Research Paper

Protective Effect and Mechanism of Dexmedetomidine on Myocardial Ischemia-Reperfusion Injury by Regulating Titin/Myosin Heavy Chain Signal Axis

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To examine the protective impact of dexmedetomidine on myocardial ischemia-reperfusion injury, and to clarify the relationship between dexmedetomidine and titin/myosin heavy chain signal axis. Rat cardiac H9c2 cells were purchased as the research object. They were divided into three groups; blank control group, hypoxia concentration incubation group (hypoxia group) and dexmedetomidine group. The lactate dehydrogenase, cardiac troponin I and creatine kinase-myocardial band in myocardial cells of rats in hypoxia group were higher than the blank control group. The lactate dehydrogenase, cardiac troponin I and creatine kinase-myocardial band in myocardial cells of rats in differentially expressed gene were reduced than hypoxia group. Compared with the control group, in the hypoxia group, there was no discernible variation in the level of telomerase in cardiomyocytes. The telomerase level of cardiomyocytes in the differentially expressed gene was higher than the hypoxia group. Compared with the control group, the titin protein in myocardial cells of hypoxia group was decreased, the beta-myosin heavy chain protein was increased while the level of titin in cardiac myocytes of differentially expressed gene was higher than that of hypoxia group, and the beta-myosin heavy chain was reduced than hypoxia group. Compared with the blank control group, the phospho-extracellular signal regulated kinases protein in cardiac myocytes of rats in hypoxia group and differentially expressed gene was lower, and the nuclear factor kappa B protein was higher. However, the phospho-extracellular signal regulated kinases protein in cardiac myocytes of rats in differentially expressed gene was higher than in hypoxia group and the nuclear factor kappa B was reduced than hypoxia group. The phospho-phosphatidylinositol-3 kinase and phospho-protein kinase B in cardiac myocytes of rats in hypoxia group and differentially expressed gene were higher than the blank control group, and the phospho-phosphatidylinositol-3 kinase and phospho-protein kinase B in rats in differentially expressed gene were higher than hypoxia group. The protective effect of dexmedetomidine on myocardial ischemia-reperfusion injury may be related to reducing cardiomyocyte apoptosis and inhibiting titin differentially expressed gene radiation by activating extracellular signal regulated kinases 1/2 and phosphatidylinositol-3 kinase signaling pathways.

Key words: Dexmedetomidine, titin/myosin heavy chain signal axis, myocardial ischemia-reperfusion, cardiac surgery

Myocardial Ischemia-Reperfusion Injury (IRI) is a common clinical pathophysiological phenomenon and a common postoperative adverse event in patients undergoing cardiac surgery[1,2]. At present, there is no specific means to effectively avoid the occurrence of myocardial injury after cardiac surgery, so it is

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of great significance to explore effective prevention and treatment measures. Studies have found that pretreatment with Dexmedetomidine (DEX) can play a protective role in myocardial function[3]. However, the specific molecular mechanism is still not fully elucidated, so the value of DEX in myocardial injury needs to be further elucidated. Titin and beta-Myosin Heavy Chain (β-MHC) are proteins closely related to myocardial function, and their expression levels are closely related to myocardial function[4]. Previous research has found that DEX can inhibit myocardial IRI through multiple signaling pathways. However, the mechanism of titin, β-MHC protein and related signaling pathway proteins in myocardial IRI, and the effect and mechanism of DEX on cardiomyocyte function remain unclear. The protective mechanism of DEX on cardiomyocytes will be further clarified by this work, and also provide new clues for clinical reduction of myocardial function injury after cardiac surgery.

MATERIALS AND METHODS

General information:

Rat cardiogenic H9C2 cells were purchased as research subjects. They were divided into three groups; Blank Control Group (BCG), hypoxic incubation group (Hypoxic Group) ((HYG)) and DEX group. The BCG was the most basic culture environment, which was cultured in Dulbecco's Modified Eagle Medium (DMEM) low glucose medium of fetal bovine serum. The low oxygen concentration group was cultured under 5 % oxygen concentration environment. The DEX group was incubated in a culture environment in which the DEX concentration was 100 μm/ml.

