Study on mechanism of Tim-3 on immune escape in benzene-induced acute myeloid leukemia

qiong Ning

Qilu Hospital of Shandong University

xiangxin li (✉ lixiangxinvip@163.com)

Qilu hospital of Shandong university

Xiangdong Jian

Shandong University Qilu Hospital

Xiaopeng He

Shandong Provincial Hospital

Research article

Abstract

To study the mechanism of Tim-3 on immune escape in benzene-induced acute myeloid leukemia (AML), to provide potential targets of clinical monitoring and intervention of hematological toxicity in benzene-induced AML . C3H/He mice were randomly divided into control group and experimental group. Serum levels of IL-12 in the experimental group were significantly lower than that in the control group. Serum levels of TGF-β1 in the experimental group were significantly higher than that in the control group(p <0.05). The proportion of Tim-3 positive CD14 + monocytes of bone marrow and spleen in the experimental group were both significantly higher than that in the control group (p <0.05) by Flow cytometry (FCM). Compared with the control group, the expression of Tim-3 on (M1+M2) macrophages of bone marrow in the experimental group significantly increased by immunofluorescence assay. The expression of type M2 macrophages in (M1+M2) macrophages of bone marrow and spleen tissues in the experimental group were both higher than that in the control group. The expression levels of p-PI3K, p-AKT and p-mTOR in the experimental group were all significantly higher than that in the control group. Tim-3 was highly expressed in macrophages in benzene-induced AML. It promoted the activation of PI3K/AKT/mTOR signaling pathway, stimulated the secretion of anti-inflammatory cytokines, and inhibited the secretion of pro-inflammatory cytokines. High expression of Tim-3 changed the phenotype and function of macrophages by promoting the macrophages polarization, thus inducing negative immune response in the tumor microenvironment and tumor immune escape.

Background

Benzene is currently a recognized important environmental factor that can induce leukemia. Long-term chronic benzene exposure primarily damages the hematopoietic system, causing leukopenia, pancytopenia, aplastic anemia and even leukemia. Benzene exposure has been associated with increased incidence of leukemia in humans, particularly AML, which predominantly based on evidence from epidemiology (Greim, H,et al. 2014; Loomis, D,et al.2017). Leukemia treatment costs are higher, and benzene-induced leukemia causes harm to the society. A number of studies showed that the blood toxicity of benzene triggered abnormal hematopoietic processes through a variety of pathways (Bi Y, et al. 2010; McHale CM, et al. 2011; Zhou H, et al.2010). including the appearance of malignant clones and malignant proliferation, and the formation of immunosuppression which allowed them to escape immune surveillance(Li B, et al. 2009). Benzene exposure can also cause immune disorders and hematopoietic microenvironment changes (Li B, et al. 2009; Wang, BS, et al.2018). However, the pathogenesis of leukemia caused by benzene is not very clear. Tim-3 is an important negative immunological checkpoint molecule that has attracted much attention in recent years (Shin DS, et al.2015). Although the role of Tim-3 as a negative immunological checkpoint molecule in tumor immune escape is important, there are relatively few reports on macrophages and leukemia in domestic and international research. Whether benzene mediates tumor immune escape by up-regulating the expression of Tim-3 in benzene-induced leukemia had not been reported.

Macrophages play an important role in regulating the immune response and are involved in health and disease (Cassetta L, et al.2011; Wynn TA, et al.2013). Myeloid cells in the bone marrow microenvironment play an immune role by differentiating into tumor-associated macrophages (TAMs) with immunosuppressive functions. The role of macrophage polarization in tumor immune escape has attracted much attention in recent years. In the tumor microenvironment, tumor cells, fibroblasts and endothelial cells can produce a variety of chemokines which recruit TAMs precursor cells(monocytes) to tumor site. They can "domesticated" type M1 macrophages which play an important role in immunosurveillance to type M2 macrophages which play the role of immunosuppression. The process is called macrophage polarization. At the same time, we speculated that Tim-3, as an important regulator of macrophage polarization(Jiang, X, et al. 2016; Sun, J, et al. 2018; Zhang, D, et al. 2019; Yang, H, et al. 2019; Zhang, W, et al.2019), might also induce macrophage polarization in the tumor microenvironment to mediate immune escape of leukemia cells in this process. This has not been reported. Does up-regulation of the expression Tim-3 as a negative regulatory molecule induce changes in the hematopoietic microenvironment during benzene-induced AML? Then tumor cells can escape immune surveillance and immune attack from the body or even completely immunodeficiency. So Tim-3 maybe promote tumor progression and participate in immune escape. This paper will demonstrate for it.

Methods

1 Materials

1.1 Animals

The male C3H/He mice at the age of 6-8 weeks of (23 ± 2) g weight with SPF grade were selected. These mice were purchased from Beijing Wei Tong Li Hua experimental animal technology co. LTD. License number is SCXK(Jing) 2016-0011. The experiment was approved by the ethics committee of Qilu Hospital of Shandong University. All mice were raised in SPF animal house of experimental animal center of Qilu Hospital of Shandong University, with ambient temperature of 20~24°C and relative humidity of 50%~70%. Ordinary feed was purchased from experimental animal center of Shandong University.

1.2 Reagents

Pure benzene (excellent purity,>99.9%) was purchased from Sinopharm Chemical Reagent co. LTD. PE-labeled Tim-3 mouse flow cytometry antibody, perCP-Cy5.5 labeled CD14 mouse flow cytometry antibody, TGF- beta 1 Elisa kit and IL-12 Elisa kit were all purchased from eBiosicence.F4/80, CD68 and CD163 mouse immunofluorescent antibody were all purchased from Servicebio. CD206 mouse immunofluorescent antibody, Tim-3 mouse immunofluorescent antibody and PI3K mouse immunofluorescent antibody were all purchased from Bioss. AKT mouse immunofluorescent antibody was purchased from Servicebio and mTOR mouse immunofluorescent antibody was purchased from CST.

2 Methods

2.1 Animal models and groups

C3H/He mice were randomly divided into control group and experimental group (benzene-induced AML model group), there were six mice in each group.

2.2 Cytokine Detection

Each group were anesthetized by intraperitoneal injection of 10% chloral hydrate in 0.1ml/10g (ml drug per 10g mouse body weight). After administration of chloral hydrate, the mice were active at first, followed by unstable gait when crawling, and then stopped crawling, finally they lay on one side. The mice did not exhibit peritonitis or other discomfort. Mice were killed by cervical dislocation. The mice orbital blood was collected, and the blood was naturally coagulated at room temperature for 20min. Then got supernatant and centrifuged at 2000 r/min for 20min. According to the instructions of Elisa kit, the contents of IL-12 and TGF-β1 in the experimental group and the control group were detected at the wavelength of 450 nm with an enzyme marker.

2.3 FCM

Expression levels of Tim-3 on $CD14^+$ monocytes in bone marrow and spleen in the experimental group and control group were detected.

2.4 Immunofluorescence staining detection

2.4.1 Immunofluorescence assay was used to detect the expression of Tim-3 on F4/80 (M1+M2) macrophages and CD206 (M2) macrophages in F4/80 (M1+M2) macrophages of bone marrow tissues

Polychromatic immunofluorescence staining of F4/80 (red)+ Tim-3 (green) + nucleus (DAPI, blue) were performed. Immunofluorescence staining of F4/80 (red)+CD206(green) + nucleus (DAPI, blue) were performed in paraffin-embedded bone marrow pathology sections.

2.4.2 Immunofluorescence assay was used to detect the expression of CD163 (M2) macrophages in CD68(M1+M2) macrophages of spleen tissues

Immunofluorescence staining of CD68 (red)+CD163(green) + nucleus (DAPI, blue) were performed in paraffin-embedded spleen pathology sections.

