Tropical Journal of Pharmaceutical Research November 2020; 19 (11): 2309-2315
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v19i11.9**

**Original Research Article**

# Dexmedetomidine preconditioning alleviates apoptosis in rat cardiomyocytes by suppressing programmed cell death 4 (PDCD4) after myocardial ischemia-reperfusion injury

**Mengning Wan, Hong Li*, Yupei Chen, Guangming Yan, Yue Pi, Youliang Deng**
*Department of Anesthesiology, The Second Affiliated Hospital of Army Medical University, Chongqing 400037, China*

**For correspondence:* ***Email:*** *lihong00611@163.com;* ***Tel:*** *+86-23-68774992*

*Sent for review: 10 August 2020* *Revised accepted: 26 October 2020*

## Abstract

***Purpose:*** *To determine the role of dexmedetomidine (Dex) in hypoxia/reoxygenation (H/R)-induced myocardial cell injury and the possible involvement of the programmed cell death 4 (Pdcd4) gene in Dex-mediated myocardial cell apoptosis after ischemia-reperfusion (I/R) injury.*
***Methods:*** *An in vivo I/R-injured rat model and in vitro H/R rat cell model were evaluated to ascertain the role of Dex in apoptosis. Programmed cell death 4 (PDCD4) gene expression levels were measured after Dex preconditioning. The effects of Pdcd4 knockdown or overexpression on Dex-mediated apoptosis during H/R injury were determined.*
***Results:*** *Dex pretreatment alleviated myocardial infarction in rats, suppressed myocardial cell apoptosis, and inhibited PDCD4 expression ($p < 0.05$). Treatment with Dex also alleviated H/R-induced apoptosis in rat cardiomyocytes, while PDCD4 expression decreased after Dex treatment ($p < 0.05$). Moreover, PDCD4 overexpression reversed the inhibitory effect of Dex on H/R myocardial cell apoptosis.*
***Conclusion:*** *Dex alleviates myocardial infarction in rats via its effect on PDCD4 expression. Therefore, Dex can potentially be used for the treatment but this has to clinical studies.*

***Keywords:*** *Acute myocardial infarction (AMI), Myocardial ischemia and reperfusion injury (MIRI), Dexmedetomidine (Dex), Programmed cell death 4 (PDCD4), Apoptosis*



**Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts**

## INTRODUCTION

Acute myocardial infarction (AMI) for myocardial acute, persistent ischemia hypoxia caused by myocardial necrosis [1]. Acute myocardial infarction (AMI), characterized by chest pain and characteristic changes in an electrocardiogram [2], is typically followed by myocardial ischemia and reperfusion injury (MIRI) [3] resulting from restricted blood supply [4]. MIRI, caused by excessive free radical attack, calcium overload, and damage by activated neutrophils [5], results in necrotic, dysfunctional cells [6].

Dexmedetomidine (Dex) is widely used in intensive care units and in clinical settings to reduce apoptosis and inhibit inflammation [7]. Dex can also reduce postoperative heart-related complications [8]. Dex has protective effects

against heart injury, including reduction of myocardial ischemia-reperfusion injury (IRI) and heart rhythm stabilization [10]. The mechanisms of protection may include the inhibition of the central sympathetic nervous system, a decrease in heart rate, an increase in oxygen supply, and a decrease in oxygen consumption [9].

The *Pdcd4* (programmed cell death 4) gene, discovered by Shibahara *et al* in 1995, promotes apoptosis and suppresses the development, invasion, and metastasis of cancer cells [11]. Additionally, PDCD4 can regulate the transcription of multiple genes [12]. PDCD4 plays an important role in heart development [13], and its expression levels and activity are significantly increased in myocardial cell injury caused by hypoxia reperfusion (H/R). Therefore, PDCD4 may play an important role in apoptosis of myocardial cells during injury [14].

