Effect and mechanism of immune surveillance activated by autotransfusion on reducing liver cancer metastasis based on HLA-DQB1/PDCD1

Keywords
liver cancer, HLA-DQB1, autotransfusion, PDCD1, immune surveillance

Abstract
Introduction
Liver cancer is a common tumor of digestive system. Hepatectomy sometimes results in massive hemorrhage, and intraoperative autotransfusion is often required.

Material and methods
Methods: In this basic research, the liver cancer rat model was established, and the visible liver cancer tissue was surgically removed, and the blood of the mice was recovered.

Results
The results showed that the model group had the highest recurrence and metastasis of liver cancer, and the lowest was autotransfusion group. Flow cytometry showed that the number of HLA-DQB1 positive cells, the content of CD4+CD25+T cells, and the proliferation of CD4+T cells were the most in the autotransfusion group, followed by the model group, and the least in the auto+HLA-DQB1 group, which showed no difference from the control group. Immunohistochemistry and western blot analysis showed that the lowest expression of PDCD1 protein was in the autotransfusion group, which showed no significant difference from the control group, and the highest expression was in auto-HLA-DQB1, followed by the model group.

Conclusions
It elucidates the molecular mechanism that autotransfusion will activate the up-regulated expression of signal molecule HLA-DQB1, enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface molecule of liver cancer cells, and inhibit liver cancer metastasis. Autotransfusion activated the expression of signaling molecule HLA-DQB1, enhanced the immune surveillance of CD4+T cells, reduced the expression of PDCD1 on the surface of liver cancer cells, and reduced the metastasis and recurrence of liver cancer.

Explanation letter
Section Editor recommendation:
Accept after changes

Review 1:
Two questions
1. There are some spelling and grammar errors in the current version of the manuscript. Please correct them carefully and seek help from qualified colleagues or professional editing company if necessary.
Response: the spelling and grammar errors in this paper have been checked and revised carefully line by line with some English experts.
Line 51: “70%[1]” has been revised to “70% [1]”
Line 52-53: “However, the liver is rich in blood supply and complicated in anatomy, and the tumor growth sites are different and often invade the large intrahepatic vessels.” has been revised to “However, the liver is rich in blood supply and complicated in anatomy, and the tumor often invade the
large intrahepatic vessels.

Line 56: “30%-40%” has been revised to “30-40%”

Line 58: “than before, but for liver cancer” has been revised to “than before. But ”

Line 59: “is high, and blood” has been revised to “high. Therefore,”

Line 60: “signs[3]” has been revised to “signs [3]”

Line 78-79: “which is closely related to the immune surveillance function is closely related” has been revised to “which is closely related to the immune surveillance function.”

Line 97: “showed” has been revised to “show”

Line 123: “201-225g” has been revised to “201-225 g”

Line 140-142: these sentence have revised to “Normal rats and liver cancer model group 1 were intravenously injected with 1.0 mL normal saline. Autologous blood red cells (1.0 mL)”

Line 202: “The tumor recurrence rate was 80% and metastasis rate was 70% in model group.” has been revised to “The tumor recurrence rate was 80% and metastasis rate was 70% in model group, respectively. ”

Line 205: “80% and 70%” has been revised to “30% and 40%”

2. Please provide the description of the actual statistical analysis method used in the current study for each analysis. Mention only the statistical analysis software is not sufficient.

Response: thank you for your valuable suggestion. But the power analyses for the sample size assessment were not conducted. We analyzed the data using one-way analysis of variance (ANOVA) and the data were presented as the mean ± standard deviation (SD). Statistical significance was defined as P< 0.05.

Review 2:
The artical data is perfect and detailed, and statistical analysis method is accurate. These contents make the article easier to understand, and more persuasive. The data analysis of the results is compelling when the author uses the tables and graphs. And, the author uses some different Statistical analysis methods, thus, the conclusion from these data become more reasonable. Although this article has some disadvantages, it gives us a lot of useful knowledge and experience. I think this article should be accepted with a minor revision.

1. Please indicate the type of study in your abstract and methods sections.

Response: this study is a basic research, and now I have added it in the abstract.

