figure 2B). In MHCC-97H cells, the rate of ATP production by mitochondria in normal cell culture was lower than that of NK cell supernatant and IFN-γ-containing cell supernatant, but the rate of ATP production by glycolysis was higher than that of NK cell supernatant and IFN-γ-containing cell supernatant (Figure 2c). Thus, the results showed that NK cells and IFN-γ could inhibit aerobic glycolysis in MHCC-97H cells.

IFN-γ from NK cells inhibited HCC migration and invasion through down-regulating HCC aerobic glycolysis

In the previous studies, we found that both NK cell supernatant and medium containing INF-γ inhibited aerobic glycolysis of MHCC-97H cells and reduced MHCC-97H cell migration and invasion. We wonder to know whether IFN-γ affects aerobic glycolysis of cells through rate-limiting enzymes, including Hexokinase 2 (HK2) and Phosphoglycerate Kinase 1 (PGK1), which alter the metastasis of HCC cells. To further study of which IFN-γ inhibits aerobic glycolysis and then reduces HCC migration and invasion in MHCC-97H cells, we added different concentration of glucose to IFN-γ pretreated MHCC-97H cells, assayed the invasion and migration ability and protein expression levels of aerobic glycolysis rate-limiting enzymes. The results showed that the count of cells in the IFN-γ group and IFN-γ plus glucose group was significantly reduced, compared to the control group, the count of cells in the IFN-γ plus glucose group

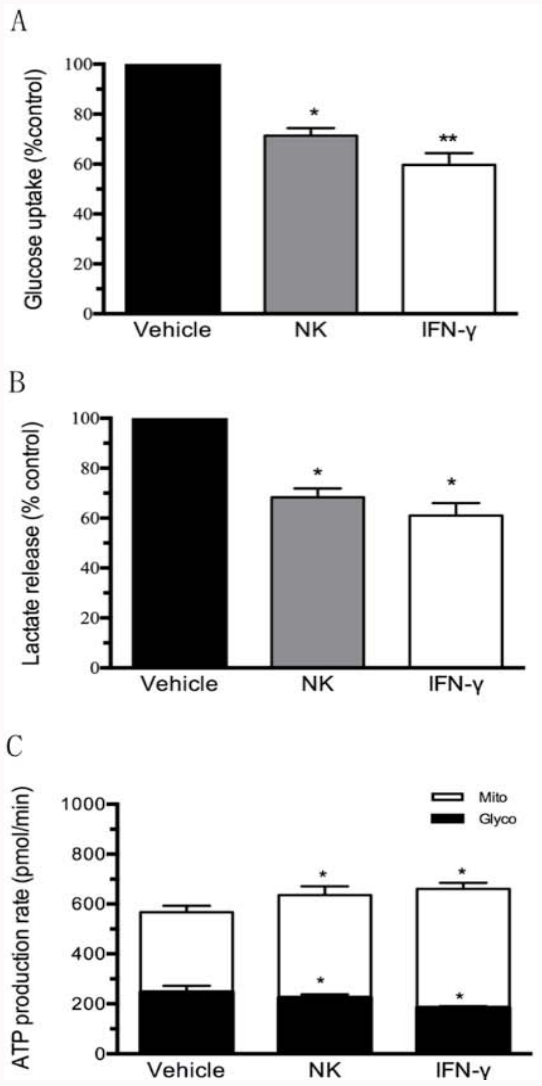

Figure 2: IFN-γ and NK cells inhibited aerobic glycolysis of HCC cells. MHCC97-H cells were cultured with complete medium (vector), NK cell culture supernatant (NK), and complete medium plus IFN-γ for 24 h. Cellular glucose uptake (A), lactate release (B), and ATP levels (C) were measured. Data are expressed as mean ± standard deviation (n=3).
* p<0.05; ** p<0.01; *** p<0.001

was significantly increased, compared to the IFN-γ group, and the number of cells in the G2 group was significantly increased compared to the G1 group (Figure 3A). West Bolt (WB) analysis further showed that compared to the control group, IFN-γ decreased the expression of HK-2 and PGK1 in MHCC-97H cells, but glucose ameliorated the inhibitory effect of IFN-γ on the expression of these two aerobic glycolytic rate-limiting enzymes and showed a grape volume dose-dependent phenomenon (Figure 3B). The above results indicated that IFN-γ inhibited the metastasis of MHCC-97H cells by down-regulating aerobic glycolytic rate-limiting enzymes.

