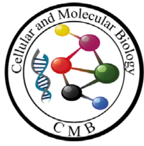
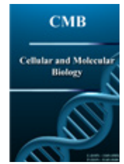
Cell. Mol. Biol. (ISSN: 1165-158X)



Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology



Original Article

Batroxase promotes the effect of NK cell adoptive therapy on lung cancer by enhancing immune cell infiltration

Xing Liu, Xiaoyu Wang, Rui Wang, Bin Shi*

Department of Respiratory Medicine, The Affiliated Suqian Hospital of Xuzhou Medical University (Nanjing Drum Tower Hospital Group Suqian Hospital), Suqian 223800, China

Article Info

Article history:

Received: March 22, 2024
Accepted: July 03, 2024
Published: August 31, 2024

Use your device to scan and read the article online

Abstract

Batroxobin, isolated from Bothrops moojeni, is a defibrinogenating agent used as a thrombin-like serine protease against fibrinogen for improving microcirculation. Here, we investigated whether, and if so, how batroxobin acts in concert with NK cells in terms of anti-tumor effects. CD3+/CD56+ NK cells were isolated and cultured from C57BL/6 mouse spleen. NK cells' viability was tested via Lactate dehydrogenase (LDH) assay. Lewis lung cancer cell (1*107 cell/ml) was used to build animal models. All animals were divided into five groups and treated with Batroxobin and NK cells respectively. HE staining was used to detect the pathological morphology of tumor tissue. The contents of fibrinogen and TNF-α in serum were determined by ELISA. The protein expression levels of MMP2, MMP9, VEGF and CD44 in tumor tissues were detected by Western Blot or immunohistochemistry. Compared with Control group, Tumor growth was not significantly affected in the group treated with Batroxobin or NK cells alone, However, tumor growth was significantly inhibited in the NK cell combined with the Batroxobin group. Serum levels of Fbg and TNF-αin mice treated with Batroxobin combined with NK cells dropped significantly, bringing them closer to normal levels. WB results showed that the expression levels of MMP2/9, VEGF and CD44 in Batroxobin combined with NK cell group also significantly decreased. Batroxobin combined with adoptive immunotherapy with NK cells significantly inhibited the growth of Lewis lung cancer in mice.

Keywords: Lewis lung cancer, Batroxobin, NK cell, Adoptive immunotherapy.

1. Introduction

In the last few years, natural killer (NK) cell-based immunotherapy has emerged as a promising therapeutic approach for solid tumors and hematological malignancies [1]. NK cells are innate lymphocytes with an array of functional competencies, including anti-cancer, anti-viral, and anti-graft-vs-host disease potential [2]. The intriguing idea of harnessing such potent innate immune system effectors for cancer treatment led to the development of clinical trials based on the adoptive therapy of NK cells or on the use of monoclonal antibodies targeting the main NK cell immune checkpoints [3, 4].

The distorted metabolic environment inside the tumor stimulates the secretion of many cytokines, which in turn stimulate the production of blood vessels inside the tumor. And the relationship between cancer and abnormal blood coagulation has been recognized for over 100 years [5-8]. Previous studies have confirmed that Coagulation Factor I is an important component of tumor extracellular matrix, which plays an important role in the process of tumor occurrence, development and metastasis [9, 10]. It can be used as a scaffold for tumor cell adhesion and shedding to promote tumor implantation, growth and metastasis [11]. Thrombosis frequently complicates the course of the disease and may be the presenting symptom. Many cancer patients, especially those with metastatic disease, have detectable blood coagulation abnormalities, including thrombocytosis, hyperfibrinogenemia and elevated markers of coagulation activation such as Fibrinopeptide-G (Fpg) [12, 13]. Although markers of clotting activation usually just non-specifically reflect the presence of intravascular coagulation, particularly high circulating levels of soluble fibrin monomer, an intermediate product of thrombin-mediated fibrin formation, which may suggest an undiagnosed malignancy [14].