Abstract

Introduction: Liver cancer is a common tumor of digestive system. Hepatectomy sometimes results in massive hemorrhage, and intraoperative autotransfusion is often required. Methods: In this basic research, the liver cancer rat model was established, and the visible liver cancer tissue was surgically removed, and the blood of the rat was recovered. Results: The results showed that the model group had the highest recurrence and metastasis of liver cancer, and the lowest was autotransfusion group. Flow cytometry showed that the number of HLA-DQB1 positive cells, the content of CD4+CD25+T cells, and the proliferation of CD4+T cells were the most in the autotransfusion group, followed by the model group, and the least in the auto+HLA-DQB1 group, which showed no difference from the control group. Immunohistochemistry and western blot analysis showed that the lowest expression of PDCD1 protein was in the autotransfusion group, which showed no significant difference from the control group, and the highest expression was in auto-HLA-DQB1, followed by the model group. Conclusion: It elucidates the molecular mechanism that autotransfusion will activate the up-regulated expression of signal molecule HLA-DQB1, enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface molecule of liver cancer cells, and inhibit liver cancer metastasis.

2. The results section should be checked. Other minor problems, please check it.

Response: the results section has been checked carefully and revised line by line in blue.

Line 204-205: “80% and 70%” has been revised to “The recurrence and metastasis rates of autotransfusion group were the lowest (30% and 40%, respectively), as shown in Table 1.”

Line 210-212: the results has been revised to “Flow cytometry results showed that the number of HLA-DQB1 positive cells was the largest in the autotransfusion group and autotransfusion could activate
HLA-DQB1 expression.

Line 212-213: “The second group was the model group, HLA-DQB1 was tumor cell specific antigen.” has been revised to “HLA-DQB1 was tumor cell specific antigen.”

3. The language is not proper and needs professional help.
Response: the spelling and grammar errors in this paper have been checked and revised carefully line by line with some English experts.

Line 51: “70%[1]” has been revised to “70% [1]”
Line 52-53: “However, the liver is rich in blood supply and complicated in anatomy, and the tumor growth sites are different and often invade the large intrahepatic vessels.” has been revised to “However, the liver is rich in blood supply and complicated in anatomy, and the tumor often invade the large intrahepatic vessels.”

Line 56: “30%-40%” has been revised to “30-40%”

4. Please state the limitations of your study and its findings.
Response: This research topic is a basic research, with a small sample size, lack of regional sample specificity investigation, and lack of in-depth verification of basic experiments, so further research is needed.

Review 3:
Generally this study design has principal mistake, and preparation of manuscript does not follow a scientific manner.

1. It should be noted that the paper is so poorly written and grammatically incorrect that it is very difficult in places to understand the presentation of the data and interpret it appropriately.
Response: the spelling and grammar errors in this paper have been checked and revised carefully line by line with some English experts.

Line 51: “70%[1]” has been revised to “70% [1]”
Line 52-53: “However, the liver is rich in blood supply and complicated in anatomy, and the tumor growth sites are different and often invade the large intrahepatic vessels.” has been revised to “However, the liver is rich in blood supply and complicated in anatomy, and the tumor often invade the large intrahepatic vessels.”

Line 205: “80% and 70%” has been revised to “30% and 40%”
2. Title: This is not a title, but is the aim of this study.
Response: I have revised the title of this research.

Line 1-3: Effect and mechanism of immune surveillance activated by autotransfusion on reducing liver cancer metastasis based on HLA-DQB1/PDCD1

3. The introduction is redundant and generally no graphic is used here.
Response: The diagram was added to illustrate the relationship between HLA-DQB1 and other proteins, as well as the design ideas of relevant studies.
Line 99-106: In addition, through the protein expression profile database, it was found that signaling molecule HLA-DQB1 had a regulatory axis relationship with protein PDCD1, as shown in Figure 1A below. This study proposed the hypothesis that "the expression of signal molecule HLA-DQB1 is upregulated to enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface of liver cancer cells, and inhibit the migration and invasion of tumor cells", as shown in Figure 1B. Meanwhile, the relevant molecular mechanism of autotransfusion to activate immune surveillance and reduce the metastasis of liver cancer was explored.

