Effects of Adenosine and Lactate Coexistence on NK92 Cell

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Authors' contributions

This work was carried out in collaboration among all authors. XX and NW contributed equally to this study. CC conceived and designed the study; XX and NW collected the data; CC and DF performed the analysis; CC and XX wrote the manuscript and performed the experiments. All of the authors have read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: This study aims to investigate the impact of lactate and adenosine, present in the tumor microenvironment (TME), on the immune cell immunity.
Methodology: Five groups included Adenosine concentration screening groups, L-lactic acid and Sodium L-lactate concentration groups, Adenosine + L-lactic acid and Adenosine + sodium L-lactate concentration groups were chosen to evaluate the NK92 cell functions. The proliferation ability and morphological observations of NK92 cells were assessed using a hemocytometer. The CCK-8 assay measured the inhibition of NK92 cell activity in the treatment group, while the crystal violet method evaluated the effect of NK92 cells on the killing ability of A549 cells.
Results: A concentration of 50 µM adenosine served as a reference for high adenosine experimental concentrations, demonstrating a significant impact on NK92 immune cells within the TME. The functional entity "lactic acid" revealed independent effects of lactate [La-] and hydrogen ions [H+]. Lactate enhanced cell viability but reduced NK92 cytotoxicity. Conversely, lactic acid containing hydrogen ions caused a sharp decrease in cell viability and cytotoxicity to tumor cells.
Conclusion: Elevated adenosine concentration and acidification of the tumor microenvironment significantly inhibit the ability of NK cells to kill tumor cells.

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1. INTRODUCTION

Cancer initiation and progression involve intricate cellular interactions between precancerous/malignant cells, immune cells, stromal cells, and blood vessels. Cellular interactions are regulated by levels of tissue oxygen, metabolic byproducts, nutrients, and hormones, which subsequently influence tumor progression [1]. The tumor microenvironment (TME) is characterized by a complex environment, where tumor cells gain a growth advantage through aerobic glycolysis or the "Warburg effect" [2]. Consequently, this leads to increased pericellular accumulation of organic acids, such as lactic acid, and decreased extracellular pH [3]. Additionally, Adenosine triphosphate (ATP) is released during local tumor cell death caused by oxygen and nutrient deprivation [4], which is then catalyzed by CD73 and CD39 on various cells to produce adenosine [5]. These factors are abundant in the TME and play a role in governing the cytotoxicity of immune cells [6]. Previous studies have demonstrated that adenosine and lactic acid alone can affect the immune regulation of immune cells, such as adenosine receptors [7], lactate receptors [8], and acidosis [9,10]. However, the coexistence of both factors can also influence immune cell regulation, although their precise biological functions in immune cells are yet to be fully understood.

Studies have shown that the immunosuppressive microenvironment poses significant challenges to the characteristics of natural killer (NK) cells, resulting in hypofunction, exhaustion, and impaired anti-tumor activity [11]. NK cells are a crucial component of the innate immune system and possess non-specific recognition of tumor cells. They have a short lifespan in the body and minimal potential side effects, making them an emerging cell type in immunotherapy [12]. NK92 cells, derived from peripheral blood mononuclear cells of a patient, are an IL-2-dependent NK cell line with comparable functionality to NK cells and exhibit potent killing activity against target cells [13].

In this experiment, NK92 cells were employed as the subject of investigation. The effects of adenosine or lactate alone on NK92 cells were examined, followed by exploring the impact of simultaneous adenosine and lactate exposure on NK92 cell viability and cytotoxicity. The objective was to provide experimental support for the influence of lactate and adenosine on immune cell immunity in the TME.