The latest experimental studies show that Batroxobin has an obvious defibrination effect, and it has a significant inhibitory function on tumor growth and metastasis [15, 16]. In addition, by combining it with the NK cell adoptive therapy and using the arterial infusion method, a high concentration of Batroxobin is injected into the tumor in a short time during the NK cell immunotherapy, so that the efficacy of Batroxobin reaches its peak in the shortest time. In this way, the anti-tumor effect of Batroxobin itself can be maximized. It can also improve the microcirculation by reducing the local and plasma FIB levels of the tumor so that the NK cells can fully contact the tumor cells, so as to give full play to its maximum effectiveness [17]. The-

* Corresponding author.
E-mail address: shibin2023@yeah.net (Bin Shi).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.8.23

refore, the combination of NK cell adoptive therapy and Batroxobin infusion can enhance the therapeutic effect of each other and have a significant synergistic effect. However, there have been numerous reports of bartoxase inhibiting tumor growth and metastasis. In addition, the efficacy and advantages of NK cells combined with Batroxobin perfusion in the treatment of tumor are still only preliminary inferential and need further clinical confirmation and research.

2. Materials and methods

2.1. Animals

55 Mice on a BALB/c background aged at 6 weeks were purchased from xxxx,all mice were kept on a 12-h light/dark cycle in a temperature-controlled room with free food and water.

2.2. Animal models

Forty SPF male, 6-week-old C57BL/6 healthy mice were purchased from Henan Skibes Biotechnology Co., LTD., Experimental Animal License No. : SCXK (Henan) 2020-0005. In addition to the normal control group, mice in model group were subcutaneously inoculated with 0.1 ml Lewis lung cancer cell suspension in the right axilla, which was a lung cancer mouse model.

(1) Normal control group (n=8): The same volume of normal saline was injected intraperitoneally once every other day.

(2) Model control group (n=8): The same volume of normal saline was injected intraperitoneally once every other day.

(3) Batroxobin group (n=8): 8 BU/kg Batroxobin was intraperitoneally injected once every other day.

(4) NK cell group (n=8): 1×10^7 NK cells were injected through the tail vein every 4 days.

(5) Batroxobin combined with theNK cell group (n=8): 8 BU/kg Batroxobin was intraperitoneally injected once every other day, and 1×10^7 NK cells were injected through the tail vein at an interval of 4 days.

2.3. NK cell extraction

Fifteen mice were purchased from Hangzhou Medical College (Production), Laboratory Animal License No: SCXK (Zhejiang) 2019-0002. The formal experiment began after all the experimental animals were reared for a week. After the mice were sacrificed with broken necks, they were immersed in 75% ethanol solution for 10 min. The spleen of mice was removed from a super clean table and cut into small pieces. The tissue blocks were placed on a 70 μm cell screen, and homogenate rinsing solution (0.1g /5 ml) was added while grinding so that all cells were dripped through the screen into the centrifugal tube. The screen was discarded, the abrasive liquid was centrifuged for 10 min at 450 g, and the supernatant was discarded. The cell suspension was carefully absorbed with a straw and added to the surface of the separation solution, 400 ~ 500 g, and centrifuged for 20 ~ 30 min. After centrifugation, the second layer was a ring opalescent NK cell layer.

2.4. Western blot

Samples were lysed in RIPA with PMSF, and BCA assay detected protein concentrations. Next, total protein was separated on a 10% SDS–PAGE and transferred onto PVDF membrane. The membranes were incubated with primary antibodies at 4°C overnight followed washed with TBST, and incubated with secondary antibodies at room temperature for 1 h. Finally, the membranes were detected by chemiluminescence (ECL, Thermo Fisher, Waltham, MA, USA). Anti-GAPDH antibody was used as a control.

2.5. ELISA

The frozen mouse serum samples were removed from the low-temperature refrigerator at -80°C 12 h in advance and rewarmed at 4°C for 12 h. The samples were used in mouse Fbg kit (Shanghai Tongwei Industrial Co., LTD., Shanghai, China, TW12870) and mouse tumor necrosis factor α (TNF-α) kit (Beijing Sulebo, Shanghai, China, SEKM-0034) Detection indicators.

2.6. HE & IHC

The tumors were separated from each group. After dewaxed and hydrated, the tumor's sections were incubated in hematoxylin solution for 15 min followed washed with PBS. Then, the slices were differentiated by differentiation fluid for 15 s followed incubated in eosin solution for 20 s. In the end, the tumor's sections were dehydrated, transparentized, and sealed, the photos were captured by the microscope (Olympus, Tokyo, Japan).