4. To the best of my cancer research experience, xenograft on wild type rat, but not on immune deficiency mouse, is almost impossible.
Response: the rats have been revised to mouse.

5. Injection or bleeding of 10 ml normal saline or blood from rat is unacceptable from animal ethic aspect.
Response: Thank you very much for your value suggestions. “10 ml normal saline or blood” is an error and has been revised.
Line 140-142: these sentence have revised to “Normal rats and liver cancer model group 1 were intravenously injected with 1.0 mL normal saline. Autologous blood red cells (1.0 mL)”

6. Autotransfusion of blood red cells is useless.
Response: Thank you very much for your value suggestions. I think autotransfusion will activate the upregulated expression of signal molecule HLA-DQB1, enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface molecule of liver cancer cells, and inhibit liver cancer metastasis.

7. your IHC staining seems like non-specific.
Response: Thank you very much for your value suggestions. The PDCD1 protein expression detection by immunohistochemistry. Immunohistochemical analysis of PDCD1 protein showed brownish yellow color. We can observe PDCD1 protein expression according to the depth of yellow color.
Review 4:
Accept
response.doc
Effect and mechanism of immune surveillance activated by autotransfusion on reducing liver cancer metastasis based on HLA-DQB1/PDCD1

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Abstract

Introduction: Liver cancer is a common tumor of digestive system. Hepatectomy sometimes results in massive hemorrhage, and intraoperative autotransfusion is often required. Methods: In this basic research, the liver cancer rat model was established, and the visible liver cancer tissue was surgically removed, and the blood of the mice was recovered. Results: The results showed that the model group had the highest recurrence and metastasis of liver cancer, and the lowest was autotransfusion group. Flow cytometry showed that the number of HLA-DQB1 positive cells, the content of CD4+CD25+T cells, and the proliferation of CD4+T cells were the most in the autotransfusion group, followed by the model group, and the least in the auto+HLA-DQB1 group, which showed no difference from the control group. Immunohistochemistry and western blot analysis showed that the lowest expression of PDCD1 protein was in the autotransfusion group, which showed no significant difference from the control group, and the highest expression was in auto-HLA-DQB1, followed by the model group. Conclusion: It elucidates the molecular mechanism that autotransfusion will activate the up-regulated expression of signal molecule HLA-DQB1, enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface molecule of liver cancer cells, and inhibit liver cancer metastasis.

Keywords: liver cancer; autotransfusion; HLA-DQB1; PDCD1; immune surveillance

Introduction

Liver cancer is a common tumor of digestive system, and whether hepatectomy can be performed largely determines its prognosis. According to statistics, the 5-year survival rate of liver cancer patients undergoing hepatectomy can reach more than 70% [1]. However, the liver is rich in blood supply and complicated in anatomy, and the tumor often invade the large intrahepatic vessels. Therefore, the implementation of standard hepatectomy often encounters the problem of massive hemorrhage during the surgery. It has been reported that the incidence of massive hemorrhage in patients undergoing hepatectomy is as high as 30-40% [2]. Despite the continuous progress of surgical techniques and instruments, the incidence of massive hemorrhage in hepatectomy is less
than before. But for liver cancer surgery with large vascular invasion or large tumor, the risk of massive hemorrhage is high. Therefore, blood transfusion is still a commonly used method to maintain the stability of intraoperative vital signs [3].

At present, the most commonly methods of blood transfusion include allogeneic transfusion and autotransfusion. Allogeneic transfusion may cause allergic and rejection reactions, as well as immunosuppression and tumor proliferation [4-5]. Autotransfusion refers to the infusion of a patient's own blood that has been stored in advance or recovered from blood loss when needed. Autotransfusion includes storage autotransfusion, dilution autotransfusion, and intra-operative cell salvage [6,7]. Autotransfusion has been paid more and more attention because it can effectively avoid the occurrence of infectious diseases and transfusion adverse reactions caused by allogeneic transfusion. Autotransfusion has been widely used in major surgical operations at home and abroad.