Aerobic glycolysis of HCC inhibits IFN-γ secretion from NK cells

Although HCC patients have abundant NK cells in peripheral blood and around HCC lesions, there are still many intra- and extra-hepatic metastases. More and more studies suggest that NK cells in the HCC patients cannot kill tumor cells because the tumor microenvironment favors tumorigenesis and progression.

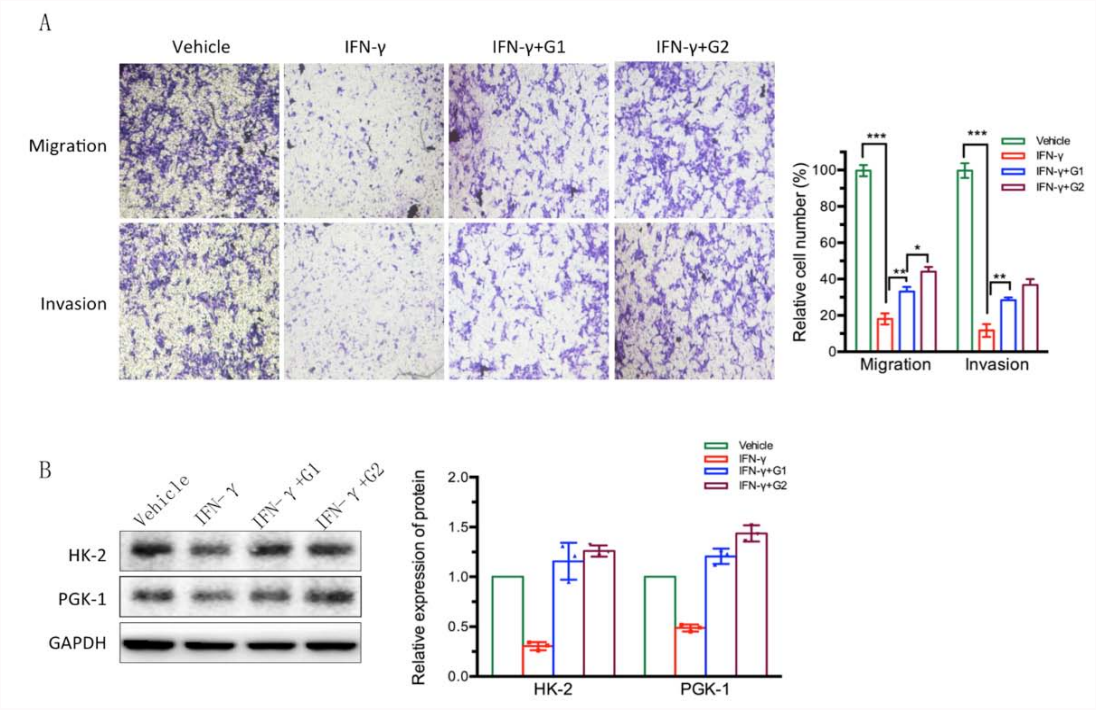

Figure 3: IFN-γ inhibited HCC migration and invasion by regulating HCC aerobic glycolysis.
(A) Transwell assay examined the migration and invasion ability of HCC cells treated with IFN-γ and glucose. Invasion was assessed in the same way as migration. (B) Immunoblot analysis of changes in expression of gluconeogenesis-associated rate-limiting proteins in IFN-γ and IFN-γ inhibitor-treated HCC cells. Protein bands were quantified and normalized in histograms. Histograms depict the fold changes of vehicle, IFN-γ, IFN-γ+G1, and IFN-γ+G2, respectively. Data are expressed as mean ± standard deviation (n=3).
* p<0.05; ** p<0.01; *** p<0.001