The sections were then incubated by the primary antibody against VEGF and CD44 (1:500, Abcam, Cambridge, MA, USA) overnight at 4°C. After stained using a secondary antibody (30-40 μL, Abcam, Cambridge, MA, USA) at room temperature for 30 min, the positive signals of VEGF and CD44 were developed using a commercial kit (Thermo, Waltham, MA, USA) according to the manufacturer's instructions. The nuclei were stained by hematoxylin solution. The images were determined under light microscopy (Olympus, Tokyo, Japan).

2.7. Statistical analysis

All experiments were performed independently at least three times. The data are presented as the mean ± standard deviation (SD). Statistical significance between two groups was analyzed using Student's unpaired t-test. ANOVA one-way analysis was used to measure the significance for more than two groups. *$P< 0.05$ was considered as significant.

3. Results

3.1. Morphology observation and cellular phenotype of NK cells

It could be observed under the microscope (Figure 1A) that the NK cells in the spleen tissue of mice were round or oval in shape, and most of them adhered to the wall after 1 day, showed clumping growth after 5 days, and the clumping phenomenon further increased after 7

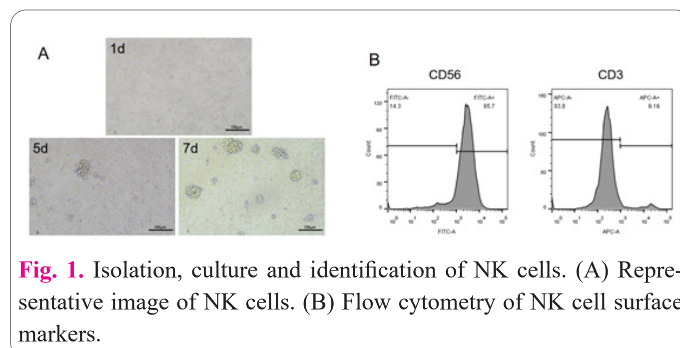


Fig. 1. Isolation, culture and identification of NK cells. (A) Representative image of NK cells. (B) Flow cytometry of NK cell surface markers.

days. The results of flow cytometric identification of NK cells in mouse spleen were shown in Figure 1B. NK cells were CD56-positive cells and CD3-negative cells. Flow cytometry showed that CD56 positive cells accounted for 85.7% of the total cells, and CD3 negative cells accounted for 93.8%.

3.2. Combination of Batroxobin with adoptive NK cells transfer significantly inhibits the growth of lung carcinoma *in vivo*

To determine if there is a synergistic effect between Batroxobin and adoptive NK cell immunotherapy, we established a lung cancer model in mice using Lweis cells. We first used YAC-1 cells to detect NK cells isolated from mouse spleens by LDH assay (Table 1). After confirming the viability of NK cells, In Lewis lung cancer carcinoma model, we performed combined infusion therapy. We found that the Batroxobin slightly limited tumor growth compared with model group. NK cell adoptive therapy alone had slight antitumor activity compared with model group. On the contrary, significant antitumor activity was seen after combination therapy compared with the model group (Figure 2A-C). Besides, in combination treatment, thymus index and spleen index of tumor-bearing mice were more close to the normal level compared with the single treatment group (Figure 2D-E). In vivo experiments were repeated twice. Similar results were obtained in three parallel experiments all of which suggested that synergetic antitumor effects were obtained by combining Batroxobin and NK cells adoptive therapy.

3.3. Batroxobin decreases microvascular density and promotes tumor vessel normalization.

The formation of blood vessels in the tumor is crucial to the growth of the tumor, and hypoxic-inducing factors will stimulate the formation of blood vessels in the tumor and stimulate the growth of the tumor in the hypoxic environment. Batroxobin is often used clinically to treat ischemic diseases of cardiovascular system, which can significantly improve the microcirculation in the ischemic site. To this end, after the batroxylase intraperitoneal injection treatment, we detected the expression levels of MMP3/9, VEGF and CD44 in the tumor tissue (Figure 3B). As expected, the expression levels of various factors in the Batroxobin treatment group were significantly reduced. HE staining results Under light microscope, it was observed that the tumor cells in Model group were densely distributed and deeply stained. Compared with Model group, the cell density of tumor tissue in Batroxobin group, NK group and Batroxobin combined with theNK group all decreased and the necrotic area increased relatively, among which the necrotic area in Batroxobin combined with the NK group was relatively obvious, and the necrotic area