2. MATERIALS AND METHODS

2.1 Cell Lines and Reagents

NK92 cells and the lung cancer cell line A549 were obtained from the China Center for Type Culture Collection (CCTCC, Wuhan, China). NK92 cells were cultured in MEM-α (Gibco) supplemented with 12.5% fetal calf serum (Sigma-Aldrich) and 12.5% horse serum (Gibco). A549 cells were maintained in 10% Ham's F-12K medium. Adenosine (purity ≥99%) was supplied by Sigma Aldrich (Merck KGaA, Darmstadt, Germany). A total of 1.3362 g adenosine (ADO) was dissolved in 100 mL sterilized distilled water to produce a stock solution with a theoretical final concentration of 0.05 M. Dosing solutions were prepared by serially diluting the stock solution with sterilized MEM-α medium. Final concentrations were set as 500 μM, 50 μM, and 5 μM. The control group contained 0 μM adenosine solution. L-lactic acid (L-LA) and sodium L-lactate (L-NaL), supplied by Aladdin (Shanghai, China), were dissolved in 10 mL of sterilized MEM-α medium to create a 0.1 M stock solution. Dosing solutions were prepared by serially diluting the stock solution with sterilized MEM-α medium. Final concentrations were set as 40 mM, 30 mM, 20 mM, and 10 mM. The highest concentration of 40 mM L-lactic acid was chosen based on previous research [14]. All other chemicals and solvents used were of analytical or pharmaceutical grade.

Five groups were chosen to evaluate the NK92 cell functions. (1) Adenosine concentration screening groups included an adenosine treatment group (5, 50, and 500 μM, dissolved in MEM-α medium), a blank control group (equal volume of MEM-α medium), and a positive control group (equal volume of NK92 cell complete culture medium). (2) L-lactic acid concentration groups included a L-lactic acid group (10, 20, 30, and 40 mM L-lactic acid dissolved in NK92 cell basal medium), a blank group (NK92 cell basal medium), and a positive control group (NK92 cells fully cultured in the basal medium). (3) Sodium L-lactate concentration groups included a sodium L-lactate group (10, 20, 30, and 40 mM L-sodium lactate dissolved in NK92 cell basal medium), a blank

group (NK92 cell basal medium), and a positive control group (NK92 cell complete culture medium). (4) Adenosine + L-lactic acid concentration groups included an Adenosine + L-lactic acid group (adenosine with a final concentration of 50 µM and final concentrations of 10, 20, 30, and 40 mM L-lactic acid dissolved in MEM-α medium), a blank group (NK92 cell basal medium), and a positive control group (equal volume of NK92 cell complete medium). (5) Adenosine + sodium L-lactate concentration groups included an Adenosine + sodium L-lactate group (adenosine with a final concentration of 50 µM and final concentrations of 10, 20, 30, and 40 mM sodium L-lactate dissolved in MEM-α medium), a blank control group (NK92 cell basal medium), and a positive control group (equal volume of NK92 cell complete medium).

To prevent the rapid metabolism or transport of adenosine in the environment, EHNA [erythro-9-(2-hydroxy-3-nonyl)adenine] with a final concentration of 10 µM and 0.5 µM dipyridamole were added to the experimental groups related to adenosine treatment.

2.2 Cell Proliferation and Morphological Observation

NK92 cells in good growth status were collected and counted. The number of NK92 cells per well was 4×10^5, and they were cultured in a 6-well cell culture plate with 2 mL of volume per well. The chemicals to be tested were added as described above. The cells were counted every other day, and the culture medium in the well plate was half-replaced. The fresh complete culture medium added also contained the tested chemicals as 50 µM adenosine solutions, 20 mM L-lactic acid and 20 mM sodium L-lactate solutions. The control cells were not treated. Each group had three replicate wells. The cells were cultured in a pre-set cell culture incubator with specified parameters. On the sixth day, each well was observed and photographed.

2.3 Cell Viability Assay

NK92 cells were seeded in 96-well plates at a density of 2×10^4 cells/100µL. The cells were then treated with adenosine solution, L-lactic acid, and sodium L-lactate solutions. Adenosine solutions were used at final concentrations of 5, 50, and 500 µM. L-lactic acid and sodium L-lactate solutions were used at final concentrations of 10, 20, 30, and 40 mM. The control cells (CK) were

not treated. After 12 hours of incubation, 10 µL of CCK8 solution (Beyotime, Ltd., China) was added to each well of the 96-well plate. The plate was incubated at 37°C in a 5% CO_2 environment for 2 hours. The absorbance at 450 nm was measured using a FlexA-200 microplate reader (Aosheng, Ltd., China).