The effect of blood transfusion on the immune system is known as transfusion-related immunomodulation, which involves both an enhanced and suppressed immune response. In recent years, most studies believe that the immunomodulation induced by blood transfusion is mainly suppression, mainly manifested as non-specific immunosuppression and specific immunosuppression [8]. The mechanism of immunosuppression induced by blood transfusion is complex, and the exact mechanism has not been agreed yet. Some studies have suggested that protein PDCD1 has a regulatory effect on T cell proliferation, which is closely related to the immune surveillance function.

PDCD1 is an immunoglobulin that is mainly expressed on the surface of activated CD4+/CD8+ T cells, B lymphocytes and myeloid cells [9]. So far, more and more evidence indicates that PDCD1 is also widely expressed in tumor cells, and it can help tumor cells evade immune surveillance, thus improving the migration and invasion ability of tumor cells [10,11]. Previous studies have shown that inhibition of PDCD1 can inhibit the proliferation and metastasis of gastric cancer cells [12].

Human leukocyte antigen (HLA)-DQB1 allele polymorphism has been confirmed to be involved in human tumors [13]. Human leukocyte antigen (HLA) is associated with almost all immune-related diseases to varying degrees, especially autoimmune diseases, tumors and infectious diseases [14]. HLA-DQB1 expression is associated with tumor immunity. HLA-DQB1 is an HLA Class II β chain analogue that is essential for immune
response. Class II molecules are expressed by antigen-presenting cells and play a central role in the immune system by presenting peptides from extracellular proteins. Previous studies on HLA-DQB1 mainly focused on polymorphism and tumor susceptibility [15,16]. However, the relationship between HLA-DQB1 expression level and tumor prognosis in lung cancer has rarely been reported. It is noteworthy that in our study, HLA-DQB1 protein is expressed on tumor cells and affects anti-tumor immunity issues. Tumors with higher HLA-DQB1 levels show greater infiltration of CD4+ and CD8+ positive T lymphocytes [17].

In addition, through the protein expression profile database, it was found that signaling molecule HLA-DQB1 had a regulatory axis relationship with protein PDCD1, as shown in Figure 1A below. This study proposed the hypothesis that "the expression of signal molecule HLA-DQB1 is upregulated to enhance the immune surveillance of CD4+T cells, reduce the expression of PDCD1 on the surface of liver cancer cells, and inhibit the migration and invasion of tumor cells", as shown in Figure 1B. Meanwhile, the relevant molecular mechanism of autotransfusion to activate immune surveillance and reduce the metastasis of liver cancer was explored.

![Figure 1. Hypothesis diagram. A: Protein expression profile database; B: Diagram of molecular mechanism hypothesis of autotransfusion to reduce liver cancer metastasis based on HLA-DQB1/PDCD1.](image)

2. Materials and methods

2.1 Materials
HepG2 cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences; DMEM medium, FBS and trypsin were purchased from Sigma. CFDA-SE (C-1157) was purchased from Molecular Probes. CD4-PE antibody, CD25-FITC antibody were purchased from Sigma. FITC-CD4 antibody, PE-CD25 antibody, PDCD1, ECM1, E-cadherin antibody were purchased for proteintech. Diaminobenzidine (DAB) were purchased from Innochem. ELISA Kit was purchased form Beyotime Biotechnology. Domestic self-2000 blood recovery machine (BW-8100A) was purchased from Beijing Wandong Medical Equipment Co., LTD. Western blotting instruments (Eppendorf); Flow meter BD; Ultra-thin slicer (LKB - V;JEOL Co.Japan). Healthy SD mice, grade SPF, 7 weeks, 30-40 g, were purchased from Beijing Weitonglihua Experimental Animal Technology Co., LTD., Quality certificate No. : SCXK(Beijing) 2016-006. All mice were kept in a constant temperature and humidity animal house, and were fed with clean grade special feed. All animal experiments have passed the review of ethics Committee of Harbin Medical University (No. 20210224).

2.2 Methods

2.2.1 Establishment of live cancer rat model
Sixty SD mice were randomly divided into 4 groups. One group was model group. HepG2 cells were injected subcutaneously into the other 3 groups. The tumorigenesis of mice was observed and recorded every 3 days. The rat model of liver cancer was established for 42 days. The experimental groups were numbered as normal group, liver cancer model group 1, liver cancer model group 2, and liver cancer model group 3.