## EXPERIMENTAL

### Animal studies

This study was conducted using the National Institutes of Health Laboratory Animal Care and Use Guidelines [15] and was approved by the Ethics Committee of The Second Affiliated Hospital of Army Medical University (approval no. AMUWEC2020213). For *in vivo* heart models, male Sprague Dawley (SD) rats (300 g) were injected intraperitoneally with pentobarbital (80 mg/kg). The animals were randomly divided into Sham, Sham + Dex, I/R, and I/R + Dex groups. For Dex administration (100 μg/kg, 30 min before I/R injury), a catheter was installed in the left jugular vein. For I/R injury, the chest was opened via left thoracotomy in the fourth or fifth intercostal space with retraction of the ribs to expose the heart. To perform transient regional ischemia in the left ventricle, a 6-0 prolene loop was placed from the emergence of the first branch of the anterior descending coronary artery (ADCA) to the ends of the suture and looped with polyethylene tubing to construct a ring for reversible occlusion. Heparin was administered at 100 U/kg (IV) after the injection of Dex and before the ischemia. The heart was then subjected to regional ischemia for 30 min before reperfusion of the ADCA region for 120 min. To confirm ischemia, cyanosis of the myocardial surface was observed. Reperfusion was confirmed by an epicardial hyperemia response and disappearance of cyanosis.

### Cell culture

Rats were sacrificed by dislocation of the spine. The hearts were excised and then incubated in cold PBS. The ventricles were sliced into 1-3 $mm^3$ sections and coated with 0.1% collagenase type II (Sigma, St. Louis, MO, USA) for digestion (37 °C, 10 min). Lysates were centrifuged (10 min, 1,500 rpm) and pellets were resuspended in DMEM-F12 (Gibco) with 15 % FBS. To detach fibroblasts from cardiomyocytes, the differential wall adhesion method was used. Cardiomyocytes were maintained in a culture hood for 2 h, the non-adhesive cells were extracted, and cell number was assessed with a hemocytometer. Cardiomyocytes, at an appropriate cell density, were seeded onto plates for experiments, and 5-bromodeoxyuridine (5-BrdU, 0.1 mM, Sigma) was added to suppress fibroblast proliferation.

For *Pdcd4* knockdown strains, cardiomyocytes were transfected with *Pdcd4* shRNA plasmids (Merck) via Lipofectamine™ 2000 (Invitrogen, MA, USA), according to the manufacturer's instructions. Plasmids pEGFP-C1 and pEGFP-C1-PDCD4 were a kind gift of Dr. Olubunmi Afonja (New York University, New York, NY, USA).

### H/R injury model

The H/R cell model was based on the addition of $Na_2S_2O_4$ (Sigma) to primary rat neonatal cardiomyocytes to induce hypoxia without harm to the membranes followed by reoxygenation through the addition of fresh medium. For the I/R model, cells were pretreated with 1.0 $\mu$M Dex for 1 h, then treated with 4.0 mM $Na_2S_2O_4$ for 1 h, and finally transferred normal culture medium for an additional 12 h of recovery.

### Measurement of myocardial infarct size

After reperfusion, isolated hearts were immediately moved to a -80°C freezer for approximately 7 min, sliced into 2-3 mm thick sections along the sagittal axis of the heart, and then stained with a 1% 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) solution (37°C, pH 7.4) for 25 min. Sections were fixed in 10% formaldehyde solution overnight. The infarct area was analyzed with ImageJ software.

### TUNEL assay and flow cytometry

Apoptosis was measured by TUNEL staining, which is based on fluorescence detection of DNA strand breaks, using a Cell Death Detection Kit (Roche) according to the manufacturer's instructions. Frozen heart slices were fixed for 1 h at 25°C, immersed in PBS containing 0.1% Triton X-100 on ice, and then stained with the reaction mixture in the kit for 1 h at 37°C in the

dark. The number of TUNEL-positive cells was determined by fluorescence microscopy. For detection by flow cytometry, cells were digested with trypsin and washed with PBS and then treated with the Annexin V/propidium iodide (PI) staining kit (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions.

### Quantitation of mRNA by RT-qPCR

RNA was extracted from cells using TRIzol reagent (Invitrogen) and RNA was reverse transcribed to make cDNA using M-MLV reverse transcriptase (Promega) according to the manufacturer's instructions. To detect transcript levels of *Pdcd4*, cDNA was used as the template for quantitative PCR (qPCR) with Takara SYBR Premix Ex TaqTM (TliRNaseH Plus, Japan). The internal control for total RNA was 18S rRNA. The primer sequences were listed in Table 1.