2.4 Crystal Violet Assay

The cytotoxicity of lactate-co-cultured NK92 cells against tumor cells was assessed using a modified crystal violet assay described in a previous study [15]. The assay measures the maintained adherence of cells through staining the attached cells with crystal violet dye that binds to proteins and DNA. Briefly, A549 cells were seeded onto 96-well plates at a density of 1×10^4 cells/100µL per well and allowed to adhere for 12 hours. Prior to co-culturing with NK92 cells, the supernatant medium in the 96-well plate was removed. Additionally, NK92 cells were treated with five groups for 12 hours as described above, respectively. Then, 100 µL of treated NK92 cells at a concentration of 2×10^5 cells/mL were added to each well and incubated for 6 hours at 37°C in a 5% CO_2 environment. A positive control and a blank control were set up, and all treatments were repeated three times. After 6 hours of incubation, the medium was aspirated and each well was cleaned twice with 200 µL PBS buffer. The plate was tapped on filter paper to remove any remaining liquid. Then, 100 µL of 0.5% crystal violet staining solution (Sangon Ltd., Shanghai, China) was added to each well and incubated for 15 minutes at room temperature. Each well was washed twice with PBS buffer, and the plate was allowed to air dry for 2 hours at room temperature without its lid. Absorbance (A) was detected at a wavelength of 590 nm using a microplate reader. The results were expressed as the cytotoxicity percentage of NK92 cells using the formula: cytotoxicity percentage = [100% - ($A_{experiment}$ - A_{blank}) / ($A_{positive}$ - A_{blank}) × 100%], where $A_{experiment}$ refers to the absorbance value of the experimental group, $A_{positive}$ refers to the absorbance value of the positive group, and A_{blank} refers to the absorbance value of the blank control group.

2.5 Statistical Analysis

Two groups of data were analyzed using a t-test, while multiple groups of data were analyzed using one-way analysis of variance (ANOVA) with Tukey's test. All analyses were conducted in

the latest version of Data Processing System (DPS) software [16]. All data were presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

3. RESULTS

3.1 Proliferation Ability and Morphology of NK92 Cells

To evaluate the long-term effect of 50 µM adenosine, 20 mM lactic acid, and 20 mM sodium lactate on the proliferation of NK92 cells, a hemocytometer counting method was employed (Fig. 1 A). The proliferation trends in the adenosine group and the control group were similar, but the total number of cells in the L-lactic acid and sodium L-lactate groups significantly decreased on the 6th day compared to the control group. Notably, the control group

and lactate-treated group exhibited cell doubling every two days, while the sodium L-lactate group showed minimal growth from the 2nd to 6th day. This finding suggests that long-term continuous treatment with L-lactic acid or sodium L-lactate reduces the total number of NK92 cells.