2.4.3 Immunofluorescence was used to detect the protein expressions of p-PI3K, p-AKT, p-mTOR in bone marrow macrophages

The protein expressions of p-PI3K, p-AKT and p-mTOR in bone marrow macrophages were detected by immunofluorescence method in the experimental group and the control group respectively.

Immunofluorescence staining of F4/80 (red)+p-PI3K(green) and nucleus (DAPI, blue), F4/80 (red)+p-AKT (green) and nucleus (DAPI, blue), F4/80 (red)+ p-mTOR(green) and nucleus (DAPI, blue) were performed

in paraffin- embedded bone marrow pathology sections.

3 Statistical Analysis

The data were preliminarily processed by Excel 2003, then the results were expressed as M±S, and SPSS 22.0 software was used for statistical analysis. $p<0.05$ was considered as statistically significant difference.

Results

1 Peripheral blood immune cytokines IL-12、TGF-β1

1.1 Cytokines IL-12

Serum levels of IL-12 in the experimental group were significantly lower than that in pre-benzene exposure, (128.65±62.06) pg/mL and (240.46±130.98) pg/mL respectively($p<0.05$). Serum levels of IL-12 in the experimental group significantly reduced than that in the control group, (128.65±62.06) pg/mL and (216.71±70.16) pg/mL respectively($p<0.05$) (Table 1).

1.2 Cytokines TGF-β1

Serum levels of TGF-β1 in the experimental group were significantly higher than that in pre-benzene exposure, (103.59±36.13) pg/mL and (54.03±21.07) pg/mL respectively($p<0.05$). Serum levels of TGF-β1 in the experimental group were significantly higher than that in the control group, (103.59±36.13) pg/mL and (76.84±15.73) pg/mL respectively($p<0.05$) (Table 2).

2 FCM

2.1 Expression of Tim-3 on CD14⁺ monocytes in bone marrow was detected by FCW

The proportion of Tim-3 positive CD14$^+$ monocytes of bone marrow in the experimental group were significantly higher than that in the control group, (76.81±6.50) and (60.91±6.18) respectively ($p<0.05$) (Figure 1).

2.2 Expression of Tim-3 on CD14⁺ monocytes in spleen was detected by FCW

The proportion of Tim-3 positive CD14$^+$ monocytes of the spleen in the experimental group were significantly higher than that in the control group, (67.71±18.76) and (12.01±7.46) respectively ($p<0.05$) (Figure 2).

3 Immunofluorescent staining technique

3.1 The expression of Tim-3 on F4/80 (M1+M2) macrophages in bone marrow was detected by immunofluorescence assay

Compared with the control group, the expression of Tim-3 on F4/80 (M1+M2) macrophages in the experimental group significantly increased (Figure 3).

3.2 Immunofluorescence analysis of polarization in bone marrow

macrophages and spleen macrophages

The results showed that the expression of CD206 macrophages in bone marrow macrophages significantly increased in the experimental group than that in the control group (Figure 4). And the expression of CD163 macrophages in spleen macrophages significantly increased in the experimental group than that in the control group (Figure 5).

3.3 The protein expressions of p-PI3K, p-AKT and p-mTOR in bone marrow macrophages were detected by immunofluorescence

The results showed that the protein expression levels of p-PI3K, p-AKT and p-mTOR of bone marrow macrophages in the experimental group were significantly higher than that in the control group (Figure 6-8).

Discussion

Benzene is the simplest aromatic hydrocarbon compound with a molecular formula of C_6H_6. It is a colorless and transparent oily liquid with a special aromatic taste. It is one of the basic raw materials of the organic chemical industry and a carcinogen, which can lead to one or multiple types of leukemia (Belingheri M, et al. 2019; Zhang L,et al. 2012; McHale CM,et al.2012). Tim-3 is mainly expressed in various immune cells such as differentiated mature Th1 cells, CD8$^+$ T cells, monocytes, regulatory T cells (Treg) and other immune cells. Tim-3 binds to its ligand galectin-9 and mediates immunosuppression in the tumor microenvironment through different mechanisms. In this study, the expression levels of Tim-3 on the surface of CD14$^+$ monocytes in bone marrow and spleen were detected for the first time in the animal model group of benzene-induced AML. The results showed that the expression levels of Tim-3 on the surface of CD14$^+$ monocytes in bone marrow and spleen in the experimental group were significantly higher than that in the control group. Moreover, the expression level of Tim-3 on mononuclear macrophages in the bone marrow samples was higher than that in spleen, so verified that Tim-3 could be used as one of the markers of toxicity in bone marrow tissues of benzene poisoning. And the percentage average of Tim-3 positive CD14$^+$ monocytes of the bone marrow in the control group was also very high, which was 60. It significantly increased than that in the control group of spleen samples. It is considered that Tim-3 is not only expressed in various immune cells, but also ectopically expressed in a series of normal tissues. And its expression is associated with poor prognosis. In addition, the experimental mice were C3H/He mice with susceptible to leukemia, so the expression level of Tim-3 in bone marrow

monocytes in the late stage of this type of mice significantly increased. At the same time, the immunofluorescence staining results showed that the expression of Tim-3 in bone marrow macrophages in the experimental group was also significantly higher than that in the control group, which further verified that benzene exposure could promote the expression of Tim-3 in macrophages. Therefore, Tim-3 is considered as a potential anti-AML therapeutic target. The results in the early stage of this study showed that there was disturbance of immune regulatory function of mononuclear macrophage system in occupational benzene poisoning patients. And it also showed that Tim-3 expression could serve as a promising diagnostic biomarker for discriminating AML from controls (Xiangxin Li, et al.2016). It also further indicates that Tim-3 can be used as a therapeutic target and predictor of AML. It was reported that Tim-3 expression was correlated with T cells, AML, tumor immune escape and clinicopathological prognosis stratification (Li C, et al.2014). These are in agreement with the results of our study.

In this study, bone marrow and spleen macrophages derived from control group and experimental group. Macrophage polarization types were identified from serum cytokine secretion levels and macrophage surface markers, demonstrating macrophages polarization was involved in the immunomodulation of benzene-induced AML. Type M1 and M2 macrophages play an immunomodulatory role by secreting different cytokines. IL-12 and TGF-β1 mediate positive and negative regulation respectively. Type M1 macrophages can secrete inflammatory cytokines such as IL-1β, TNF-α, IL-12, IL-18 and IL-23 (Benoit M, et al. 2008; Murray PJ, et al. 2011; Mills CD, et al.2014). Type M2 macrophages secrete large amounts of immunoregulatory cytokines IL-10, TGF-β and the metabolic conversion of arginine to ornithine and polyamines (Van den Bossche J, et al.2012). The results showed that the serum IL-12 level in the experimental group was significantly lower than that in the control group, and the level of TGF-β1 in the experimental group was significantly higher than that in the control group. The serum expression level of these factors can be used as a biomarker of the aggravating of AML. By monitoring the changes of these molecular markers in different stages of leukemia patients, the severity and development trend of the disease can be predicted and diagnosed.

In order to confirm the phenotype of TAMs, the study founded that the expression levels of CD206 macrophages in bone marrow macrophages significantly increased in the experimental group compared with the control group. And the expression levels of CD163 macrophages in spleen macrophages significantly increased in the experimental group compared with the control group. CD206 and CD163 macrophages represent type M2 macrophages, thus indicating that macrophages began to polarize to type M2 during the pathogenesis of benzene leukemia. At the same time, the study also observed that Tim-3 was highly expressed in bone marrow macrophages in experimental group. Therefore, we believe that up-regulation of Tim-3 expression in macrophages of experimental group may accelerate macrophages polarized to type M2, indicating that the polarization of macrophages was involved in the pathogenesis of benzene leukemia.