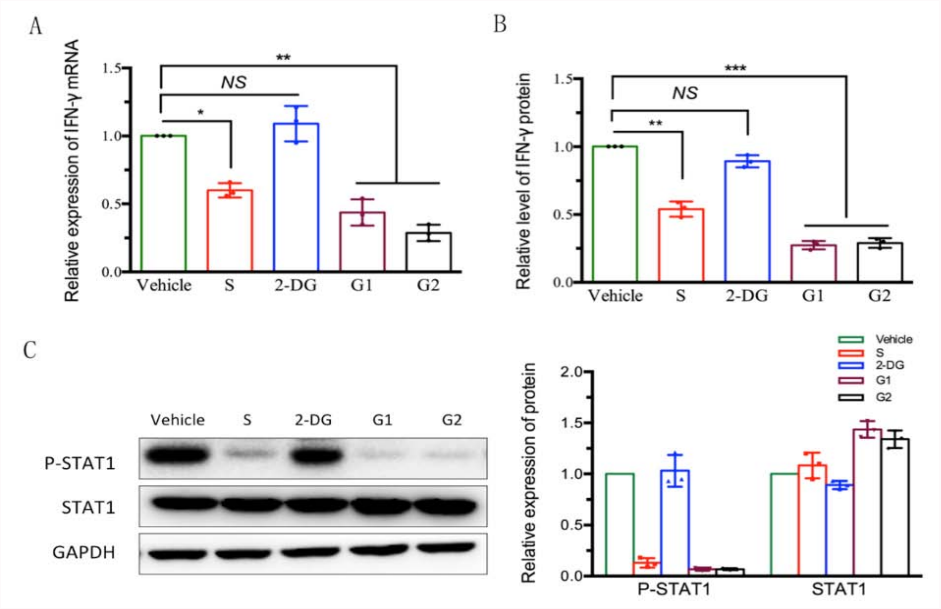

Figure 4: Aerobic glycolysis of HCC inhibited IFN-γ secretion from NK cells.
NK cells were cultured in normal medium (Vehicle), MHCC-97H culture Supernatant (S), MHCC-97H + 2-DG supernatant (2-DG), MHCC-97H + G1 supernatant (G1), and MHCC-97H + G2 supernatant (G2), respectively. (A) RT-qPCR was used to detect IFN-γ mRNA expression, and (B) ELISA was used to detect IFN-γ protein expression. Relative IFN-γ expression = [(IFN-γ expression in each group)/(IFN-γ expression in vehicle group)]. (C) Immunoblot analysis of STAT1 pathway protein expression changes in NK cells treated with different conditioned media. Protein bands were quantified and normalized in a histogram. Each experiment included experimental and control groups and was repeated at least three times. Data are expressed as mean ± standard deviation (n=3).
ns = non-significant; * P<0.05; ** P<0.01; *** P<0.001; 2-DG: 2-Deoxy-D-Glucose

Therefore, these NK cells are not functional enough in monitoring and clear tumor cells. We wanted to know whether HCC regulates the function of NK cells by regulating the secretion of IFN-γ. The Supernatant (S) of MHCC-97H cells, the supernatant of 2-Deoxy-D-

glucose (2-DG), a glucose analogue that is a glycolysis inhibitor, and the supernatant of glucose (G1 and G2) were co-cultured with NK cells, and the secretion of INF-γ in NK cells was detected by Q-PCR and ELISA, respectively. The results showed that the INF-γ secretion was significantly decreased in mRNA and protein level in NK cells, which treated with supernatant of MHCC-97H cells and supernatant of glucose compared with normal medium (Vehicle), but there was no change in IFN-γ secretion with supernatant of 2-deoxy-D-glucose compared with normal medium (Figure 4A, 4B), and this result indicated that the secretion, regulated by aerobic glycolysis of HCC, of MHCC-97H cells down-regulated INF-γ secretion by NK cells. In addition, a mass of evidence suggests that STAT1 is an important protein linking signal transduction between cell membrane receptors and effectors. STAT1 can be phosphorylated and aggregated into homodimers or heterodimers upon stimulation by extracellular signals, which then enter the nucleus and promote transcription of target genes. Phosphorylated STAT1 (p-STAT1) positively regulates the secretion of IFN-γ by NK cells [17]. Here we analyzed the protein level of p-STAT1 on NK cells treated with S, 2-DG, G1, and G2 groups, respectively. The result indicated that the expression of p-STAT1 was decreased in S, G1, and G2 groups, but no change in the 2-DG group compared with the Vehicle group. Interesting, it is consistent with IFN-γ secretion in these groups. The link between p-STAT1 expression in NK cells and aerobic glycolysis in HCC cells attracts us to further study.