The growth status of NK92 cells was also observed under an inverted microscope on the 6th day (Fig. 1 B). In the control group, cells grew mainly in groups with good growth status (Fig. 1 B-a). The adenosine-treated group showed increased cell fragments but still grew in groups (Fig. 1 B-b). In the L-lactic acid-treated group, some cells formed clumps, but there were also more single cells (Fig. 1 B-c). In the sodium L-lactate-treated group, cells grew mostly as single cells, with few clumps (Fig. 1 B-d). This indicates that long-term continuous treatment with adenosine, L-lactic acid, or sodium L-lactate affects the growth status of NK92 cells.
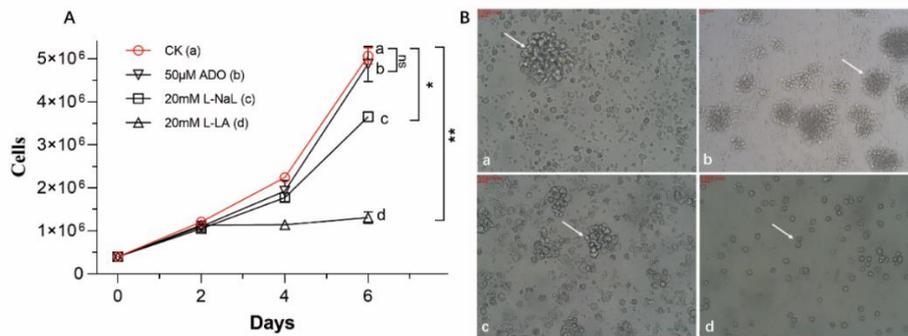
Fig. 1. NK92 cell proliferation ability (A) and morphological observation (B). (a) Untreated group of NK92 cells, (b) NK92 cells treated with 50 µM adenosine, (c) NK92 cells treated with 20 mM L-lactic acid, (d) NK92 cells treated with 20 mM sodium L-lactate. Bar=0.02 mm
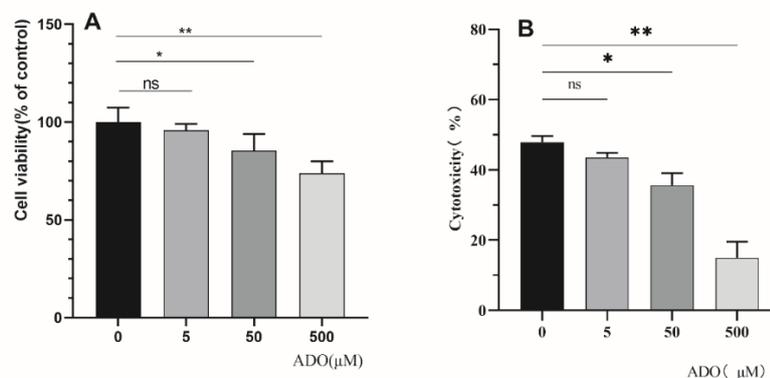
Fig. 2. Effects of adenosine on NK92 cell activity and cell killing. (A) Inhibitory effect of ADO on NK92 cells; (B) Killing effect of NK92 cells on A549 cells. "ns" indicates P ≥ 0.05, and " ** " indicates P < 0.01

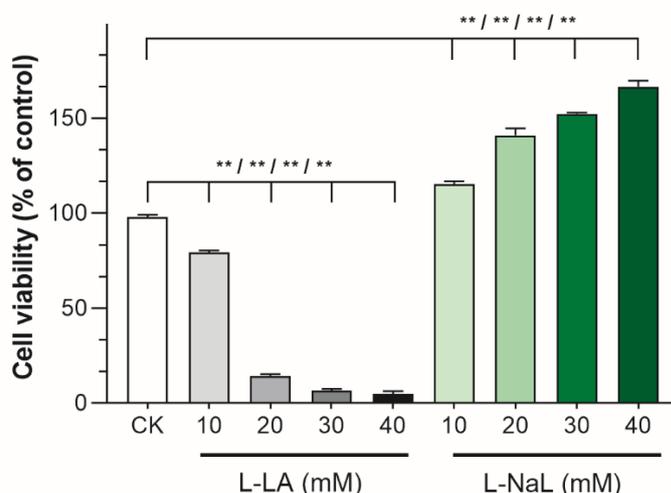## 3.2 Effects of Adenosine on NK92 Cell Viability and Cytotoxicity

To investigate the effect of adenosine on NK92 cell proliferation, a CCK8 reagent was used to measure relative activity rates after 12 hours (Fig. 2A). At adenosine concentrations of 5, 50, and 500 µM, the relative activity rates of NK92 cells were 95.9%, 85.5%, and 73.9%, respectively. Higher adenosine concentrations resulted in more pronounced inhibitory effects. Additionally, crystal violet staining was conducted to evaluate the effect of adenosine on NK92 cell toxicity. The killing rates of NK92 cells against A549 cells were 47.9%, 43.5%, 35.6%, and 14.9% at adenosine concentrations of 0, 5, 50, and 500 µM, respectively. Although the killing ability of NK92 cells did not significantly decrease at 5 µM adenosine, there was a downward trend. Moreover, a concentration of 50 µM adenosine significantly reduced the killing ability of NK92 cells against A549 cells compared to the control group. These findings suggest that adenosine significantly inhibits the cytotoxicity of NK92 cells against tumor cells, with a more pronounced effect at higher concentrations.