Then, we further studied the specific regulation mechanism of up-regulation of Tim-3. Current studies had shown that the PI3K/AKT/mTOR signaling pathway was a signaling pathway closely related to tumor development, involving various tumor chemotherapy resistance and tumor cell survival. Besides breast

cancer, small cell lung cancer and urinary system tumors, the occurrence and development of leukemia were also closely related to this signal transduction pathway (Liu LL, et al. 2013; Zhou ZW, et al.2015; Hao, Y, et al. 2019). So is the occurrence of benzene-induced AML related to the signaling pathway? Previous studies had founded that Tim-3 was highly expressed in human AML cells, triggered growth factor-like effects by activating the PI3K/AKT/mTOR signaling pathway, and promoted hypoxia-induced neovascularization and activation by activating HIF-1 signaling pathway (Prokhorov A,et al.2015). Activation of PI3K/AKT/mTOR signaling pathway can effectively promote intracellular glucose uptake, improve hexokinase activity and glycolysis process, and enhance the glycolysis ability of cells, which are very important for maintaining the survival and function of type M2 macrophages (Covarrubias AJ, et al.2015). A number of studies had also confirmed that the PI3K/AKT/mTOR signaling pathway was related to macrophage function regulation which involved in the proliferation and development of macrophages (Xu Q, et al.2003). Activation of this pathway promoted the conversion of macrophages from type M1 to M2 (Troutman, TD; et al. 2012; T. Fukao, et al. 2003; Liu R, et al. 2017; Tian Y, et al. 2015; Ruckerl D, et al.2012).

The activity of the PI3K/AKT/mTOR signaling pathway was determined by detecting the phosphorylation levels of PI3K, AKT and mTOR. The results showed that benzene poisoning could promote the expression of p-PI3K, p-AKT and p-mTOR in bone marrow macrophages. This experiment research results demonstrated that the immunosuppression microenvironment of leukemia cells programed the phenotype and function of macrophages by various channels, including higher expression level of Tim-3 and higher expression of phosphorylation of PI3K, AKT and mTOR in bone marrow macrophages, which promote macrophage polarization from type M1 to M2. The ability of macrophages in recognizing and swallowing antigens decreased. So suppression of the immune system played an important role in the benzene leukemia tumor environment. At the same time, with the deepening of molecular biology and proteomics research, more abnormalities of PI3K/AKT/mTOR pathway molecules involved in the pathogenesis of leukemia will be revealed, providing more reliable and specific targets for the emerging research on tumor targeted therapy.

Tim-3 was highly expressed in macrophages in benzene leukemia. It promoted the activation of PI3K/AKT/mTOR signaling pathway, stimulated the secretion of anti-inflammatory cytokines, and inhibited the secretion of pro-inflammatory cytokines. And the up-regulation of Tim-3 expression changed the phenotype and function of macrophages by promoting type M2 polarization of macrophages, thus inducing negative immune response in the tumor microenvironment, making tumor cells escape from immune surveillance and attack of the body or even immunodeficiency, accelarating cancer progression and immune escape. It may be one of the mechanisms of benzene-induced AML. And Tim-3 is therefore considered a potential anti-AML therapeutic target.

However, in this study, the research on the specific regulation mechanism of Tim-3 in this pathway was not deep and was lack of further confirmation of clinical samples. In future studies, we will conduct in-depth discussion and research.

Conclusions

1. Tim-3 as a immunosuppressive molecule was highly expressed on CD14$^+$ monocytes in bone marrow and spleen in experimental group, and the Tim-3 expression level of bone marrow macrophages in the experimental group was also significantly higher than that in the control group. The results showed that benzene exposure can promote the expression of Tim-3 in macrophages.

2. In the benzene-induced AML model group, with the up-regulation of Tim-3 expression, the serum level of IL-12 as type M1 macrophage functional marker decreased, and the serum level of TGF-β1 as type M2 macrophage functional marker increased. The expression of M2 macrophages in the bone marrow and spleen macrophages in the experimental group was significantly higher than that in the control group, suggesting that benzene exposure can induce the macrophages polarization to type M2.

3. The protein expressions of PI3K/AKT/mTOR signal pathway in bone marrow tissue macrophages increased. So this signaling pathway protein may be involved in the macrophages activation process, and promote the macrophages polarization from type M1 to type M2 and that led to AML.

Declarations

Abbreviations:

Not applicable.

Ethics Approval and Consent to Participate

The animal experiment complied with the declaration of Helsinki.

Consent for publication

Not Applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing Interest

The authors declare that they have no conflict of interest.

Funding

Authors' contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by Q. N. X X. L was the corresponding author and performed the final approval of the manuscript. Material preparation, data collection and analysis were performed by XD. J and XP. H. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

References

Greim, H; Kaden, DA; Larson, RA; et al. The bone marrow niche, stem cells, and leukemia: impact of drugs, chemicals, and the environment. Ann N Y Acad Sci.2014;1310:1-6. https://doi.org/10.1111/nyas.12362.

Loomis, D; Guyton, KZ; Grosse, Y; International Agency for Research on Cancer Monograph Working Group, et al. Carcinogenicity of benzene. Lancet Oncol.2017;18(12):1574-1575. https://doi.org/10.1016/S1470-2045(17)30832-X.

Bi Y, Li Y, Kong M, et al. Gene expression in benezene-exposed workers by microarray analysis of peripheral mononuclear blood cells:induction and silencing of CYP4F3A and regulation of DNA-dependent protein kinase catalytic submit in DNA double strand break repair. Chem Biol Interact. 2010;184(1-2):207-211. https://doi.org/10.1016/j.cbi.2009.12.024.

McHale CM, Zhang L, Lan Q, et al. Global gene expression profiling of a population exposed to a range of benzene levels. Environ Health Perspect. 2011;119(5):628-634. https://doi.org/10.1289/ehp.1002546.

Zhou H, Dehn D, Kepa JK, et al. NAD(P)H: quinone oxidoreductase 1-compromised human bone marrow endothelial cells exhibit decreased adhesion molecule expression and CD34$^+$ hematopoietic cell adhesion. J Pharmacol Exp Ther. 2010;334(1):260-268. https://doi.org/10.1124/jpet.110.167841.

Li B, Li YQ, Yang LJ, et al. Decreased T-cell receptor excision DNA circles in peripheral blood mononuclear cells among benzene-exposed workers. Int J Immunogenet.2009;36(2):107-111. https://doi.org/10.1111/j.1744-313X.2009.00832.x.

Wang, BS; Han, L; Yang, DD; et al. Impact of Enterprise Ownership and Size on Registered Hematotoxicity in Benzene-exposed Workers. Biomed Environ Sci.2018;31(12):917-921. https://doi.org/10.3967/bes2018.125.

Shin DS, Ribas A.The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol. 2015;33:23-35. https://doi.org/10.1016/j.coi. 2015.01.006.

Cassetta L, Cassol E, Poli G. Macrophage polarization in health and disease. Scientific World Journal. 2011;11:2391-2402.

Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature.2013;496(7446): 445−455. https://doi.org/10.1038/nature12034.

Jiang, X; Zhou, T; Xiao, Y; et al. Tim-3 promotes tumor-promoting M2 macrophage polarization by binding to STAT1 and suppressing the STAT1-miR-155 signaling axis. Oncoimmunology.2016;5(9): e1211219. https://doi.org/10.1080/2162402X.2016. 1211219.

Sun, J; Huang, Q; Li, S; et al. MiR-330-5p/Tim-3 axis regulates macrophage M2 polarization and insulin resistance in diabetes mice. Mol Immunol.2018;95:107-113. https://doi.org/10.1016/j.molimm.2018.02.006.