2.2.2 Autotransfusion in mice
After the liver cancer tissue was resected in the model mice, the operative wound was sutured with surgical suture, and blood was recovered from the operative field of each mouse by the domestic autologous 2000 blood recovery machine. Normal mice and liver cancer model group 1 were intravenously injected with 1.0 mL normal saline. Autologous blood red cells (1.0 mL) were collected from each mouse in the model groups 2 and 3 by tail vein infusion. The model group 3 was injected with HLA-DQB1 antibody for 8 weeks. The experimental group was renumbered as control, model, autotransfusion, and autotransfusion+HLA-DQB1 (auto+HLA-DQB1).
2.2.3 Tumor recurrence and metastasis rates in mice

After 8 weeks of feeding, the mice were anesthetized by chloral hydrate and sacrificed by neck amputation. The liver tissues of the mice in each group were collected, and the recurrence and metastasis of the mice in each group were observed and recorded. The recurrence rate was calculated by the number of animals with tumor recurrence. Tumor metastasis rate: distant metastasis rate was calculated by counting tumor nodules on the surface of liver tissue. Liver tissue was fixed with Bouin solution, and liver tissue was observed the yellow tumor metastases and white after 24-48 h. The number of tumor metastases was calculated by naked eye.

2.2.4 Number of HLA-DQB1 positive cells

CFDA-SE was dissolved with DMSO (5 mmol/L) and stored at -20°C, then balance to room temperature before use. 2.5 μmol CFDA-SE was thoroughly mixed with HLA-DQB1 protein. The number of HLA-DQB1 positive cells in the blood of each group was detected by flow cytometry.

2.2.5 Content of CD4+CD8+T cells

Abdominal aorta blood and peripheral blood mononuclear cells were collected from each group, then PBS was added and the cell density was adjusted to $1 \times 10^6$ /L. The cells were incubated with FITC-CD4 antibody and PE-CD25 antibody. The content of CD4+CD8+T cells was detected by flow cytometry.

2.2.6 Proliferation of CD4+T cells

Abdominal aorta blood of mice in each group was collected, cells were centrifuged, and CD4+T cells were labeled with CFSE. The cells were suspended to $1 \times 10^6$ cells/mL with RPMI-1640 and stained with 1 μL staining solution. The solution was incubated at 37°C with 5% CO$_2$ for 20 min. Centrifugation was performed at 1200 rpm for 5 min, and supernatant was discarded. The proliferation of CD4+T cells in blood of each group was detected by flow cytometry.

2.2.7 PDCD1 protein expression detection by immunohistochemistry

Paraffin samples were prepared from the liver tissues of each group. Each paraffin tissue was cut into 2 pieces with a thickness of about 5 μm and used for PDCD1 protein staining. The paraffin sections were washed repeatedly with ionized water, and the blocking antibody was added into the milk liquid for 5 min. PDCD1 antibody were added
and incubated at 37°C for 2 h. The samples were washed with PBS for three times. Fluorescent staining labeled secondary antibody was added and incubated at 4°C for 30 min. Diaminobenzidine (DAB) chromogenic agent was added after PBS washing. The slices were dehydrated by gradient ethanol, sealed by neutral gum and observed under microscope.

2.2.8 Detection of PDCD1, ECM1, E-cadherin protein expression by western blot

Tissue (20 mg) is centrifuged and added lysate (RIPA: PMSF = 99:1, 200 μL). 3 magnetic beads are added to lysis tissue (4°C, 45 Hz, 60 s). Then BCA kit is used to measure the protein concentration, and 5×SDS-PAGE protein plus sample buffer is added. After being dried in hot bath at 100°C for 15 min, samples are stored in -80°C for subsequent running electrophoresis. A total of 50 μg of protein from each sample is loaded and transferred to the PVDF membrane. The primary antibodies used for immunoblotting are as follows: PDCD1, ECM1, E-cadherin1 (1:1000), GAPDH (1:1000). After the second antibody is incubated, the cells are slowly shaken and incubated at room temperature for 1 h. Image J software is used to analyze the gray levels of protein bands in each group.