Methods:

Cell culture and passage: The frozen storage tube of the cell line was removed from the refrigerator at -80° and rapidly melted in a water bath at 37°. The frozen cells were absorbed into a clean centrifuge tube under aseptic conditions, and an appropriate amount of fresh medium was added to mix gently. After a series of treatments, the cells were cultured, and the fresh medium was changed for passage after the cells were completely adherent to the wall.

Cell grouping and DEX co-culture: Confluent adherent cells cultured under conventional conditions were divided into three groups; BCG, HYG and Differentially Expressed Gene (DEG). The BCG was the most basic culture environment, which was cultured in DMEM low glucose medium of fetal bovine serum. The low oxygen concentration group was cultured under 5 % oxygen concentration environment. The DEX group was incubated for 24 h in a culture environment perfused with DEX concentration of 100 μm/ml.

Observation indicators:

Cell Counting Kit–8 (CCK-8) was used to measure cell viability: A 100 l cell suspension is prepared in a 96-well plate and then pre-cultured. After 24, 48 or 72 h of incubation, 10 μl of CCK-8 is added to each sample and incubated for 4 h. Absorbance at 450 nm is measured by a spectrophotometer.

Detection of apoptosis rate: After 24 h of culture, trypsin was used to digest each group's cells without the use of Ethylenediaminetetraacetic Acid (EDTA), centrifuged, and then single-cell suspension was prepared by adding 500 μl binding buffer. The suspension was mixed and incubated for 10 min at room temperature. Finally, using flow cytometry, the apoptosis rate of the cells in each group was found.

Determination of cardiomyocyte activity (myocardial zymogram, telomerase activity): The 6-well plate was taken out of the incubator for observation, and the ice box and cell scraper for protein extraction were prepared in advance. The 6-well plate was placed on the ice box, and 2 ml Phosphate Buffered Saline (PBS) was taken from each well 3 times. Cells were lysed with Radio-Immunoprecipitation Assay (RIPA) protein lysate, and the activities of myocardial zymography (cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH), Creatine Kinase-Myocardial Band (CK-MB)) were measured by Bicinchoninic Acid (BCA) assay method. Telomerase in plasma and cardiac nucleus was detected by Enzyme-Linked Immunosorbent Assay (ELISA). The procedure was carried out exactly as the reagent's instructions required. A microplate reader was used to measure the Optical Density (OD) value of telomerase in each group within 5 min of the reaction's completion in order to assess each group's activity.

Cardiomyocyte contractile related proteins titin, β-MHC and signal pathway proteins: After 24 h of treatment, the cells were washed with PBS, and the total protein was extracted. The titin and β-MHC were determined by Western blot method. According to literature reports, the myocardial infarction is closely related to the effect on myocardial cells of signaling

pathways including Extracellular Signal Regulated Kinases (ERK1/2) and Phosphatidylinositol-3 Kinase (PI3K) protein, Therefore, the levels of ERK1/2 and PI3K proteins were determined by Western blot.

Data processing:

The statistical software Statistical Package for the Social Sciences (SPSS) 20.0 was employed to conduct the analysis, specifically utilizing the Chi-square (χ^2) test for count data, and was used for measurement data. The t-test was employed to conduct a comparison between two distinct groups. In comparison to the control group, statistical significance was observed ($p<0.05$).

RESULTS AND DISCUSSION

Compared with the BCG, the survival rate of cardiomyocytes in HYG was decreased; Compared with HYG, the survival rate of cardiomyocytes in DEG was increased as shown in Table 1. The LDH, CTnI and CK-MB in myocardial cells of rats in HYG were higher than BCG, and the LDH, CTnI and CK-MB in myocardial cells of rats in DEG were reduced than HYG as shown in Table 2. There was no discernible difference between the hypoxic group and the control group in terms of the amount of telomerase present in the cardiomyocytes. The telomerase level of cardiomyocytes in the DEG was higher than the HYG as shown in Table 3. The apoptosis rate of cardiomyocytes in HYG was higher than BCG, and the apoptosis rate of cardiomyocytes in DEG was reduced than HYG as shown in Table 4. Compared with the BCG, the titin protein was decreased and β-MHC protein was increased in the HYG, while the expression of titin and β-MHC in the DEG was higher than the HYG as shown in Table 5.