Discussion

The morbidity and mortality are high in HCC [18]. The development of distant metastases in HCC patients is closely related to immune imbalance and is an essential cause of treatment failure [19,20]. Our study showed that IFN-γ secretion by NK cells inhibited the migration and invasion of MHCC-97H cells by down-regulating aerobic glycolysis. NK cells are known to play an important role in fighting against tumors. NK cells can directly kill tumor cells through perforin and granzyme upon contact with HCC cells. NK cells can also suppress tumors by secreting cytokines such as IFN-γ and tumor necrosis factor [21,22]. Interestingly, Kai Su et al. showed that fibrinogen-like protein 2 contributes to the hypercoagulable state of tumors and induces tumor metastasis *via* IFN-γ. Hiroto Kayashima noted that IFN-γ inhibited recurrence after liver HCC transplantation in mice. Meanwhile, a large number of studies have shown that aerobic glycolysis is prevalent in a variety of tumors, including prostate cancer [23], gastric cancer [24], lung cancer [25], pancreatic cancer [26], renal cell carcinoma [27], and breast cancer [28]. Bustamante E, Beyolu D [29], and Li S [30] demonstrated that inhibition of aerobic glycolysis in HCC cells promotes cell death. There are three rate-limiting enzymes in aerobic glycolysis, including HK2, PGK1, and pyruvate kinase. HK2 catalyzes the conversion of glucose to glucose-6-phosphate [31]. HK2 is highly expressed in HCC tissues and directly correlates with the pathological stage [32]. It is more efficient than other isomers in promoting aerobic glycolysis [33]. PGK1 is another important rate-limiting enzyme that controls aerobic glycolysis in HCC cells. Huijun Xie et al. reported that PGK1 drives HCC metastasis by enhancing metabolic processes [34]. Hongli Hu et al. noted that acetylation of PGK1 promoted the proliferation and tumorigenesis of hepatocellular carcinoma cells [35]. Our study showed that IFN-γ secreted by NK cells could inhibit the aerobic glycolysis of HCC cells and suppress the migration and invasion of HCC by down-regulating HK-2 and PGK-1. It suggests that IFN-γ secreted by NK cells can regulate aerobic glycolysis of HCC cells, thereby reducing HCC metastasis.

Surprisingly, in our study, aerobic glycolysis of MHCC-97H cells, in turn, reduced IFN-γ secretion from NK cells by inhibiting STAT1 activation, mitigating the effects of metastasis inhibition from NK cells. Numerous studies have shown that HCC cells survive by changing themselves to evade the immune system when attacked by immune cells.

There are many explanations for how HCC evades immune surveillance through aerobic glycolysis. Aerobic glycolysis can promote the metastasis of HCC to the following evidence. (1) ATP production during aerobic glycolysis, allows tumors to adapt to a microenvironment lacking energy resources [36]. (2) The enhancement of aerobic metabolism promotes the increase of metabolic intermediates. The production of these intermediates can enter other metabolic pathways for nucleotides and protein synthesis that promote cancer cell proliferation [37]. (3) Production of lactate and Hydrogen ions (H+) inhibits immune cell function [38]. Many studies have found that migration and invasion of HCC are associated with enhanced aerobic glycolysis of HCC. Li et al. found that different HCC cell lines, including MHCC-97H, and HCC-LM3, showed high levels of aerobic glycolysis. It is consistent with our results [39-41].

Impaired NK cells are an important factor in tumor development. It has reported that patients with liver metastases from rectal and gastric cancers have a low number of lethal NK cells in the liver. Patients with HCC have significantly reduced functional receptors for NK cells and a significantly weaker secretion of cytokines for IFN-γ. p-STAT1 expression in NK cells is necessary for NK cell activation [42]. Our study showed that the supernatant of HCC cells inhibited the secretion of IFN-γ in NK cells, and aerobic glycolysis of MHCC-97H cells was negatively correlated with IFN-γ secretion by NK cells. However, what component of the supernatant of MHCC-97H reduces p-STAT1 in NK cells requires further investigation.

In this study, we investigated the mechanism of NK cells that inhibited HCC metastasis. The results showed that IFN-γ secretion by NK cells inhibited aerobic glycolysis of HCC and reduced the metastasis of HCC cells. HCC cells, in turn, inhibited IFN-γ secretion by NK cells through inhibition of the STAT1 signaling pathway. However, the mechanisms by which IFN-γ inhibits aerobic glycolysis in HCC and down-regulates p-STAT1 expression in NK cells in HCC culture supernatant need further exploration. We believe that our study could provide a scientific basis for future clarification of HCC cell evasion of immune surveillance and prevention of HCC metastasis.

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