**Tab le 1:** Protein sequences

| Name | Primer | Sequence (5'-3') |
|---|---|---|
| PDCD4 | Forward | TGAGCACGGAGATACGAACGA |
| | Reverse | GCTAAGGACACTGCCAACACG |
| 18S | Forward | TCAAGAACGAAAGTCGGAGG |
| | Reverse | GGACATCTAAGGGCATCAC |

### Immunoblot assay

Proteins were extracted from cells using RIPA buffer (Beyotime, Beijing, China) and protein concentrations were determined using BCA assays. Proteins were separated by 8% SDS-PAGE and transferred to PVDF membranes. After blocking with 5% milk in TBS + 0.5% Tween 20 at room temperature for 2 h, membranes were incubated at room temperature for 2 h with primary antibodies against PDCD4 (1:1000, Abcam), caspase 3 (1:2000, Abcam), cleaved caspase 3 (1:1000, Abcam), or b-actin (1:3000; Cell Signaling) and then incubated with an HRP-conjugated secondary antibody. Antibodies were detected using an ECL kit (Novex™ ECL Chemiluminescent Substrate Reagent kit; Thermo Fisher Scientific) according to the manufacturer's instructions.

### Statistical analysis

Data analysis was performed using GraphPad Prism 6 software (La Jolla, CA, USA). All data are given as mean ± SD. The Student *t*-test was used for analysis of statistical significance between two groups. $P < 0.05$ was the threshold set for statistical significance.

## RESULTS

### Dex preconditioning relieved apoptosis of cardiomyocytes and inhibited PDCD4 expression following I/R injury

Dex treatment decreased the large heart infarct that was induced by I/R injury in rats (Figure 1 A). After reperfusion, TUNEL staining of cardiomyocytes revealed increased apoptosis. Dex pretreatment also suppressed apoptosis efficiently in I/R myocardial cells (Figure 1 B). Since PDCD4 was upregulated in I/R-injured cells, the level of PDCD4 in the Sham, Sham + Dex, I/R, and I/R + Dex groups was measured. I/R injury increased *Pdcd4* mRNA and protein levels, while Dex preconditioning inhibited PDCD4 expression caused by I/R injury (Figure 1 C and D).

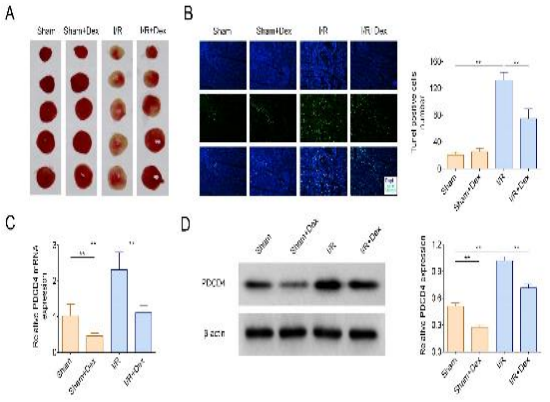


**Figure 1:** Dex preconditioning relieved apoptosis in cardiomyocytes and inhibited PDCD4 expression after I/R injury. (A) TTC staining revealed that DEX preconditioning reduced infarct size when compared to I/R groups in the *in vivo* models. (B) TUNEL staining showed reduced cell apoptosis after exposure to Dex in I/R injured rats. (C) RT-qPCR and (D) immunoblot detected PDCD4 levels in sham, sham + Dex, I/R, and I/R + Dex groups. Data are mean ± SD (n=5 per group); $**p < 0.01$ versus control group

### Dex preconditioning inhibited PDCD4 levels in a rat cardiomyocyte H/R model

Our *in vitro* H/R model utilized $Na_2S_2O_4$ treatment of cultured primary cardiomyocytes to induce hypoxia, resulting in increased apoptosis. Following Dex treatment, apoptosis was reduced, suggesting a protective effect for Dex (Figure 2 A). H/R injury induced expression of PDCD4 in cardiomyocytes, and Dex pretreatment of cells reduced PDCD4 expression in both the H/R model and the control (Figures 2 B and C). Dex pretreatment also led to an increase in cleaved caspase 3 in cells after H/R injury (Figure 2 C). Collectively, these data suggest that Dex

preconditioning substantially suppressed H/R-mediated apoptosis and increased PDCD4 expression in cardiomyocytes.