Compared with the BCG, the p-ERK protein in cardiomyocytes of rats in the HYG and DEG was decreased, and the Nuclear Factor Kappa B (NF-κB) protein was increased, while the p-ERK in cardiomyocytes of rats in DEG was higher than the HYG, and the NF-κB was reduced than the HYG as shown in Table 6. The p-PI3K and phospho-Protein kinase B (p-Akt) in myocardial cells of rats in HYG and DEG were higher than those in BCG, and the p-PI3K and p-Akt in DEG were higher than HYG as shown in Table 7.

TABLE 1: COMPARISON OF CARDIOMYOCYTE SURVIVAL RATE

Group	n	Survival rate of cells
BCG	10	98.65±0.21
HYG	10	46.38±4.27[a]
DEG	10	75.39±5.62[ab]
F		26.544
p		<0.001

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 2: COMPARISON OF MYOCARDIAL ZYMOGRAM LEVELS OF CARDIOMYOCYTES (x̄±s)

Group	n	LDH (KU/L)	CTnI (ng/ml)	CK-MB (U/ml)
BCG	10	0.25±0.03	36.87±6.52	15.46±2.35
HYG	10	1.46±0.13[a]	1365.48±21.57[a]	104.68±7.11[a]
DEG	10	0.77±0.11[ab]	349.65±21.54[ab]	83.97±6.47[ab]
F		14.683	365.574	25.621
p		<0.001	<0.001	<0.001

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 3: COMPARISON OF MYOCARDIAL ZYMOGRAM LEVELS OF CARDIOMYOCYTES (x̄±s)

Group	n	Telomerase (ng/mg)
BCG	10	56.38±3.65
HYG	10	60.64±4.82[a]
DEG	10	83.27±3.68[ab]
F		14.624
p		<0.001

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 4: COMPARISON OF CELL APOPTOSIS IN EACH GROUP (x̄±s)

Group	n	Apoptosis
BCG	10	2.83±0.23
HYG	10	37.95±3.54[a]
DEG	10	18.23±2.11[ab]
F		16.582
p		<0.001

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 5: COMPARISON OF CONTRACTILE PROTEIN EXPRESSION IN CARDIOMYOCYTES OF RATS (x̄±s)

Group	n	Titin	ß-MHC
BCG	10	1.03±0.05	1.03±0.03
HYG	10	0.68±0.04[a]	1.13±0.03[a]
DEG	10	0.83±0.07[ab]	1.05±0.04[b]
F		5.642	0.357
p		<0.01	0.751

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 6: COMPARISON OF PROTEIN EXPRESSION LEVELS OF ERK SIGNALING PATHWAY IN MYOCARDIUM OF RATS (x̄±s)

Group	n	p-ERK	ERK	NF-κB
BCG	10	1.03±0.06	1.02±0.08	1.03±0.04
HYG	10	0.57±0.05[a]	0.98±0.02[a]	1.68±0.21[a]
DEG	10	0.79±0.06[ab]	0.97±0.03[ab]	1.23±0.12[ab]
F		6.572	0.462	7.675
p		<0.01	0.658	<0.01

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

TABLE 7: COMPARISON OF PI3K/AKT PATHWAY PROTEIN EXPRESSION LEVELS IN CARDIOMYOCYTES OF RATS (x̄±s)

Group	n	p-PI3K	p-AKT
BCG	10	0.26±0.04	0.23±0.03
HYG	10	0.36±0.06[a]	0.37±0.04[a]
DEG	10	0.63±0.05[ab]	0.59±0.05[ab]
F		6.543	5.567
p		<0.01	<0.01

Note: Compared with BCG, [a]p<0.05 and compared with HYG, [b]p<0.05

DEX hydrochloride is a novel, highly selective and highly effective Alpha (α) 2 adrenergic receptor agonist with sedative, anti-sympathetic excitability, analgesic and mononuclear properties[5,6]. DEX, a clinically highly selective α2 adrenergic receptor agonist, has been used in cardiac surgery to attenuate cardiovascular responses. DEX attenuates hemodynamic response to endotracheal intubation in patients undergoing cardiac surgery. Intravenous administration of DEX before cardiopulmonary bypass can attenuate the cardiovascular response to skin excisions and sternal splits[7]. DEX has been reported to stabilize hemodynamics during cardiac surgery in both adults and children. Although DEX has side effects of causing bradycardia and hypotension, it has a significant effect on reducing catecholamine secretion and maintaining hemodynamic stability during cardiac surgery[8]. Pasero *et al.*[9] found that intra-coronary infusion of DEX could significantly improve the myocardial contractility and reduce the plasma concentration of norepinephrine after ischemia-reperfusion in