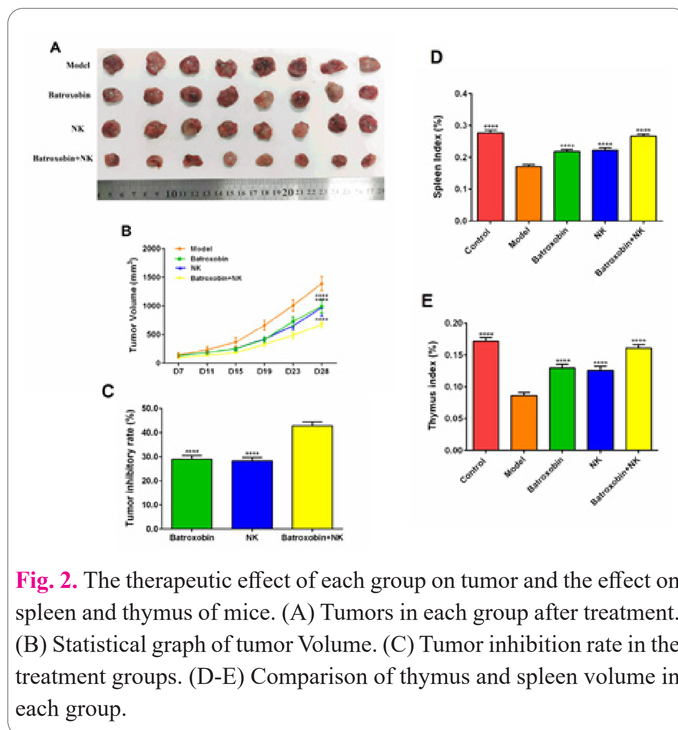


Fig. 2. The therapeutic effect of each group on tumor and the effect on spleen and thymus of mice. (A) Tumors in each group after treatment. (B) Statistical graph of tumor Volume. (C) Tumor inhibition rate in the treatment groups. (D-E) Comparison of thymus and spleen volume in each group.

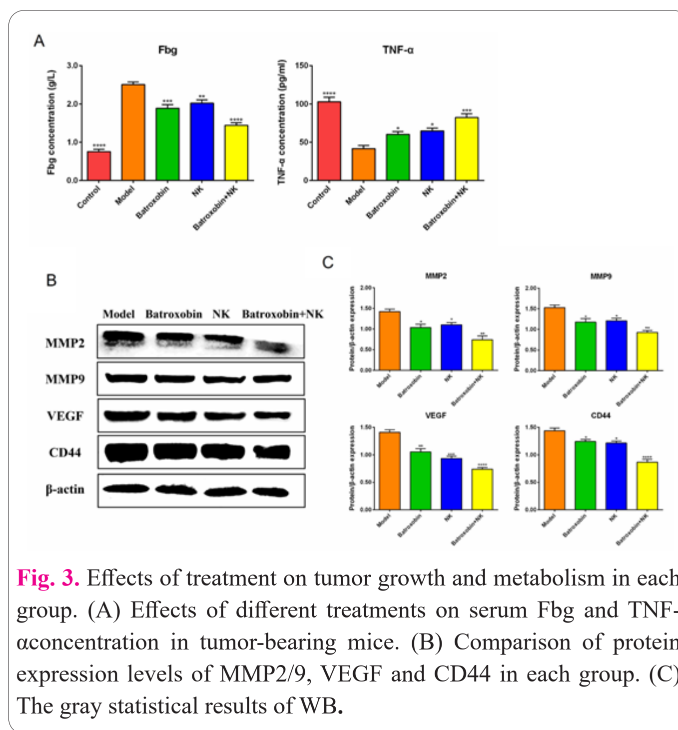


Fig. 3. Effects of treatment on tumor growth and metabolism in each group. (A) Effects of different treatments on serum Fbg and TNF-αconcentration in tumor-bearing mice. (B) Comparison of protein expression levels of MMP2/9, VEGF and CD44 in each group. (C) The gray statistical results of WB.

Table 1. NK cell cytoactive analysis.