2.2.9 Serum levels of inflammatory cytokines IL-10 and IFN-γ

After anesthesia, blood was taken from the abdominal aorta of mice, centrifuged (3600 r • min\(^{-1}\) × 10 min), and the serum was frozen at -80°C. After balancing the reagents and serum samples to room temperature, the procedure was performed according to the ELISA kit instructions. The absorbance was detected at 450 nm and 630 nm, and calculate the sample OD value according to the standard curve.

2.3 Statistical analysis

All experiments were replicated independently at least three times. The data were analyzed using one-way analysis of variance (ANOVA) and are presented as the mean ± standard deviation (SD). Statistical significance was defined as P < 0.05.

3. Results

3.1 Tumor recurrence and metastasis rates in mice

The tumor recurrence rate was 80% and metastasis rate was 70% in model group, respectively. In the auto+HLA-DQB1 group, the recurrence rate was 70% and the
metastasis rate was 60%. The recurrence and metastasis rates of autotransfusion group were the lowest (30% and 40%, respectively), as shown in Table 1.

<table>
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<th></th>
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<th>Number of cases</th>
<th>Recurrence rate</th>
<th>Number of cases</th>
<th>Metastasis rate</th>
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<td>10</td>
<td>7</td>
<td>70%</td>
<td>6</td>
<td>60%</td>
</tr>
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</table>

### 3.2 Number of HLA-DQB1 positive cells

Flow cytometry results showed that the number of HLA-DQB1 positive cells was the largest in the autotransfusion group and autotransfusion could activate HLA-DQB1 expression. HLA-DQB1 was tumor cell specific antigen. The least number of HLA-DQB1 positive cells was in the auto+HLA-DQB1 group, which showed no difference from the control group, as shown in Figure 2.

**Figure 2.** The number of HLA-DQB1 positive cells was detected by flow cytometry.
3.3 Content of CD4+CD8+T cells

The maximum content of CD4+CD8+T cells was in the autotransfusion group (98.4%), indicating that autotransfusion could activate immune system expression. The second group was the model group (96.6%), and the CD4+CD8+T content was the least in the auto+HLA-DQB1 group (92.5%), which showed no difference from the control group, as shown in Figure 3.

![Figure 3](image_url)

Figure 3. The content of CD4+CD25+T cells was analyzed by flow cytometry.

3.4 Proliferation of CD4+T cells

The proliferation of CD4+T cells in rat blood was the largest in the autotransfusion group, indicating that autotransfusion can activate the immune system. The second group was the model group, and CD4+T proliferation was the least in the auto+HLA-DQB1 group, which showed no difference from the control group, as shown in Figure 4.
Figure 4. The proliferation of CD4+T cells was analyzed by flow cytometry.

3.5 PDCD1 protein expression detection by immunohistochemistry

Immunohistochemical analysis of PDCD1 protein showed brownish yellow color. The expression of PDCD1 protein was the lowest in the autotransfusion group, and there was no significant difference between the normal group and the autotransfusion group. The expression of PDCD1 protein was highest in auto-HLA-DQB1, followed by the model group, as shown in Figure 5. This is because the immunosuppression of auto-HLA-DQB1 group leads to the high expression of PDCD1 protein, which leads to immune escape of tumor cells and tumor proliferation.
3.6 Western blot analysis of PDCD1, ECM1, E-cadherin protein expression

The expressions of PDCD1, ECM1, E-cadherin proteins in liver tissue of mice were showed in Figure 6. The expressions of PDCD1, ECM1, E-cadherin protein were highest in auto+HLA-DQB1 group, and the second was the model group. There was a significant difference, compared with the Control group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Compare with model group, ###$P < 0.001$, ##$P < 0.01$. 

Figure 5. Immunohistochemical staining (× 200).
Figure 6. Protein levels of PDCD1, ECM1, E-cadherin in different groups determined by western blot (Mean±SD, n=3). *P<0.05, **P<0.01, ***P<0.001 compared with control group; ###P<0.001, ##P<0.01 compared with model group.