Zhang, D; Ren, L; Zhao, M; et al. Role of Tim-3 in Decidual Macrophage Functional Polarization During Abnormal Pregnancy With Toxoplasma gondii Infection. Front Immunol.2019;10:1550. https://doi.org/10.3389/fimmu.2019.01550.

Yang, H; Xie, T; Li, D; et al. Tim-3 aggravates podocyte injury in diabetic nephropathy by promoting macrophage activation via the NF-κB/TNF-α pathway. Mol Metab.2019;23:24-36. https://doi.org/10.1016/j.molmet.2019.02.007.

Zhang, W; Zhang, Y; He, Y; et al. Lipopolysaccharide mediates time- dependent macrophage M1/M2 polarization through the Tim-3/Galectin-9 signalling pathway. Exp Cell Res.2019;376(2):124-132. https://doi.org/10.1016/j.yexcr.2019.02.007.

Belingheri M, Fustinoni S, De Vito G, et al. Benzene and leukemia: from scientific evidence to regulations. A historical example. Med Lav. 2019;110(3):234-240. https://doi.org/10.23749/mdl.v110i3.7995.

Zhang L, Lan Q, Ji Z, et al. Leukemia-related chromosomal loss detected in hematopoietic progenitor cells of benzene-exposed workers.Leukemia. 2012;26(12):2494-2498. https://doi.org/10.1038/leu.2012.143.

McHale CM, Zhang L, Smith MT. Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis.2012;33(2):240-252. https://doi.org/10.1093/carcin/bgr297.

Xiangxin Li, Xiaopeng He, Chunhong Ma, et al. Tim-3 as a leukemia stem cell specific marker expressing on bone marrow mononuclear cells is a factor for prognosis evaluation in patients with acute myelogenous leukemia. Int J Clin Exp Med. 2016;9(6):10979-10986.

Li C, Chen X, Yu X, et al. Tim-3 is highly expressed in T cells in acute myeloid leukemia and associated with clinicopathological prognostic stratification. Int J Clin Exp Pathol. 2014;7(10):6880-6888.

Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. J Immunol.2008;181(6): 3733-3739.

Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol.2011;11(11): 723-737. https://doi.org/10.1038/nri3073.

Mills CD, Thomas AC, Lenz LL, Munder M. Macrophage: SHIP of Immunity. Front Immunol.2014;5:620. https://doi.org/10.3389/fimmu.2014.00620.

Van den Bossche J, Lamers WH, Koehler ES, et al. Pivotal Advance: Arginase-1-independent polyamine production stimulates the expression of IL-4-induced alternatively activated macrophage markers while inhibiting LPS-induced expression of inflammatory genes. J Leukoc Biol. 2012;91(5):685-699. https://doi.org/ 10.1189/jlb.0911453.

Liu LL, Long ZJ, Wang LX, et al. Inhibition of mTOR pathway sensitizes acute myeloid leukemia cells to aurora inhibitors by suppression of glycolytic metabolism. Mol Cancer Res. 2013;11(11):1326-1336. https://doi.org/10.1158/1541-7786.MCR-13-0172.

Zhou ZW, Li XX, He ZX, et al. Induction of apoptosis and autophagy via sirtuin1 and PI3 K/Akt/mTOR-mediated pathways by plumbagin in human prostate cancer cells. Drug Des Devel Ther.2015;12(9):1511-1554. https://doi.org/10.2147/DDDT.S75976.

Hao, Y; Yin, H; Zhu,C; et al. Matrine inhibits proliferation and promotes autophagy and apoptosis in non-small cell lung cancer cells by deactivating PI3K/AKT/mTOR pathway. Nan Fang Yi Ke Da Xue Xue Bao. 2019;39(7):760-765. https://doi.org/10.12122/j.issn.1673-4254.2019.07.02.

Prokhorov A, Gibbs BF, Bardelli M,et al. The immune receptor Tim-3 mediates activation of PI3 kinase/mTOR and HIF-1 pathways in human myeloid leukemia cells. Int J Biochem Cell Biol.2015;59:11-20. https://doi.org/10.1016/j.biocel.2014.11.017.

Covarrubias AJ, Aksoylar HI, Horong T. Control of macrophage metabolism and activation by Mtor and Akt signaling. Semin Immunol. 2015;27(4):286-296. https://doi.org/10.1016/j.smim.2015.08.001.

Xu Q, Simpson SE, Scialla TJ, et al. Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood.2003;102(3):972-980. https://doi.org/10.1182/blood-2002-11-3429.

Troutman, TD; Bazan, JF; Pasare, C. Toll-like receptors, signaling adapters and regulation of the pro-inflammatory response by PI3K.Cell Cycle.2012;11(19):3559-3567. https://doi.org/10.4161/cc.21572.

363. Fukao, S. Koyasu. PI3K and negative regulation of TLR signaling. Trends Immunol. 2003;24(7):358-363. https://doi.org/10.1016/s1471-4906(03)00139-x.

Liu R, Fan T, Geng W, et al. Negtive Immune Regulator TIPE2 Promotes M2 Macrophage Differentiation through the Activation of PI3K/AKT Signaling Pathway. PLoS One.2017;12(1): e0170666. https://doi.org/10.1371/journal.pone.0170666.

Tian Y, Piras BA, Kron IL, et al. Adenosine 2B Receptor Activation Reduces Differentiation via PI3K/AKT Pathway. Oxid Med Cell Longev.2015;2015:585297. https://doi.org/10.1155/2015/585297.

Ruckerl D, Jenkins SJ, Laqtom NN, et al. Induction of IL-4Ralpha-dependent microRNAs identifies PI3K/Akt signaling as essential for IL-4-driven murine macrophage proliferation in vivo. Blood.2012;120(11): 2307-2316. https://doi.org/10.1182/blood-2012-02-408252.

Tables

Table 1. Serum levels of peripheral blood IL-12

group	case	IL-12（pg/mL）
0 day	6	240.46±130.98
control group	6	216.71±70.16
experimental group	6	128.65±62.06[ab]

Note: [a] Compared with pre-infection, $p < 0.05$; [b] Compared with the control group, $p < 0.05$.

Table 2. Serum levels of peripheral blood TGF-β1

group	case	TGF-β1（pg/mL）
0 day	6	54.03±21.07
control group	6	76.84±15.73
experimental group	6	103.59±36.13[ab]