Sample	Wells	OD Values	Mean	NK activity (%)
YAC-1-1	B2	0.186		3.09%
YAC-1-2	B3	0.175	0.172	0.72%
YAC-1-3	B4	0.154		-3.81%
YAC-1+NK-1	B5	0.328		33.69%
YAC-1+NK-2	B6	0.291	0.307	25.72%
YAC-1+NK-3	B7	0.302		28.09%
YAC-1+NP-40-1	B8	0.620		96.62%
YAC-1+NP-40-2	B9	0.648	0.636	102.66%
YAC-1+NP-40-3	B10	0.639		100.72%

showed large sheets (Figure 4A). Immunohistochemical staining of VEGF in the tumor tissue of mice in the group showed that the positive expression of VEGF was brown and yellow under light microscope (Figure 4B). Compared with Model group, Batroxobin group, NK group, Batroxobin group and Batroxobin combined with the NK group became lighter brown and yellow, and the positive expression of VEGF was relatively reduced, and the positive expression of VEGF was relatively reduced in Batroxobin combined with the NK group.CD44 showed the same results (Figure 4C).

On the other hand, Compared with the model group, the serum concentration of coagulation factor Fbg was significantly decreased in the Batroxobin treatment group, and the serum level of TNF-α was increased (Figure 3A), indicating that the body's killing ability to tumor cells was enhanced after the improvement of the hypoxic environment inside the tumor. All these are related to the improvement of blood supply and hypoxic environment in tumor by Batroxobine.

3.4. Batroxobin augments the infiltration of NK cells and the intratumoral NK cells are increased after the combination therapy

The following experiment examined the accumulation of NK cells in Lewis lung carcinoma xenograft. In order to analyze NK cells antitumoral activity in vivo, tumor necrosis was tested by H&E stain. H&E stain section from control tumor showed no regions of necrosis. Sections from Batroxobin or NK cells treated tumors showed minor degree of necrosis. However, tumor from combinational group showed large area of liquefactive necrosis surrounded by viable tumor.

4. Discussion

Adoptive immune cell therapy technology collects the body's immune cells, through in vitro culture, so that the number of thousands of times increases, or increases the targeted killing function, and then back into the patient's body, so as to kill pathogens in the blood and tissue, cancer cells, mutated cells [18, 19]. Adoptive immune cell therapy does not attack cancer cells directly, but it does so by growing and treating immune cells in the patient's body, which is very different from conventional therapy. It can be divided into two types: non-specific therapy (non-specific therapy without clear immune cell target, which is to improve human immunity and alleviate tumor symptoms on the whole, such as NK cell therapy) [20, 21] and specific therapy (with clear target and mechanism, which can activate or inhibit specific target to realize immune activation of tumor by the immune system, such as TCR and CAR therapy) [22, 23]. Natural Killer (NK) cells are characterized by their potential to kill tumor cells by different means without previous sensitization and have, therefore, become a valuable tool in cancer immunotherapy [24]. Natural killer (NK) cells can come from several different sources. Including genetically engineered chimeric antigen receptor (CAR) NK cells, but also cord blood, peripheral blood, NK-92 cells, and induced pluripotent stem cell (iPSC) derived natural killer cells. Compared with other tumor immunotherapy methods, adoptive immunotherapy will not cause obvious side effects or GVHD due to the characteristics of NK cells themselves [25]. However, their efficacy against solid tumors is still poor and further studies are required to improve it.

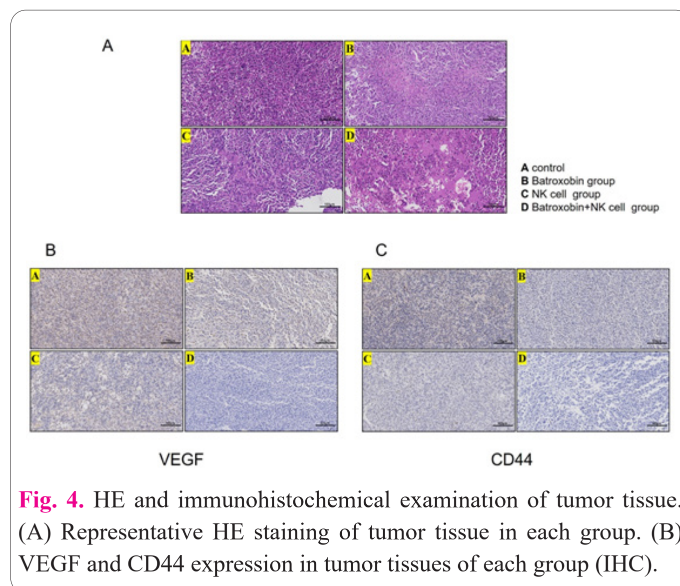


Fig. 4. HE and immunohistochemical examination of tumor tissue. (A) Representative HE staining of tumor tissue in each group. (B) VEGF and CD44 expression in tumor tissues of each group (IHC).