the innervated area of the left anterior descending coronary artery of porcine, which may be related to stimulation of cardiac presynaptic α2-adrenoceptor and decrease the concentration of norepinephrine in the ischemic area[10]. Previous research has found that ketamine combined with DEX has a myocardial protective impact compared with sevoflurane with sufentanil. DEX can reduce myocardial damage after cardiac surgery, however, the specific mechanism of the myocardial effect of DEX is still unclear[11].

The structural protein titin gene and the contractile protein β-MHC gene of cardiomyocytes are important functional protein genes regulating the contractile function of cardiomyocytes. During the development of cardiomyocytes, titin is expressed earlier than myosin and cTn[12,13]. Related experiments have also shown that Bone Marrow-Derived Mesenchymal Stem Cells (BMMSCs) can form connections with cardiomyocytes in mixed culture with cardiomyocytes, and the expression of titin is significantly enhanced. Some cells have sarcomele-like structures, indicating that titin plays an essential role in the early differentiation of mesenchymal stem cells into cardiomyocytes[14]. Titin is intimately associated with several different heart functions, including coagulation, myocardial contraction, myocardial fiber development, myocardial hypertrophy, myocardial tissue morphogenesis and other biological processes. Previous studies have shown that titin fragment in plasma is upregulated when it dissociates from cardiomyocytes during myocardial infarction caused by long-term ischemia, and when myocardial tissue dies, the contraction of striated myocardial tissue changes. This process is probably due to the degradation of actin by Matrix Metalloproteinase (MMP)-12[15]. Titin, especially exon N2B, can be used as a new biomarker specifically associated with cardiac injury in serum[16]. In this study, it was found that the level of titin in rat cardiomyocytes in DEG was higher than that in HYG, revealing that DEX could reduce the degradation of titin and play an essential role in cardiomyocytes. However, the specific changes in actin phosphorylation and actin splicing require further investigation to elucidate the specific role leading to actin modification[17].

Myosin is a characteristic protein of cardiomyocytes and a major component of thick myofilaments[18]. Before induction, MSCS showed weak expression of β-MHC gene, and the β-MHC gene was enhanced 1 d, 4 d, 7 d and 14 d after induction[19]. Studies have shown that when vitamin C induces Embryonic stem cells (Es) to differentiate into cardiomyocytes, β-MHC gene expression is also present before induction, and the expression gradually increases after induction, reaching the peak at 10 d, and then gradually decreases[20]. After the interference of titin gene expression, the distribution of β-MHC was dispersed, and the expression of the β-MHC gene did not alter considerably from before the interference, and the arrangement of myofibrils was disordered[21].

Myocardial zymography is an index for differentiating myocardium, skeletal muscle and brain injury. CK-MB is a dimer composed of brain-type subunits and myotype subunits. When patients have myocardial injury, the level of CK-MB increases significantly, which is one of the sensitive indicators of myocardial tissue injury[22]. CTnI is a regulatory protein of myocardial muscle contraction. Under normal circumstances, the level of CTnI is very low, but when myocardial injury, the level of CTnI is significantly increased. CTnI is an important index to evaluate myocardial injury. LDH exists in almost all tissues, most abundant in heart, skeletal muscle and kidney, and is a reducing oxidase of pyruvate, the final product of glucose colysis[23]. In this study, all indicators of myocardial enzymes after hypoxic culture showed serious injury of cardiomyocytes, but all indicators tended to be improved after DEX addition. One of the traditional Mitogen-Activated Protein Kinases (MAPKs) signaling pathways, the ERK signaling pathway is essential for the growth and differentiation of cardiomyocytes. Abnormal ERK signaling pathway can lead to coronary atherosclerosis, myocardial infarction, myocardial hypertrophy and other cardiovascular diseases[24,25]. Stress in and out of cardiomyocytes can affect the activation process of ERK signaling pathway. Activation of ERK signaling pathway can significantly increase the phosphorylation level of ERK protein, and then affect the expression of downstream target protein NF-kB, and regulate cell proliferation and apoptosis[26,27]. An essential channel for membrane receptor signal transmission into cells, the PI3K/Akt signaling pathway controls various cellular processes, including cell proliferation, death, metastasis, and other cellular processes[28]. Recent studies have found that DEX can alleviate LPS-induced lung cell apoptosis and mitochondrial apoptosis signal activation by activating PI3K/Akt signaling pathway, and then improve acute lung