Note: [a] Compared with pre-infection, $p < 0.05$; [b] Compared with the control group, $p < 0.05$
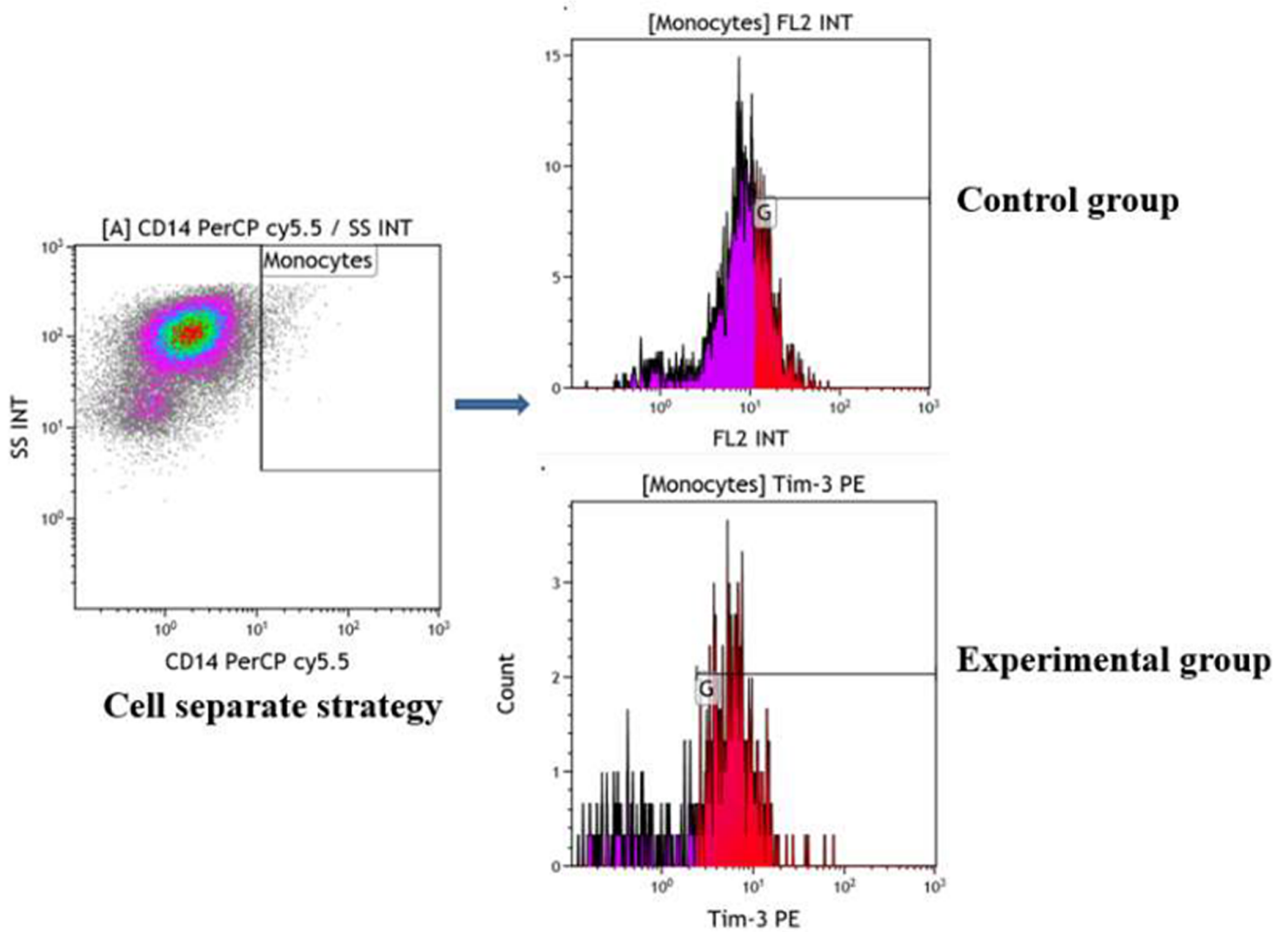
Figures

Figure 1

Benzene-induced AML promoted Tim-3 expression in CD14+ monocytes of bone marrow: The proportion of Tim-3 positive CD14+ monocytes of bone marrow in the experimental group were significantly higher than that in the control group.
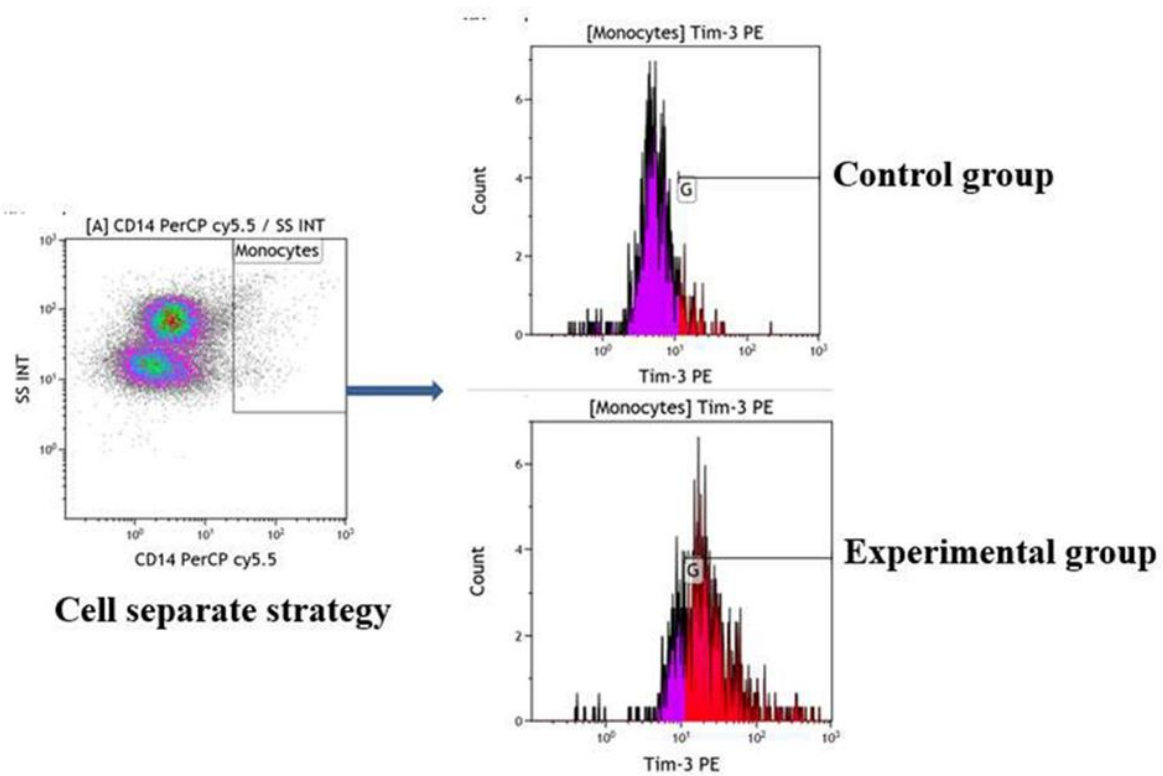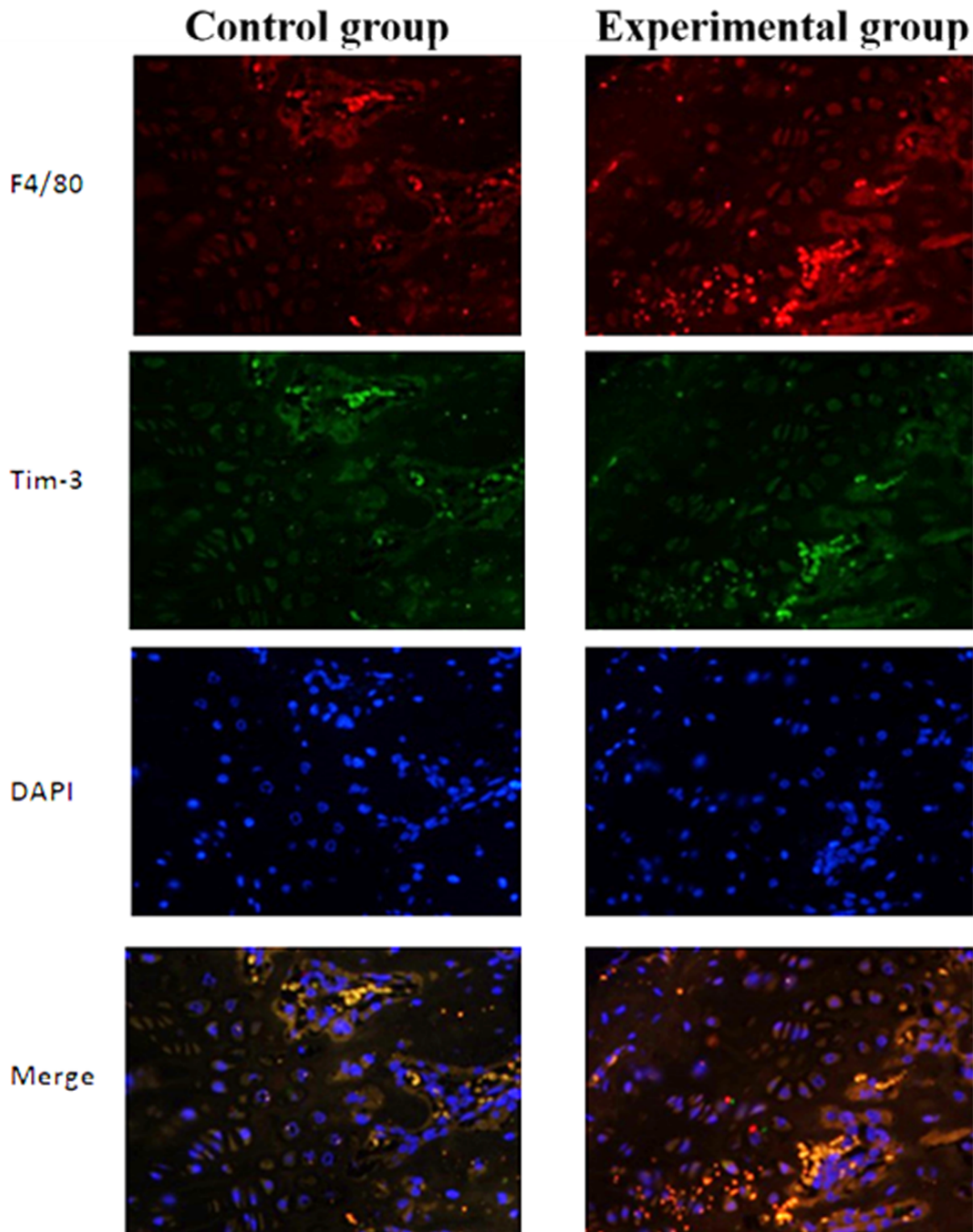
Figure 2