TME (Tumor Micro-Environment) is characterized by a heterogeneous mix of immunosuppressive metabolites, glucose and amino acid deprivation, hypoxia, and acidity [26, 27]. Hypoxia is a common driver of immune cell dysfunction in solid tumors(28). It has been shown that NK cell function is impaired in anoxic TME, mainly due to the increase of inhibitory cells and the direct effects of hypoxia [29, 30]. Abnormal metabolic behavior of tumors leads to high levels of lactic acid, consumption of important nutrients, and increased concentrations of toxic decomposition metabolites, adenosine and reactive oxygen species [31, 32]. Uncontrolled proliferation, dysfunctional vasculature, and immunosuppressive cell subsets all suppress immune effector cells. There are usually two ways to overcome the current difficulties: one is to change the composition of metabolites in the tumor to improve the hypoxia state inside the tumor; The other is that it is genetically engineered to be immune to the inhibitory metabolites in TME [33]. However, the cost and technical difficulty of the second method is relatively high, so the first method has become the best choice at present.

The ability of NK cells to transport and penetrate the tumor bed is a key prerequisite for effective anti-tumor immunity, and NK cells are guided to the tumor site through the dynamic interaction of chemokine receptors and their homologous ligands secreted in TME [34, 35]. But there are several important obstacles. First, chemotactic gradients depend on adequate perfusion, while microthrombus induction in tumor and tumor itself will cause circulation obstruction and hinder perfusion. Secondly, chemokine release in TME follows different tumor-specific dynamics and may require artificial enhancement of chemokine levels, like CCL3, CCL4, CCL5, and XCL1concentration, within the tumor [36].

Batroxobin has an obvious defibrination effect. Usually, Batroxobin is combined with interventional technology in clinical practice to reduce the high concentration of Batroxobin into the local tumor in a short time, so that the drug effect can reach its peak in the shortest time [37-39]. In this way, by reducing the local tumor and the level of coagulation factor in plasma, the microcirculation can be improved, and the tumor drugs can be fully infiltrated in the tumor body, thus giving full play to the maximum effectiveness. First, we demonstrated that there is no inter-

ference between Batroxobin and the therapeutic function of NK cells; Second, our experimental results showed that the tumor growth of mouse Lweis lung cancer was significantly inhibited when Batroxobin was combined with the adoptive treatment of NK cells. Immunohistochemical detection also found that the infiltration degree of NK cells in tumor tissues was significantly increased under the combined treatment condition compared with the treatment group of Batroxobin or NK cells alone. The expression levels of hypoxia-inducing factors in tumor tissues were significantly decreased, indicating that Batroxobin could well solve the shortcomings of tissue hypoperfusion in the adoptive NK cell therapy and the severe hypoxia environment in the tumor that affected the killing function of NK cells. On the other hand, the expression level of VEGF in tumor was observed to be significantly reduced in our experiment which indicates that the angiogenesis and remodeling in tumor tissue were inhibited which is essential to tumor growth.

In conclusion, we conducted this experiment to treat Lweis lung cancer mice with intraperitoneal injection of allogeneic NK cells combined with Batroxobin, in order to study and solve the problem of poor efficacy caused by insufficient perfusion in the clinical NK cell adoptive therapy. Although the sample size of this exploratory trial is limited, the positive results of this study will be worthy of clinical reference.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

All procedures performed in the studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee of The Affiliated Suqian Hospital of Xuzhou Medical University (Nanjing Drum Tower Hospital Group Suqian Hospital).

Informed consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions

Bin Shi: supervision and resources; Xin Liu: paper writing and revising; Xiaoyu Wang and Rui Wang: Experiments and data collation. All authors read and approved the final manuscript.

Funding

This work was supported by the Suqian Science and Technology Project (K202007).