injury[29]. In this research, it was discovered that the p-ERK, p-PI3K and p-Akt in rat cardiomyocytes in DEG were higher than HYG, indicating that DEX could activate ERK1/2 and PI3K signaling pathways to protect cardiomyocytes after ischemia-reperfusion.

In conclusion, DEX's ability to prevent cardiac IRI may be due to its ability to activate the ERK1/2 and PI3K signaling pathways, which prevents cardiomyocyte apoptosis and titin degradation.

Authors' contributions:

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Conflicts of interests:

The authors declared no conflict of interests.

REFERENCES

1. Yi Q, Tan F, Zhang G. Application and evaluation of optical fiber oxygen measurement system in rat model of myocardial ischemia/reperfusion injury. Chin Pharmacol Bull 2020;36(1):155-6.
2. Yang L, Yuan Y, Liu X. Compound Musk injection has protective effect on myocardial ischemia/reperfusion injury in rats by inhibiting endoplasmic reticulum stress. Chin J Integr Tradit Western Med 2020;27(5):4-9.
3. Xing X, Tang B, Deng H. Effect of dexmedetomidine combined with sevoflurane postconditioning on myocardial ischemia-reperfusion injury in patients with diabetes mellitus. J Clin Anesthesiol 2021;37(9):913-8.
4. Li S, Gao D, Ismail L. Effect of titin on diastolic dysfunction of diabetic heart by resistance and aerobic exercise. China Sports Sci Technol 2021;57(11):7.
5. Tonner PH. Additives used to reduce perioperative opioid consumption 1: Alpha2-agonists. Best Pract Res Clin Anaesthesiol 2017;31(4):505-12.
6. Wang Y, Li S, Gao Y. Protective effect of dexmedetomidine on oxygen and glucose deprivation injury in PC12 cells. J Zhengzhou Univ 2019;54(4):611-4.
7. Isaiah A, Kiss E, Olomu P, Koral K, Mitchell RB. Characterization of upper airway obstruction using cine MRI in children with residual obstructive sleep apnea after adenotonsillectomy. Sleep Med 2018;50:79-86.
8. Kumar G, Dubey PK, Sanjeev OP. Effect of midazolam and dexmedetomidine sedation on the onset and duration of supraclavicular brachial plexus block: A randomised comparative study. Turk J Anaesthesiol Reanim 2018;46(3):201-7.
9. Pasero D, Sangalli F, Baiocchi M, Blangetti I, Cattaneo S, Paternoster G, *et al.* Experienced use of dexmedetomidine in the intensive care unit: A report of a structured consensus. Turk J Anaesthesiol Reanim 2018;46(3):176.
10. Dharmarao PS, Holyachi R. Comparative study of the efficacy of dexmedetomidine and fentanyl as adjuvants to ropivacaine in ultrasound-guided supraclavicular brachial plexus block. Turk J Anaesthesiol Reanim 2018;46(3):208-13.
11. Sriganesh K, Saini J, Theerth K, Venkataramaiah S. Airway dimensions in children with neurological disabilities during dexmedetomidine and propofol sedation for magnetic resonance imaging study. Turk J Anaesthesiol Reanim 2018;46(3):214-21.
12. Rennie MY, Stovall S, Carson JP, Danilchik M, Thornburg KL, Rugonyi S. Hemodynamics modify collagen deposition in the early embryonic chicken heart outflow tract. J Cardiovasc Dev Dis 2017;4(4):24.
13. Dong B, Zhou S, Yu S. The role of NOD-like receptor thermoprotein domain 3 inflammasome in myocardial ischemia-reperfusion injury. J Central South Univ 2017;42(7):848-53.