3.3 Effects of L-Lactic Acid and Sodium L-Lactate on NK92 Cell Viability and Cytotoxicity

To assess the effects of L-lactic acid and sodium L-lactate on NK92 cell proliferation, a CCK8 reagent was also used after 12 hours (Fig. 3). The relative activity rates of NK92 cells decreased to 97.9%, 79.3%, 14.3%, 6.7%, and 4.8% at L-lactic acid concentrations of 0, 10, 20, 30, and 40 mM, respectively, indicating that higher concentrations of L-lactic acid resulted in more pronounced inhibition of NK92 cell activity. To examine the influence of hydrogen ions in L-lactic acid on the inhibition of NK92 cell activity, a similar experiment was conducted using sodium L-lactate. The relative activity rates of NK92 cells at sodium L-lactate concentrations of 0, 10, 20, 30, and 40 mM were 97.9%, 115.2%, 141.0%, 152.3%, and 166.6%, respectively. The activity of NK92 cells increased significantly with higher concentrations of sodium L-lactate. These results suggest that sodium L-lactate inhibits cell activity in the short term, while sodium L-lactate promotes NK cell activity.

To determine the effects of L-lactic acid and sodium L-lactate on the cytotoxicity of NK92 cells, crystal violet staining was used (Fig. 4). At L-lactic acid concentrations of 0, 10, 20, 30, and 40 mM, the average killing rates of NK92 cells against A549 cells were 91.9%, 77.5%, 59.0%, 53.6%, and 38.7%, respectively. For sodium L-lactate concentrations of 0, 10, 20, 30, and 40 mM, the average killing rates of NK92 cells were 91.9%, 91.4%, 91.9%, 93.7%, and 91.4%, respectively. These findings indicate that L-lactic acid significantly inhibits the cytotoxicity of NK92 cells against A549 cells, with a more pronounced effect at higher concentrations. In contrast, sodium L-lactate had no significant effect on the cytotoxicity of NK92 cells.

Fig. 3. Effects of NK92 cell activity treated with L-lactic acid and sodium L-lactate. CK indicates "control". " ** " indicates P < 0.01

3.4 Effects of L-Lactic Acid and Sodium L-Lactate Coexisting with High-concentration Adenosine on NK92 Cell Viability and Cytotoxicity

Fig. 5 illustrates the impact of different concentrations of sodium L-lactate coexisting with high-concentration adenosine on the activity of NK92 cells. After a 12-hour treatment with 50 μM adenosine and sodium L-lactate concentrations of 0, 10, 20, 30, and 40 mM, the relative activity rates of NK92 cells were 90.3%, 76.1%, 10.9%, 6.3%, and 4.6% respectively. As the sodium L-lactate concentration increased, the inhibition of NK92 cell activity became more pronounced. Conversely, when the sodium L-lactate concentration was 0, 10, 20, 30, and 40 mM, and the adenosine concentration remained at 50 μM, the relative activity rates of NK92 cells after treatment were 90.3%, 113.5%, 134.5%, 143.2%, and 153.6%, respectively. The activity of NK92 cells increased significantly with the rise in sodium L-lactate concentration.