Benzene-induced AML promoted Tim-3 expression in CD14+ monocytes of spleen: The proportion of Tim-3 positive CD14+ monocytes of spleen in the experimental group were significantly higher than that in the control group.
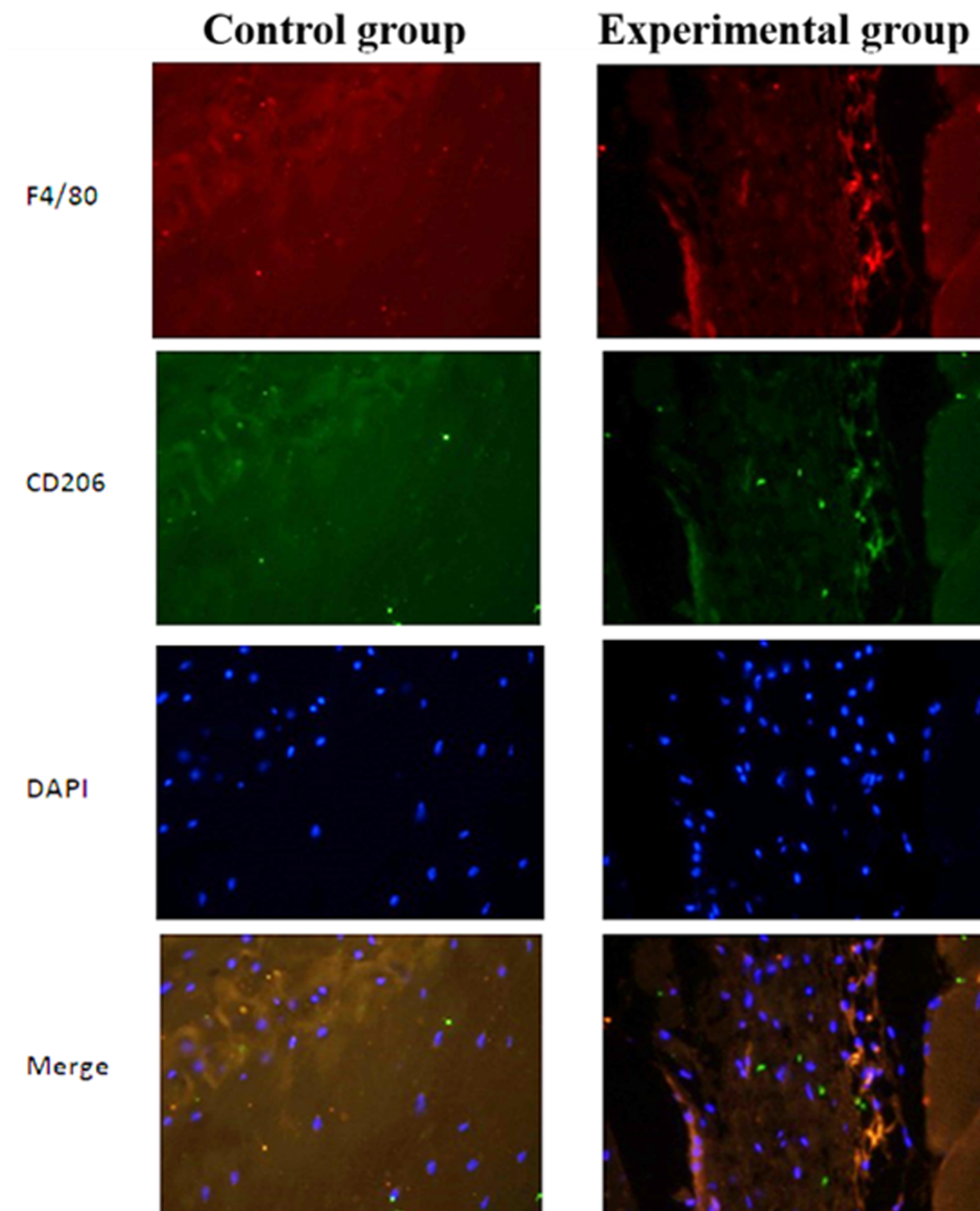
Figure 3

Benzene-induced AML promoted Tim-3 expression in the bone marrow macrophages (Red: F4/80, Green: Tim-3, Blue: DAPI, Immunofluorescence 400x): Compared with the control group, the expression of Tim-3 on F4/80 (M1+M2) macrophages of bone marrow in the experimental group significantly increased.