14. Mohanty P, Bhatnagar S. Structure of focal adhesion kinase in healthy heart *vs.* pathological cardiac hypertrophy: A modeling and simulation study. J Mol Graph Model 2018;80:15-24.
15. Vassiliadis E, Rasmussen LM, Byrjalsen I, Larsen DV, Chaturvedi R, Hosbond S, *et al.* Clinical evaluation of a matrix metalloproteinase-12 cleaved fragment of titin as a cardiovascular serological biomarker. J Transl Med 2012;10:140.
16. Bogomolovas J, Gasch A, Bajoras V, Karciauskaite D, Serpytis P, Grabauskiene V, *et al.* Cardiac specific titin N2B exon is a novel sensitive serological marker for cardiac injury. Int J Cardiol 2016;212:232-4.
17. LeWinter MM, Granzier H. Cardiac titin: A multifunctional giant. Circulation 2010;121(19):2137-45.
18. Shchepkin DV, Matyushenko AM, Bershitsky SY, Kopylova GV. Effect of interchain disulfide crosslinking in the tropomyosin molecule on actin-myosin interaction in the atrial myocardium. Bull Exp Biol Med 2019;167:65-8.
19. Mijailovich SM, Stojanovic B, Nedic D, Svicevic M, Geeves MA, Irving TC, *et al.* Nebulin and titin modulate cross-bridge cycling and length-dependent calcium sensitivity. J Gen Physiol 2019;151(5):680-704.
20. Seara FA, Barbosa RA, Santos MV, Domingos AE, Monnerat G, Carvalho AB, *et al.* Paradoxical effect of testosterone supplementation therapy on cardiac ischemia/reperfusion injury in aged rats. J Steroid Biochem Mol Biol 2019;191:105335.
21. Bunch TA, Kanassatega RS, Lepak VC, Colson BA. Human cardiac myosin–binding protein C restricts actin structural dynamics in a cooperative and phosphorylation-sensitive manner. J Biol Chem 2019;294(44):16228-40.
22. Pyati AK, Devaranavadagi BB, Sajjannar SL, Nikam SV, Shannawaz M, Patil S. Heart-type fatty acid-binding protein, in early detection of acute myocardial infarction: Comparison with CK-MB, troponin I and myoglobin. Indian J Clin Biochem 2016;31(4):439-45.
23. Ding M, Dong Q, Liu Z, Liu Z, Qu Y, Li X, *et al.* Inhibition of dynamin-related protein 1 protects against myocardial ischemia–reperfusion injury in diabetic mice. Cardiovasc Diabetol 2017;16(1):19.
24. Tsai JJ, Chen JH, Chen CH, Chung JG, Hsu FT. Apoptosis induction and ERK/NF-κB inactivation are associated with magnolol-inhibited tumor progression in hepatocellular carcinoma *in vivo*. Environ Toxicol 2020;35(2):167-75.
25. Das AA, Chakravarty D, Bhunia D, Ghosh S, Mandal PC, Siddiqui KN, *et al.* Elevated level of circulatory sTLT1 induces inflammation through SYK/MEK/ERK signalling in coronary artery disease. Clin Sci 2019;133(22):2283-99.

26. Guo CX, Li ZY, Niu JB, Fan SC, Yan SY, Lu PP, *et al.* Qishen capsule safely boosts cardiac function and angiogenesis via the MEK/ERK pathway in a rat myocardial infarction model. J Geriatr Cardiol 2019;16(10):764-74.
27. Fan YZ, Huang H, Wang S, Tan GJ, Zhang QZ. Effect of lncRNA MALAT1 on rats with myocardial infarction through regulating ERK/MAPK signaling pathway. Eur Rev Med Pharmacol Sci 2019;23(20):1041-9.
28. Subramonian D, Phanhthilath N, Rinehardt H, Flynn S, Huo Y, Zhang J, *et al.* Regorafenib is effective against neuroblastoma *in vitro* and *in vivo* and inhibits the RAS/MAPK, PI3K/Akt/mTOR and Fos/Jun pathways. Br J Cancer 2020;123(4):568-79.
29. Chen S, Zhang Y, Liu J. Dexmedetomidine improves LPS-induced acute lung injury in rats by activating PI3K/Akt pathway. Mod Med Clin Pract 2020;35(11):8-9.