To assess the impact of different concentrations of sodium L-lactate and high-concentration adenosine on NK92 cytotoxicity, crystal violet staining was employed. The killing results are depicted in Fig. 6. With L-lactic acid concentrations of 0, 10, 20, 30, and 40 mM, the average killing rates of NK92 cells were 48.1%, 29.2%, 13.5%, 9.9%, and 3.3% respectively. The corresponding average killing rates with sodium L-lactate concentrations of 0, 10, 20, 30, and 40 mM were 48.1%, 46.0%, 49.1%, 50.3%, and 50.1% respectively. Under high concentrations of adenosine, sodium L-lactate significantly inhibited the cytotoxicity of NK92 cells towards A549 cells, while it had no significant effect on NK92 cell cytotoxicity. This aligns with the previous findings of reduced killing ability of A549 cells following treatment with different concentrations of adenosine by NK92 cells. These results demonstrate that both adenosine and L-lactic acid can inhibit NK92 cytotoxicity, and the inhibitory effect becomes more significant with increasing concentration.
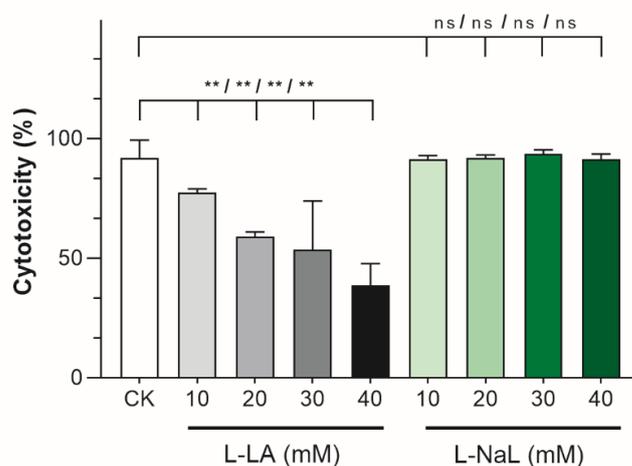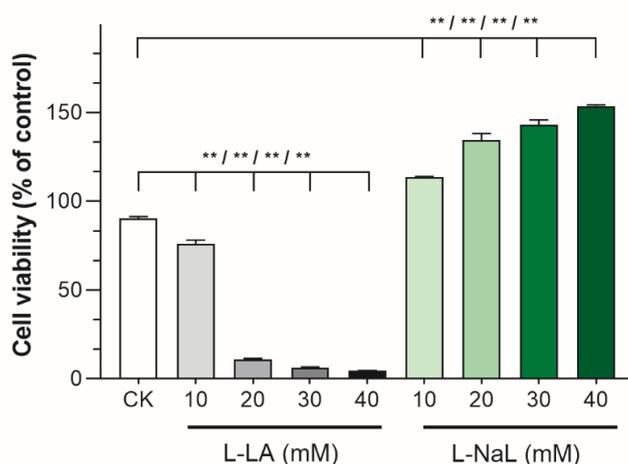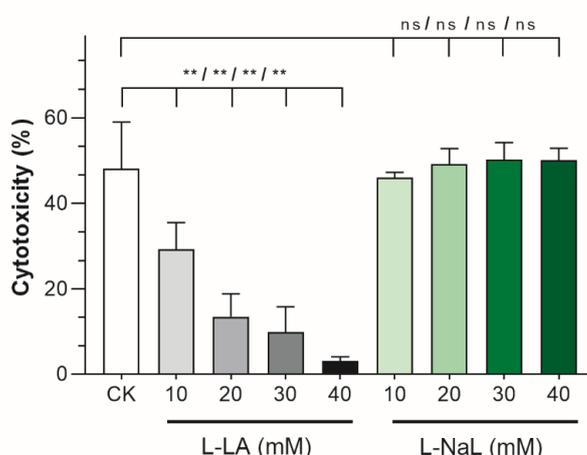

Fig. 4. Effects of NK92 cell on the killing ability of A549 cells treated with L-lactic acid and sodium L-lactate. CK indicates "control". "ns" indicates P ≥ 0.05, and " ** " indicates P < 0.01

Table 1. Variance analysis results

		Cell viability (%)		Cytotoxicity (%)	
Adenosine	0μM	87.60±58.57 a	F=43.57	78.11±20.60 a	F=559.33
	50μM	82.34±55.38 b	P=0.0001	34.75±19.06 b	P=0.0001
Lactate	L-NaL	130.82±24.70 a	F=13256.83	70.38±22.44 a	F=231.84
	L-LA	39.12±39.39 b	P=0.0001	42.47±29.21 b	P=0.0001
Concentrations	0mM	94.10±4.33 a	F=116.55	69.98±24.24 a	F=20.63
	10mM	96.04±19.30 a	P=0.0001	61.04±25.92 b	P=0.0001
	20mM	75.19±65.56 c		53.39±29.33 bc	
	30mM	77.13±73.89 c		51.86±32.31 c	
	40mM	82.40±81.33 b		45.86±33.14 c	

Fig. 5. Effects of NK92 cell activity treated with L-lactic acid and sodium L-lactate coexisting with 50 µM adenosine. CK indicates "control". " ** " indicates P < 0.01