3.7 Serum levels of inflammatory cytokines IL-10 and IFN-γ

The content of IL-10 was highest in auto+HLA-DQB1 group (114.80±12.16 ng/L). The second was model group (82.27±13.48 ng/L). The content of IL-10 in autotransfusion group (34.01±11.75 ng/L) was similar to that in the control group (33.06±7.23 ng/L), as shown in Figure 7A. The content of IFN-γ and IL-10 showed the same trend. The control group was 1130.50±261.97 ng/L, model group was 3288.45±615.18 ng/L, Autotransfusion group was 1075.09±196.79 ng/L, Auto+HLA-DQB1 group was 2306.32±548.25 ng/L, as shown in Figure 7B.

Figure 7. Serum levels of inflammatory cytokines IL-10 and IFN-γ; A: content of IL-10; B: content of IFN-γ.
4. Discussion

At present, the common methods of blood transfusion include allogeneic transfusion and autotransfusion. Blood tension and transmission of blood-borne diseases often exist in allogeneic transfusion. Autotransfusion has unique advantages over allogeneic blood transfusion, such as the storage time of autologous blood is shorter than that of stock blood, and the biochemical indexes are closer to the body. Blood transfusion is an irreplaceable treatment to solve this problem. Studies have found that reclaimed autotransfusion can reduce the expression of PDCD1 protein and reduce the tumor migration and recurrence rate.

PDCD1 is an immunoglobulin expressed primarily on the surface of activated CD4+/CD8+ T cells [18]. Low PDCD1 expression and enhanced immune surveillance inhibit the migration and invasion of tumor cells. The expression level of PDCD1 in peripheral blood T cells of tumor patients was higher than that of control subjects. Recent studies have shown that abnormal expression of PDCD1 is closely related to tumors of various systems [19-21]. It was found that PDCD1 expression was elevated in stage II~III colon cancer and inhibited T cell immune response to tumor [22]. Immunohistochemical results confirmed that the expression of PDCD1 protein increased in model group, and the expression of PDCD1 protein was the lowest in autotransfusion group, and the expression of PDCD1 protein was the highest in auto-HLA-DQB1.

Flow cytometry results showed that the number of HLA-DQB1 positive cells was the largest in the autotransfusion group HCC model, which proved that autotransfusion can activate HLA-DQB1 expression. The proliferation of CD4+T and the content of CD4+CD8+T in the blood of mice were the most in autotransfusion group, indicating that autotransfusion can activate the expression of immune system. In conclusion, the above two experiments demonstrated that HLA-DQB1 activated the CD4+T immune system, reduced the expression of PDCD1, and inhibited the migration and invasion of tumor cells.

In addition, contents of IFN-γ and IL-10 were increased in liver cancer models and auto+HLA-DQB1 group. This may be because IFN-γ and IL-10 are mainly secreted by helper T lymphocytes (Th) 17, which regulates the interaction between different types of
immune cells and promotes the formation of tumor microvessels, and is involved in the occurrence, invasion and metastasis of tumors[23].

5. Conclusion

Autotransfusion activated the expression of signaling molecule HLA-DQB1, enhanced the immune surveillance of CD4+T cells, reduced the expression of PDCD1 on the surface of liver cancer cells, and reduced the metastasis and recurrence of liver cancer.

Funding

This work is supported by National Natural Science Foundation of China (No. 82170225).

Ethics, consent and permissions

All animal experiments were performed according to the guidelines of the animal ethical organization and obtained the permission of The Shanghai Gongli Hospital, Naval Military Medical University.

Author’s contribution

(I) Conception and design: Laiwei You1,2#, Yu Bai3#, Ying-Hui Cui3#
(II) Administrative support: Jin-Huo Wang4, Na Yao2, Xiao-Fang Zhou4, Xiao-Fei Chen5*, Jian-Rong Guo2,4#
(III) Provision of study materials or patients: Laiwei You1,2#, Yu Bai3#, Ying-Hui Cui3#
(IV) Collection and assembly of data: Laiwei You1,2#, Yu Bai3#, Ying-Hui Cui3#
(V) Data analysis and interpretation: Laiwei You1,2#, Yu Bai3#, Ying-Hui Cui3#
(VI) Manuscript writing: All authors
(VII) Final approval of manuscript: All authors

Interest of conflict

All of the authors have no conflict of interest in this research.
Data Availability
The data are free access to available upon the corresponding author’ request.

References