Figure 4

Benzene-induced AML promoted CD206 expression in the bone marrow macrophages (Red: F4/80, Green: CD206, Blue: DAPI, Immunofluorescence 400x): The expression of CD206 macrophages in F4/80 (M1+M2) macrophages of bone marrow in the experimental group significantly increased than that in the control group.
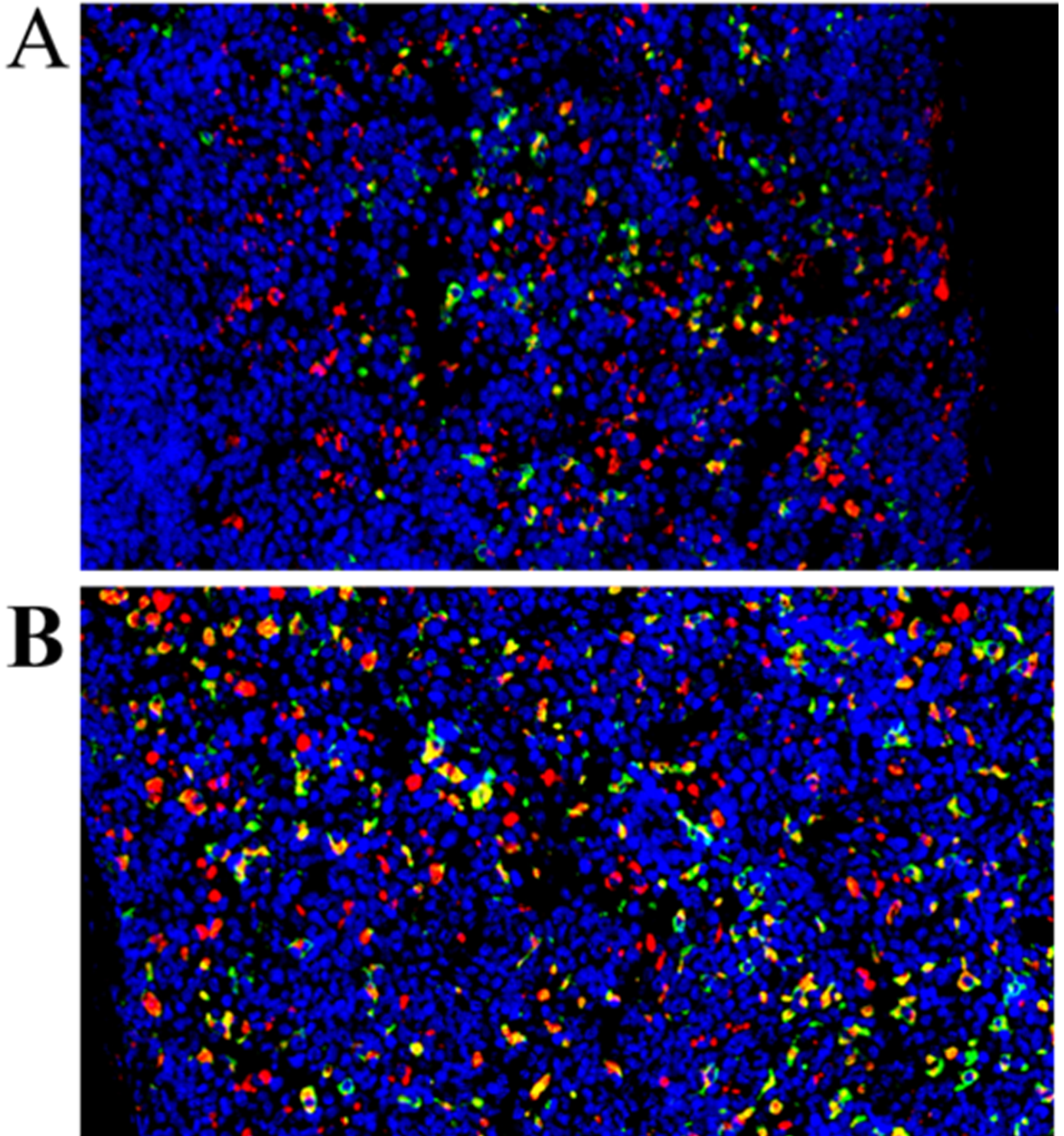
Figure 5

Benzene-induced AML promoted CD163 expression in the spleen macrophages (A: control group B: experimental group) (Red: CD68, Green: CD163, Blue: DAPI, Immunofluorescence 400x): The expression of CD163 macrophages in CD68(M1+M2) macrophages of spleen in the experimental group significantly increased than that in the control group.
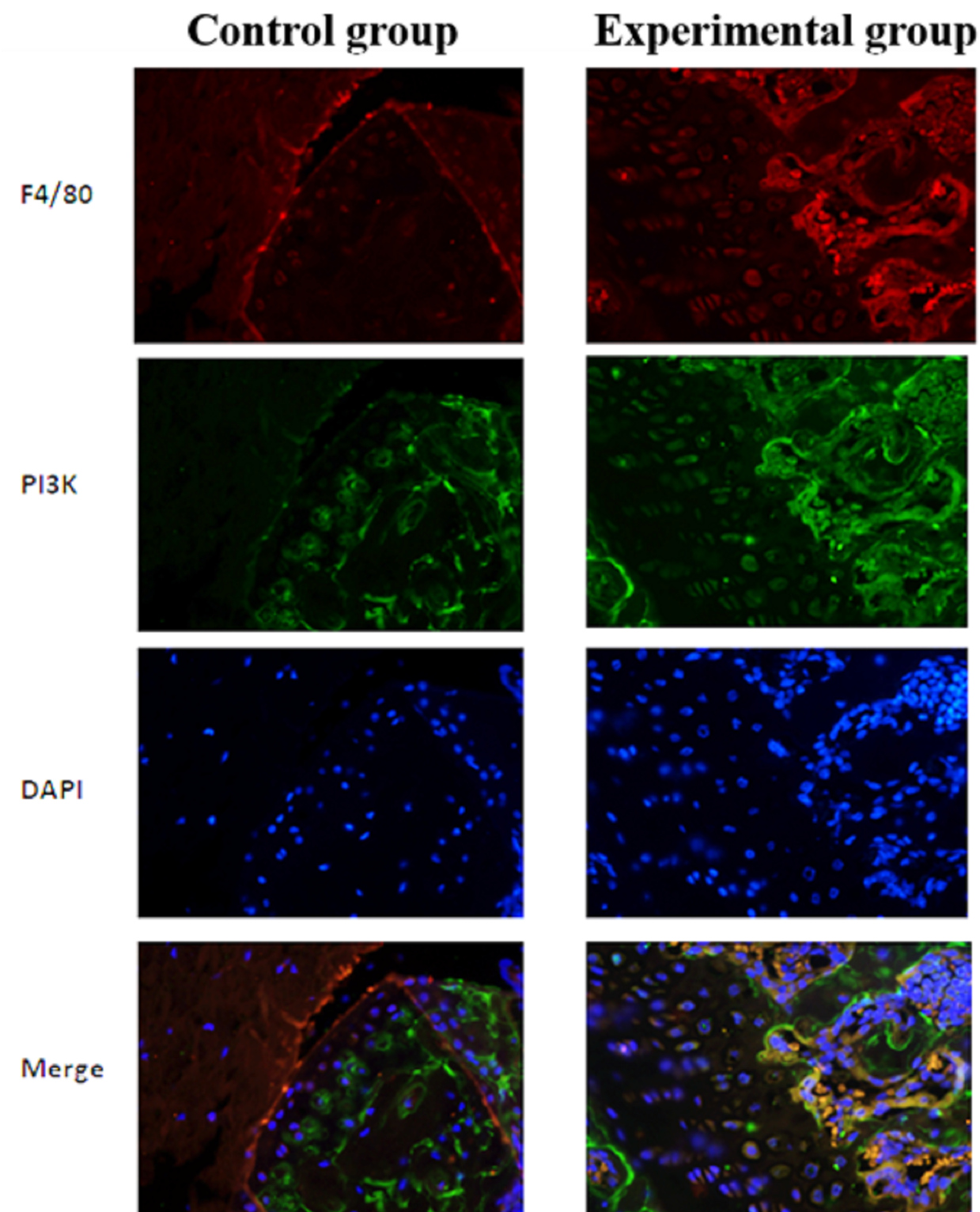
Figure 6