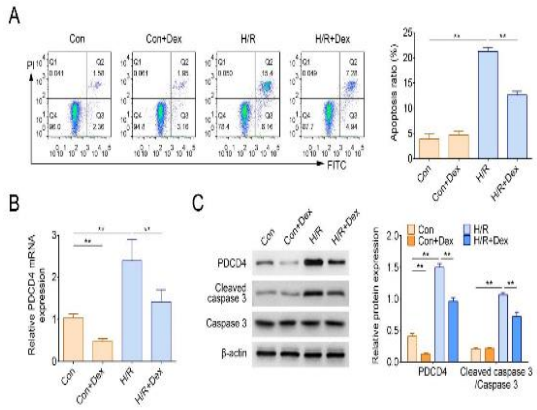


**Figure 2:** Dex preconditioning in the rat cardiomyocyte H/R model system. (A) Flow cytometric analysis of Dex-treated and untreated cells in H/R and control groups. (B) RT-qPCR quantitation of *Pdcd4* mRNA in con, con + Dex, H/R, and H/R + Dex groups. (C) Immunoblots using primary antibodies against PDCD4, caspase 3, cleaved caspase 3, and b-actin in cells for con, con + Dex, H/R, and H/R + Dex groups. Data are mean ± SD; ∗∗$p$ < 0.01 versus control group

### *Pdcd4* knockdown strains showed decreased apoptosis in rat cardiomyocytes exposed to H/R

PDCD4 is involved in the regulation of apoptosis and may be responsible for the beneficial Dex-mediated effect observed in cultured cardiomyocytes exposed to H/R. The *Pdcd4* knockdown strains, which showed decreased expression of *Pdcd4* m-RNA and protein (Figure 3 A and B), resulted in decreased apoptosis compared to both the H/R cells and the strain producing normal levels of PDCD4 (Figure 3 C). The *Pdcd4*-knockdown strain significantly reduced cleaved caspase 3 levels in cells exposed to H/R injury (Figure 3 D). These results suggest that PDCD4 is responsible for mediating H/R-induced cardiomyocyte injury.

### Overexpression of *Pdcd4* inhibited the protective effects of Dex

Overexpression of mRNA and protein for *Pdcd4* was demonstrated by RT-qPCR and immunoblot analysis, respectively (Figure 4 A and B). Dex pretreatment significantly decreased apoptosis in cells exposed to H/R (Figure 3 C); however, the protective effect decreased with PDCD4 overexpression (Figure 4C). Similarly, the Dex-mediated reduction of cleaved caspase 3 levels in H/R-injured cells was reversed by PDCD4 overexpression (Figure 4 D). These data suggest that increased PDCD4 expression negatively affected the Dex-mediated cardioprotection of cells against H/R.

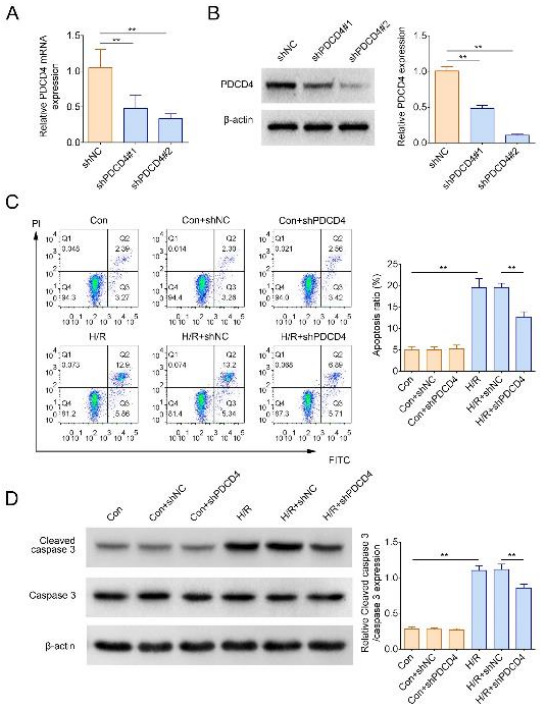


**Figure 3:** *Pdcd4* knockdown suppressed apoptosis in rat cardiomyocytes exposed to H/R. (A, B) *Pdcd4* mRNA and protein expression was detected in cells transfected with *Pdcd4* shRNA. (C) Flow cytometry showed a reduction in apoptosis in H/R-exposed cells. (D) Cleaved caspase 3 levels were inhibited in rat cardiomyocytes exposed to H/R. Data are mean ± SD. ∗∗ $p$ < 0.01 versus control group

## DISCUSSION

Dex, a selective alpha-2-adrenergic agonist, has sedative and analgesic effects [16]. Dex also provides hemodynamic stability, but can also cause bradycardia and hypotension. Additionally, it reduces the incidence of postoperative agitation, nausea, and vomiting [17]. Dex has a myocardial protective effect, induces autonomic nerve regulation to balance the oxygen supply and demand in myocardial cells, and promotes the expression of 2-adrenergic-receptor-mediated pro-myocardial viability protein kinase [18].