References

1. Abel AM, Yang C, Thakar MS, Malarkannan S (2018) Natural Killer Cells: Development, Maturation, and Clinical Utilization. Front Immunol 9:1869. doi: 10.3389/fimmu.2018.01869
2. Fang F, Xiao W, Tian Z (2017) NK cell-based immunotherapy for cancer. Semin Immunol 31:37-54. doi: 10.1016/j.smim.2017.07.009
3. Galon J, Bruni D (2019) Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 18:197-218. doi: 10.1038/s41573-018-0007-y
4. Guedan S, Ruella M, June CH (2019) Emerging Cellular Therapies for Cancer. Annu Rev Immunol 37:145-171. doi: 10.1146/annurev-immunol-042718-041407
5. Ho YJ, Wang TC, Fan CH, Yeh CK (2017) Current progress in antivascular tumor therapy. Drug Discov Today 22:1503-1515. doi: 10.1016/j.drudis.2017.06.001
6. Qian CN, Tan MH, Yang JP, Cao Y (2016) Revisiting tumor angiogenesis: vessel co-option, vessel remodeling, and cancer cell-derived vasculature formation. Chin J Cancer 35:10. doi: 10.1186/s40880-015-0070-2
7. Shigeta K, Datta M, Hato T, Kitahara S, Chen IX, Matsui A et al (2020) Dual Programmed Death Receptor-1 and Vascular Endothelial Growth Factor Receptor-2 Blockade Promotes Vascular Normalization and Enhances Antitumor Immune Responses in Hepatocellular Carcinoma. Hepatology 71:1247-1261. doi: 10.1002/hep.30889
8. Wenes M, Shang M, Di Matteo M, Goveia J, Martin-Perez R, Serneels J et al (2016) Macrophage Metabolism Controls Tumor Blood Vessel Morphogenesis and Metastasis. Cell Metab 24:701-715. doi: 10.1016/j.cmet.2016.09.008
9. Azoitei N, Becher A, Steinestel K, Rouhi A, Diepold K, Genze F et al (2016) PKM2 promotes tumor angiogenesis by regulating HIF-1alpha through NF-kappaB activation. Mol Cancer 15:3. doi: 10.1186/s12943-015-0490-2
10. Palacios-Acedo AL, Mege D, Crescence L, Dignat-George F, Dubois C, Panicot-Dubois L (2019) Platelets, Thrombo-Inflammation, and Cancer: Collaborating With the Enemy. Front Immunol 10:1805. doi: 10.3389/fimmu.2019.01805
11. Liu S, Zhang Y, Zhao X, Wang J, Di C, Zhao Y et al (2019) Tumor-Specific Silencing of Tissue Factor Suppresses Metastasis and Prevents Cancer-Associated Hypercoagulability. Nano Lett 19:4721-4730. doi: 10.1021/acs.nanolett.9b01785
12. Krenzlin H, Lorenz V, Alessandri B (2017) The involvement of thrombin in the pathogenesis of glioblastoma. J Neurosci Res 95:2080-2085. doi: 10.1002/jnr.24049
13. Kwaan HC, Lindholm PF (2019) Fibrin and Fibrinolysis in Cancer. Semin Thromb Hemost 45:413-422. doi: 10.1055/s-0039-1688495
14. Lugano R, Ramachandran M, Dimberg A (2020) Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci 77:1745-1770. doi: 10.1007/s00018-019-03351-7
15. Lan D, Song S, Liu Y, Jiao B, Meng R (2021) Use of Batroxobin in Central and Peripheral Ischemic Vascular Diseases: A Systematic Review. Front Neurol 12:716778. doi: 10.3389/fneur.2021.716778
16. Wang DS, Hanamoto M, Fang F, Ohba M, Ishii M, Kimura F et al (2001) Defibrinogenating effect of batroxobin (Defibrase) in rats and inhibition of migration of human vascular smooth muscle cells by the plasma of batroxobin-treated rats in vitro. Atherosclerosis 156:73-80. doi: 10.1016/s0021-9150(00)00628-6
17. Tieken C, Versteeg HH (2016) Anticoagulants versus cancer. Thromb Res 140 Suppl 1:S148-S153. doi: 10.1016/S0049-3848(16)30114-1
18. Lee DA (2019) Cellular therapy: Adoptive immunotherapy with expanded natural killer cells. Immunol Rev 290:85-99. doi: 10.1111/imr.12793
19. Zhu H, Kaufman DS (2019) An Improved Method to Produce

Clinical-Scale Natural Killer Cells from Human Pluripotent Stem Cells. Methods Mol Biol 2048:107-119. doi: 10.1007/978-1-4939-9728-2_12