Fig. 6. Effects of NK92 cells on the killing ability of A549 cells treated with L-lactic acid and sodium L-lactate coexisting with 50 µM adenosine. CK indicates "control". "ns" indicates P ≥ 0.05, and " ** " indicates P < 0.01

3.5 Comprehensive Analysis

Comprehensive analysis was conducted to investigate the impact of adenosine, L-lactic acid, and sodium L-lactate on NK92 cells. The results are presented in Table 1. Adenosine was found to have an inhibitory effect on the cell viability of NK92 cells. Specifically, the addition of 50 µM adenosine significantly reduced the activity of NK92 cells compared to cells without adenosine. Additionally, adenosine was found to significantly decrease the cytotoxicity of NK92 cells. Treatment with 50 µM adenosine reduced the cytotoxicity by approximately 44%, which decreased from $(78.11\pm20.60)\%$ to $(34.75\pm19.06)\%$. On the other hand, lactate was observed to enhance cell viability, with a recorded viability of $(130.82 \pm 24.70)\%$. However, under lactic acid treatment, cell viability was significantly lower at $(39.12 \pm 39.39)\%$. This suggests that the hydrogen ions present in lactic acid are detrimental to cell viability, indicating that pH value plays a crucial role in the viability of immune cells.

Interestingly, the effect of lactate on cytotoxicity was found to be insignificant. Contrary to this, treatment with lactic acid significantly reduced the cytotoxicity of NK92 cells to $(42.47 \pm 29.21)\%$, highlighting its significance.

Furthermore, it was observed that as the concentration increased, both cell viability and cytotoxicity exhibited a downward trend.

4. DISCUSSION

The tumor microenvironment is a complex and dynamic environment that includes immune cells and the extracellular matrix [17]. Previous studies have shown that the concentration of adenosine in normal tissue ranges from approximately 10 nM to 100 nM, while the concentration in the solid tumor microenvironment can reach up to 100 µM [18]. Even a concentration of 1 mM was studying the effect of adenosine on NK cell function [19]. Therefore, a concentration of 50 µM as our intermediate concentration of adenosine in the microenvironment was chosen. And one concentration above and below this average as reference points were employed to determine the adenosine concentration gradient. The results in Fig. 2 demonstrate that adenosine at a concentration of 50 µM can serve as a representative value of high adenosine concentration, which significantly affects the activity of NK92 immune cells.

The Warburg effect is a characteristic feature of tumor cell proliferation and metastasis. Even in the presence of sufficient oxygen, tumor cells still rely on glycolysis to obtain energy for their growth. The breakdown of glucose through glycolysis generates a substantial amount of lactic acid, which is then transported outside the cell, leading to the creation of an acidic microenvironment with a pH value between 6-6.5 [20]. The concentration of lactic acid can reach as high as 40 mM [21,22]. So a concentration of 40 mM lactic acid was selected as the maximum concentration, and then three lower and medium concentrations were set up as reference points. Subsequently, it was established five concentrations of lactic acid gradient for our experiments. Following a 12-hour exposure of NK92 cells to various concentrations of L-lactic acid, it was observed that as the concentration of L-lactic acid increased, the inhibition of NK92 cell activity became more prominent. To determine if the presence of hydrogen ions contributed to the reduced cell activity, it was conducted similar experiments using L-sodium lactate, a neutral salt of L-lactic acid, while keeping other conditions constant. The results revealed that as the concentration of sodium L-lactate increased, NK92 cell activity was enhanced. This finding is consistent with the experimental evidence that lactic acid, as a metabolic fuel, can promote tumor cell proliferation [23].

Lactic acid inhibits the immune function of cells and induces the transformation of immune-competent macrophages into the M2 type, resulting in immune tolerance. It also promotes the proliferation and survival of Tregs and myeloid-derived suppressor cells (MDSCs) while impairing the immune activity of NK cells and dendritic cells [20,24]. Fischer et al. discovered that the addition of lactic acid to cytotoxic T lymphocytes (CTL) cultures in vitro resulted in reduced cell proliferation, cytokine production, and a 50% decrease in cytotoxicity [25]. Similarly, Husain et al. found that lactic acid inhibited NK cytotoxic activity by reducing the expression of perforin, granzyme, and the cell-activating receptor NKp46 in NK cells [26].