In our study, Dex reduced myocardial apoptosis caused by I/R in rats, suggesting that Dex can be used to treat heart-related diseases and may improve clinical symptoms. PDCD4 is upregulated in myocardial infarction samples [19] and is a regulator of cell proliferation and apoptosis [20]. In this study, PDCD4 expression was increased both in I/R-induced rats and in

H/R-stimulated primary cardiomyocytes. Dex preconditioning effectively inhibited the upregulation of PDCD4 caused by I/R injury; thus, PDCD4 might be involved in the regulation of apoptosis.

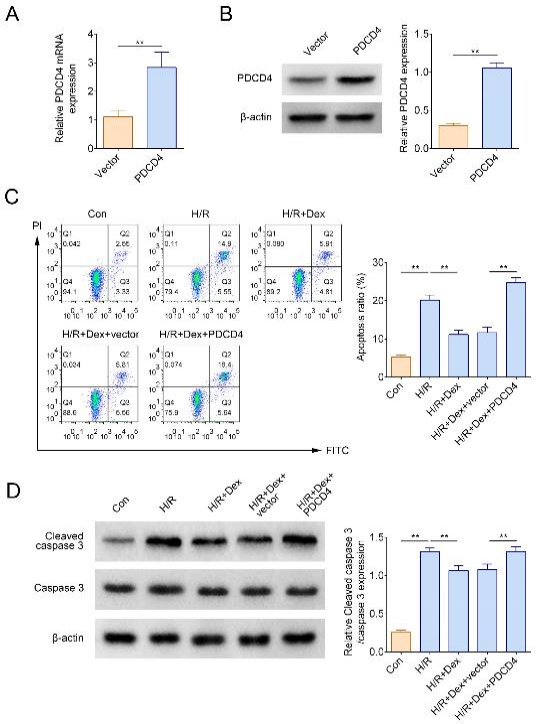


**Figure 4:** Overexpression of *Pdcd4* reversed the protective effects of Dex. (A, B) *Pdcd4* mRNA and protein expression were detected in *Pdcd4*-overexpressing cells. (C) Flow cytometry showed enhanced apoptosis in H/R and Dex-treated cells. (D) Cleaved caspase 3 protein levels were increased in rat cardiomyocytes exposed to H/R and Dex. Data are mean ± SD; $**p < 0.01$ versus control group

PDCD4 negatively regulates autophagy in both tumor and normal cells by regulating ATG5 and suppresses proliferation, migration, and invasion of endometrial cells by regulating autophagy [21]. *PDCD4* ablation significantly reduces cell sensitivity to apoptosis, and PDCD4 overexpression increases cell susceptibility to TNF-α and IFN-γ-triggered apoptosis [22]. *Pdcd4*-ablated mice are less sensitive to lipopolysaccharide-induced death [23]. In this study, high PDCD4 levels were associated with high TUNEL-positive cell numbers, a high level of apoptosis, and cleavage of caspase 3. Knockdown of *Pdcd4* expression significantly reduced apoptosis in cardiomyocytes with H/R injury, and the overexpression of *Pdcd4* reversed the inhibitory effect of Dex on H/R myocardial cell apoptosis. Collectively these data suggest that PDCD4 may regulate apoptosis in cardiomyocytes.

PDCD4 plays a critical role in a variety of physiological and pathological processes [24]. PDCD4 depletion ameliorates left-ventricular remodeling and improves insulin resistance in a type-2 diabetic cardiomyopathy rat model [25]. PDCD4 also serves as an endogenous suppressor of BDNF translation and affects stress-induced depression [26]. An effect of PDCD4 on tumorigenesis has also been reported, with high levels of expression in multiple types of tumors [22]. *PDCD4* serves as the target of multiple drugs and miRNAs [26]. In this study, Dex affected myocardial cell apoptosis through PDCD4 caused by I/R; however, understanding the molecular mechanism of its action on myocardial cells in the context of its effect on many cellular processes will require further study.