Benzene-induced AML promoted p-PI3K expression in the bone marrow macrophages (Red: F4/80, Green: p-PI3K, Blue: DAPI, Immunofluorescence 400x): Compared with the control group, the expression levels of p-PI3K of bone marrow macrophages in the experimental group significantly increased.
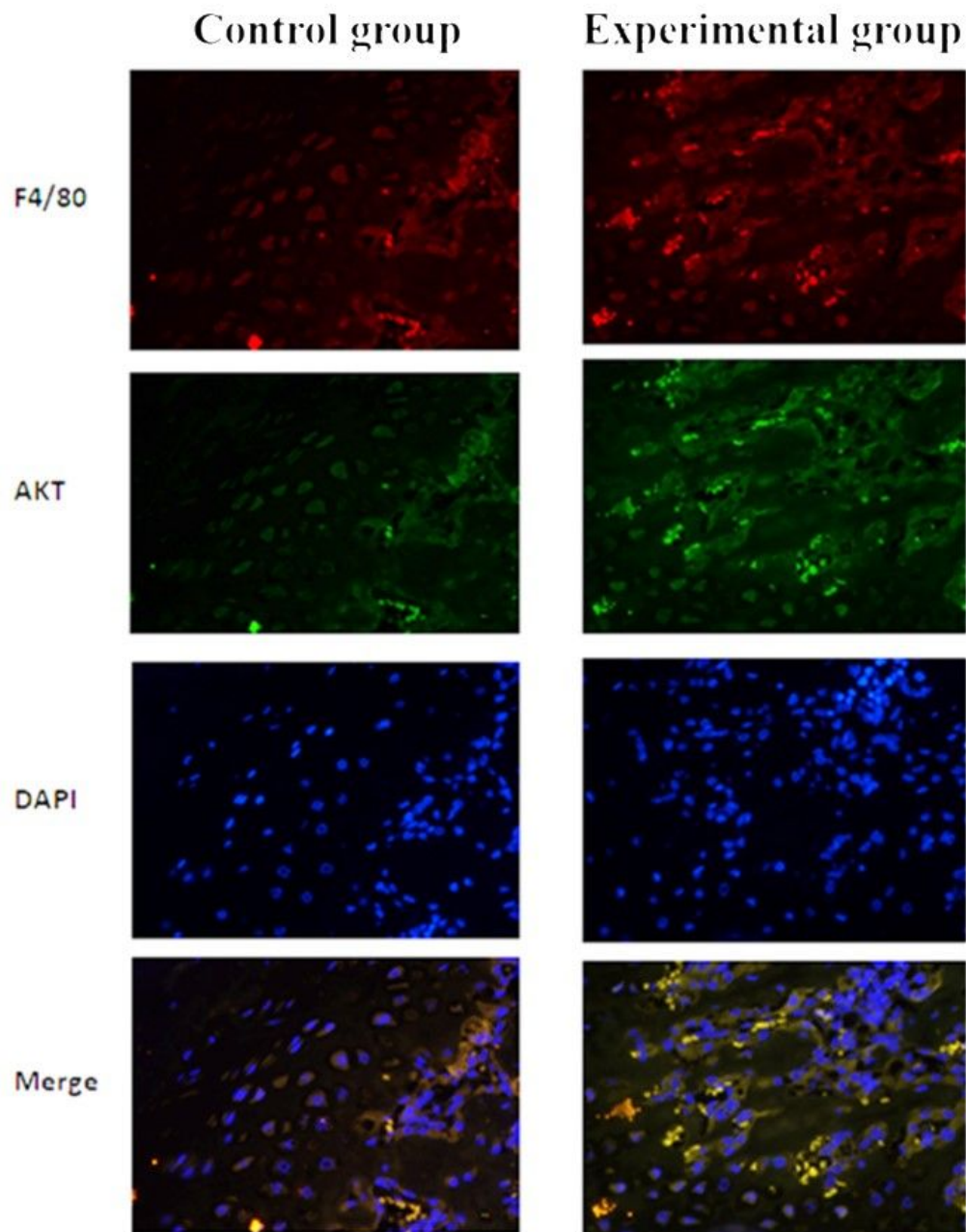
Figure 7

Benzene-induced AML promoted p-AKT expression in the bone marrow macrophages (Red: F4/80, Green: p-AKT, Blue: DAPI, Immunofluorescence 400x): Compared with the control group, the expression levels of p-AKT of bone marrow macrophages in the experimental group significantly increased.
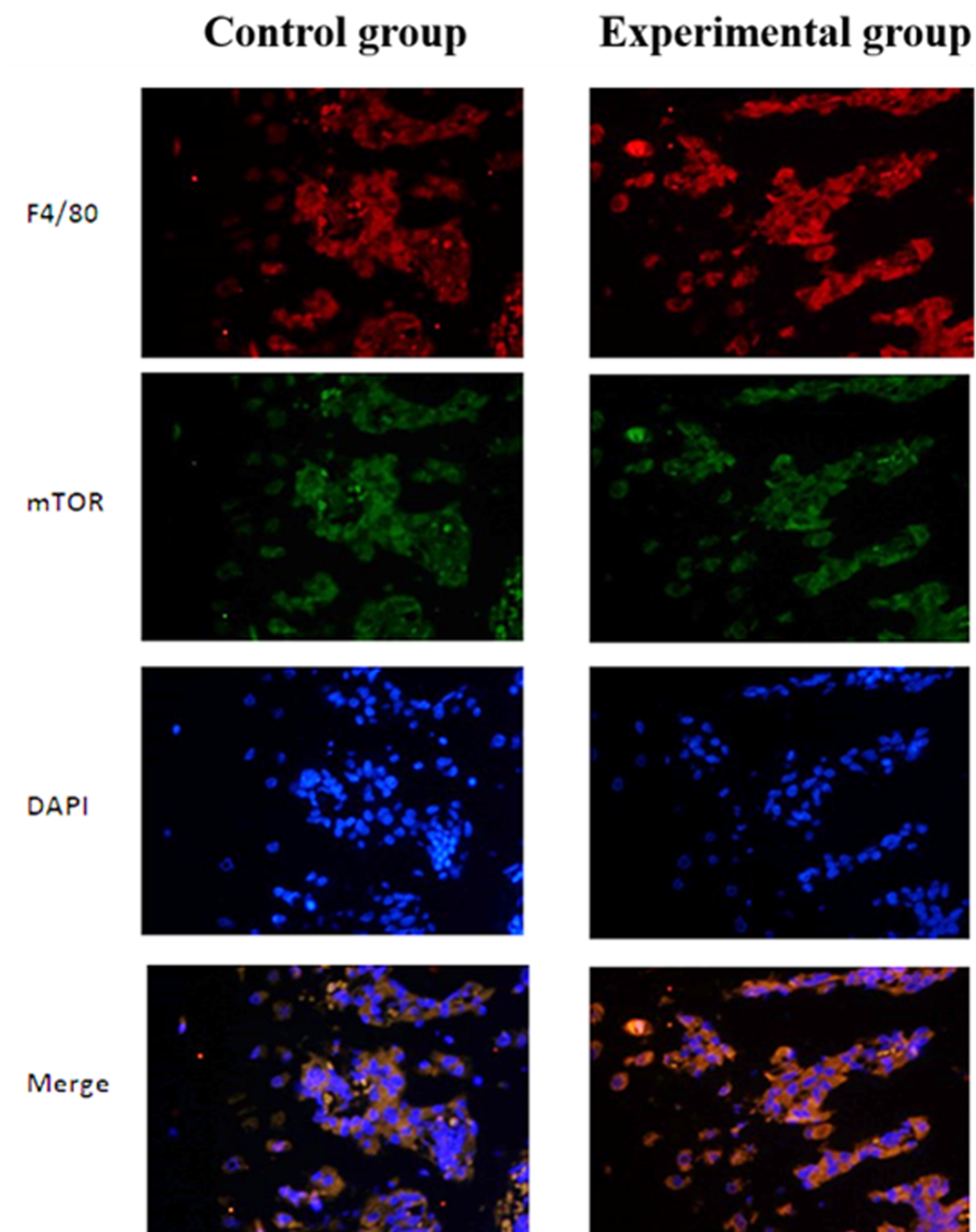
Figure 8

Benzene-induced AML promoted p-mTOR expression in the bone marrow macrophages (Red: F4/80, Green: p-mTOR, Blue: DAPI, Immunofluorescence 400x): Compared with the control group, the expression levels of p-mTOR of bone marrow macrophages in the experimental group significantly increased.