20. Ngambenjawong C, Gustafson HH, Pun SH (2017) Progress in tumor-associated macrophage (TAM)-targeted therapeutics. Adv Drug Deliver Rev 114:206-221. doi: 10.1016/j.addr.2017.04.010
21. Wang W, Jiang J, Wu C (2020) CAR-NK for tumor immunotherapy: Clinical transformation and future prospects. Cancer Lett 472:175-180. doi: 10.1016/j.canlet.2019.11.033
22. Martinez M, Moon EK (2019) CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol 10:128. doi: 10.3389/fimmu.2019.00128
23. Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G (2019) Past, Present, and Future of Regulatory T Cell Therapy in Transplantation and Autoimmunity. Front Immunol 10:43. doi: 10.3389/fimmu.2019.00043
24. Liu S, Galat V, Galat Y, Lee Y, Wainwright D, Wu J (2021) NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol 14:7. doi: 10.1186/s13045-020-01014-w
25. O'Brien KL, Finlay DK (2019) Immunometabolism and natural killer cell responses. Nat Rev Immunol 19:282-290. doi: 10.1038/s41577-019-0139-2
26. Jarosz-Biej M, Smolarczyk R, Cichon T, Kulach N (2019) Tumor Microenvironment as A "Game Changer" in Cancer Radiotherapy. Int J Mol Sci 20:3212. doi: 10.3390/ijms20133212
27. Wu T, Dai Y (2017) Tumor microenvironment and therapeutic response. Cancer Lett 387:61-68. doi: 10.1016/j.canlet.2016.01.043
28. Hinshaw DC, Shevde LA (2019) The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res 79:4557-4566. doi: 10.1158/0008-5472.CAN-18-3962
29. Hu Z, Xu X, Wei H (2021) The Adverse Impact of Tumor Microenvironment on NK-Cell. Front Immunol 12:633361. doi: 10.3389/fimmu.2021.633361
30. Melaiu O, Lucarini V, Cifaldi L, Fruci D (2019) Influence of the Tumor Microenvironment on NK Cell Function in Solid Tumors. Front Immunol 10:3038. doi: 10.3389/fimmu.2019.03038
31. Sokratous G, Polyzoidis S, Ashkan K (2017) Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme. Hum Vacc Immunother 13:2575-2582. doi: 10.1080/21645515.2017.1303582
32. Zheng X, Qian Y, Fu B, Jiao D, Jiang Y, Chen P et al (2019) Mitochondrial fragmentation limits NK cell-based tumor immunosurveillance. Nat Immunol 20:1656-1667. doi: 10.1038/s41590-019-0511-1
33. Ochoa MC, Minute L, Rodriguez I, Garasa S, Perez-Ruiz E, Inoges S et al (2017) Antibody-dependent cell cytotoxicity: immunotherapy strategies enhancing effector NK cells. Immunol Cell Biol 95:347-355. doi: 10.1038/icb.2017.6
34. Poznanski SM, Singh K, Ritchie TM, Aguiar JA, Fan IY, Portillo AL et al (2021) Metabolic flexibility determines human NK cell functional fate in the tumor microenvironment. Cell Metab 33:1205-1220. doi: 10.1016/j.cmet.2021.03.023
35. Sivori S, Pende D, Quatrini L, Pietra G, Della CM, Vacca P et al (2021) NK cells and ILCs in tumor immunotherapy. Mol Aspects Med 80:100870. doi: 10.1016/j.mam.2020.100870
36. Terren I, Orrantia A, Vitalle J, Zenarruzabeitia O, Borrego F (2019) NK Cell Metabolism and Tumor Microenvironment. Front Immunol 10:2278. doi: 10.3389/fimmu.2019.02278
37. Santibanez JF, Krstic J (2018) Transforming Growth Factor-Beta and Urokinase Type Plasminogen Interplay in Cancer. Curr Protein Pept Sc 19:1155-1163. doi: 10.2174/1389203718666171030103801
38. Song S, Wu H, Ji X, Meng R (2022) The BE COOL Treatments (Batroxobin, oxygEn, Conditioning, and cOOLing): Emerging Adjunct Therapies for Ischemic Cerebrovascular Disease. J Clin Med 11:6193. doi: 10.3390/jcm11206193
39. Tang J, Kang Y, Huang L, Feng X, Wu L, Peng Y (2021) Neuroprotective effects of Hemocoagulase Agkistrodon on experimental traumatic brain injury. Brain Res Bull 170:1-10. doi: 10.1016/j.brainresbull.2021.01.023