## CONCLUSION

Dex alleviated myocardial infarction, suppressed myocardial cell apoptosis, and inhibited PDCD4 expression in rats. *PDCD4* overexpression reversed the inhibitory effects of Dex on H/R myocardial cell apoptosis. This suggests that Dex acts by suppressing PDCD4 to alleviate apoptosis in cardiomyocytes after myocardial ischemia.

## DECLARATIONS

### *Conflict of interest*

No conflict of interest is associated with this work.

### *Contribution of authors*

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Mengning Wan and Hong Li designed the study, supervised data collection, and analyzed the data. Yupei Chen interpreted the data and prepared the manuscript for publication. Guangming Yan, Yue Pi, and Youliang Deng supervised data collection, analyzed the data, and reviewed drafts of the manuscript. All authors read and approved the manuscript for publication.

### *Open Access*

## REFERENCES


1. *Wang Z, Wang Z, Wang T, Yuan J, Wang X, Zhang Z. Inhibition of miR-34a-5p protected myocardial ischemia reperfusion injury-induced apoptosis and reactive oxygen species accumulation through regulation of Notch Receptor 1 signaling. Rev Cardiovasc Med 2019; 20(3): 187-197.*
2. *Alghamdi A, Alotaibi A, Alharbi M, Reynard C, Body R. Diagnostic Performance of Prehospital Point-of-Care Troponin Tests to Rule Out Acute Myocardial Infarction: A Systematic Review. Prehosp Disaster Med 2020: 1-7.*
3. *Zacherl MJ, Todica A, Wangler C, Schirrmacher R, Hajebrahimi MA, Pircher J, Li X, Lindner S, Brendel M, Bartenstein P et al. Molecular imaging of cardiac CXCR4 expression in a mouse model of acute myocardial infarction using a novel (68)Ga-mCXCL12 PET tracer. J Nucl Cardiol 2020.*
4. *Hao MH, Zhang F, Liu XX, Zhang F, Wang LJ, Xu SJ, Zhang JH, Ji HL, Xu P. Qualitative and quantitative analysis of catechin and quercetin in flavonoids extracted from Rosa roxburghii Tratt. Trop J Pharm Res 2018; 17(1): 71-76.*
5. *Pamukcu HE, Acikel S. Prognostic Value of Elevated Pulmonary Artery Systolic Pressure on Short Term in Patients With Acute Myocardial Infarction. Angiology 2020: 3319720941723.*
6. *Laichuthai N, Abdul-Ghani M, Kosiborod M, Parksook WW, Kerr SJ, DeFronzo RA. Newly Discovered Abnormal Glucose Tolerance in Patients With Acute Myocardial Infarction and Cardiovascular Outcomes: A Meta-analysis. Diabetes Care 2020; 43(8): 1958-1966.*
7. *Abdellatif AA, Kasem AA, Bestarous JN, Toaima TN, Ali MM, Shokri H. Efficacy of dexmedetomidine as an adjuvant to Quadratus lumborum block for paediatrics undergoing laparoscopic pyeloplasty. A prospective randomized double blinded study. Minerva Anestesiol 2020.*
8. *Wang L, Wang S, Xing Z, Li F, Teng J, Jia T. Application of Dexmedetomidine in Cardiopulmonary Bypass Prefilling. Dose Response 2020; 18(3): 1559325820939764.*
9. *Naduvanahalli Vivekanandaswamy A, Prasad Shetty A, Mugesh Kanna R, Shanmuganathan R. An analysis of the safety and efficacy of dexmedetomidine in posterior spinal fusion surgery for adolescent idiopathic scoliosis: a prospective randomized study. Eur Spine J 2020.*
10. *Hao J, Wu Z, Luo Z, Dong B. Addition of dexmedetomidine to ropivacaine for local infiltration anaesthesia improves analgesic efficacy after tonsillectomy and adenoidectomy: A randomized controlled trial. Int J Pediatr Otorhinolaryngol 2020; 137: 110168.*