During the culturing of NK92 cells, most cells tend to aggregate into clusters, while a few cells disperse. When there are numerous single cells and cell debris, these cells are more prone to cell death [27]. The cell morphology was examined using an inverted microscope. On the 6th day, the cells in the control group appeared round or nearly round, with a translucent appearance and clustering. The cells in the adenosine-treated group displayed no significant differences compared to the control group. Conversely, the cells in the L-lactic acid group lacked translucency, and most cells existed as single units. In the sodium L-lactate group, some cells formed clusters, but there was also an abundance of cell fragments. These findings illustrate that while adenosine treatment has no effect on NK92 cell proliferation, it does weaken cell viability to some extent. Prolonged exposure of NK92 cells to L-lactic acid leads to a decrease in total cell count and significantly impairs cell proliferation. Conversely, treatment with sodium L-lactate leads to a decrease in total cell count, but does not affect cell proliferation.

Additionally, the cytotoxicity of NK92 cells were assessed by using crystal violet staining. After co-culturing the treated NK92 cells with tumor cells for 6 hours, it was observed that the cytotoxicity of NK92 cells treated with L-lactic acid increased with the concentrations, whereas it decreased compared to the control group. In contrast, the cytotoxicity of NK92 cells treated with sodium L-lactate showed no significant difference. These findings confirm that lactic acid inhibits the cytotoxicity of NK92 cells, while sodium L-lactate has no apparent effect. This conclusion is consistent with previous studies demonstrating that an increase in lactate reduces the cytotoxicity of tumor-infiltrating T cells and

NK cells [28]. In the acidic tumor microenvironment, the cytotoxicity of NK cells decreases with increasing lactic acid levels, but can be restored by reversing pH [29].

When investigating the functional implications of the coexistence of adenosine and sodium L-lactate on NK92 cells, it was observed that adenosine has the ability to inhibit the activity of NK92 cells. Notably, the addition of 50 µM adenosine to NK92 cells resulted in reduced activity compared to cells without adenosine. Adenosine specifically had a significant impact on the cell killing capabilities of NK92 cells, with a concentration of 50 µM leading to a reduction in cell killing power by approximately 44%. The data is supported by Fig. 4 and Fig. 6, which demonstrate the major role of adenosine in inhibiting immune cell function within the tumor microenvironment. Furthermore, it is important to discern between lactate and hydrogen ions in research that combines them into a single functional entity referred to as "lactic acid" [30]. Our study reveals that lactate enhance cell viability, whereas cell viability significantly decreases under lactic acid treatment. This suggests that hydrogen ions in lactic acid are the primary factors impairing cell viability. However, the effect of lactate on cytotoxicity is not significant, whereas the combination of enhanced cell viability and its reduced impact on cytotoxicity implies an inhibitory effect of lactate. This inhibition may be attributed to the binding of lactate to the GPR81 receptor, which influences the decreased release of killing-related cytokines, consistent with a previous publication [8]. Conversely, the cytotoxicity of NK92 cells sharply declines following lactic acid treatment, indicating a highly significant effect. Considering its pronounced impact on cell viability, it can be speculated that the key factor affecting the cytotoxicity of lactic acid is the influence of hydrogen ions on cell viability or quantity. The impact of hydrogen ions on cytotoxicity requires further investigation, while the effect of lactate on cytotoxicity can be evaluated.

5. CONCLUSION

Our study demonstrates that adenosine plays a significant role in inhibiting the function of immune cells in the tumor microenvironment. A concentration of 50 µM of adenosine can be considered as a high adenosine experimental concentration that significantly impacts NK92 immune cells. The effects of lactate and hydrogen ions in the functional entity known as "lactic acid" are distinct. Lactate enhance cell viability, but when they interact with the GPR81 receptor, they impact the release of killing-related cytokines by NK92 cells, thereby inhibiting the killing power against tumor cells. On the other hand, lactic acid containing hydrogen ions causes a profound decrease in both cell viability and cytotoxicity to tumor cells. Therefore, high adenosine concentration and acidification of the tumor microenvironment significantly inhibit the killing of tumor cells by NK cells, thus influencing tumor progression.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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