11. *Wang S, Li G. LncRNA XIST inhibits ovarian cancer cell growth and metastasis via regulating miR-150-5p/PDCD4 signaling pathway. Naunyn Schmiedebergs Arch Pharmacol 2020.*
12. *Jiang Z, Li L, Hou Z, Liu W, Wang H, Zhou T, Li Y, Chen S. LncRNA HAND2-AS1 inhibits 5-fluorouracil resistance by modulating miR-20a/PDCD4 axis in colorectal cancer. Cell Signal 2020; 66: 109483.*
13. *Fan B, Jin Y, Zhang H, Zhao R, Sun M, Sun M, Yuan X, Wang W, Wang X, Chen Z et al. MicroRNA21 contributes to renal cell carcinoma cell invasiveness and angiogenesis via the PDCD4/cJun (AP1) signalling pathway. Int J Oncol 2020; 56(1): 178-192.*
14. *Zhang K, Pan X, Zheng J, Liu Y, Sun L. SIRT1 protects against aortic dissection by regulating AP-1/decorin signaling-mediated PDCD4 activation. Mol Biol Rep 2020; 47(3): 2149-2159.*
15. *Guide for the care and use of laboratory animals. 1996.*
16. *Coluzzi F, Angelini A, Simmaco M, Alampi D, Alessandri E, Grassi E, Monina MG, Rocco M. The effect of dexmedetomidine on status epilepticus in a patient with anti-NMDA receptor encephalitis. Can J Anaesth 2020.*
17. *Aso S, Matsui H, Fushimi K, Yasunaga H. Dexmedetomidine and Mortality From Sepsis Requiring Mechanical Ventilation: A Japanese Nationwide Retrospective Cohort Study. J Intensive Care Med 2020: 885066620942154.*
18. *Karna SR, Chambers P, Johnson CB, Singh P, Stewart LA, Lopez-Villalobos N, Kongara K. Effect of combinations of morphine, dexmedetomidine and maropitant on the electroencephalogram in response to acute electrical stimulation in anaesthetized dogs. J Vet Pharmacol Ther 2020.*
19. *Xu H, Cao H, Zhu G, Liu S, Li H. Overexpression of microRNA-145 protects against rat myocardial infarction through targeting PDCD4. Am J Transl Res 2017; 9(11): 5003-5011.*
20. *Zeng T, Zhang Q, Yu X, Gao X, Qiu Y. Inhibition of cell migration and invasion and promotion of cell apoptosis by overexpression of programmed cell death 4 (PDCD4) in cervical cancer Siha cells. Int J Clin Exp Pathol 2018; 11(9): 4676-4683.*
21. *Zhang Y, Zhu Z, Huang S, Zhao Q, Huang C, Tang Y, Sun C, Zhang Z, Wang L, Chen H et al. lncRNA XIST regulates proliferation and migration of hepatocellular carcinoma cells by acting as miR-497-5p molecular sponge and targeting PDCD4. Cancer Cell Int 2019; 19(198.*
22. *Chen C, Zheng Q, Kang W, Yu C. Long non-coding RNA LINC00472 suppresses hepatocellular carcinoma cell proliferation, migration and invasion through miR-93-5p/PDCD4 pathway. Clin Res Hepatol Gastroenterol 2019; 43(4): 436-445.*
23. *Wang X, Li Y, Wan L, Liu Y, Sun Y, Liu Y, Shi Y, Zhang L, Zhou H, Wang J et al. Downregulation of PDCD4 induced by progesterone is mediated by the PI3K/AKT*

*signaling pathway in human endometrial cancer cells. Oncol Rep 2019; 42(2): 849-856.*

24. *Sun R, Zhang L. Long non-coding RNA MALAT1 regulates cardiomyocytes apoptosis after hypoxia/reperfusion injury via modulating miR-200a-3p/PDCD4 axis. Biomed Pharmacother 2019; 111: 1036-1045.*
25. *Gu H, Liu Z, Li Y, Xie Y, Yao J, Zhu Y, Xu J, Dai Q, Zhong C, Zhu H et al. Serum-Derived Extracellular Vesicles Protect Against Acute Myocardial Infarction by Regulating miR-21/PDCD4 Signaling Pathway. Front Physiol 2018; 9: 348.*
26. *Ma J, Zhang J, Wang Y, Long K, Wang X, Jin L, Tang Q, Zhu L, Tang G, Li X et al. MiR-532-5p alleviates hypoxia-induced cardiomyocyte apoptosis by targeting PDCD4. Gene 2018; 675: 36-43.*