The Influence of L-arginine on Cell Proliferation, iNOS Expression and Cell Cycle in the Human Colon Carcinoma Cell Line LS174

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Abstract: The relationship between the L-Arg-NO pathway and tumor growth has not yet been clearly elucidated. The aim of this study was to explore the effects of L-arginine (L-Arg) on cell proliferation, inducible nitric oxide synthase (iNOS) expression and the cell cycle in the human colon carcinoma cell line LS174. LS174 cells were cultured with L-Arg at different concentrations for different times. The MTT method was employed to evaluate the level of cell proliferation. The production of nitric oxide (NO) in the culture supernatants was detected by the enzymatic reduction of nitrate. The distribution of the cell cycle was detected using flow cytometry (FCM). The expression levels of iNOS were determined by western blot and immunohistochemical staining. The growth of LS174 cells was promoted by L-Arg at low concentrations (0.125 mmol/L) and inhibited at high concentrations (0.5, 2, 8, or 32 mmol/L). The levels of NO increased with increasing concentrations of L-Arg. Compared with the control group, the ratio of cells in S phase was increased after 48 hour' treatments with high concentrations of L-Arg (0.5, 2, 8 or 32 mmol/L) ($P<0.05$, $P<0.01$); but there was no obvious difference after treatments with a low concentration (0.125 mmol/L) ($P>0.05$). With the increase in L-Arg concentrations, the expression of iNOS increased. L-Arg can induce the expression of iNOS resulting in an increase the production of NO. Low concentrations of L-Arg can promote growth, whereas high concentrations can inhibit the growth and proliferation of LS174 cells. High concentrations of L-Arg can induce S phase arrest.
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Key words: L-arginine, nitric oxide, inducible nitric oxide synthase, cell cycle, human colon carcinoma cell Line LS174, cell proliferation.

1. Introduction

L-Arginine (L-Arg), as a conditionally essential amino acid, can generate nitric oxide (NO), polyamine and L-proline through metabolic processes and is involved in the regulation of cell growth (Ying *et al.*, 2013) (García-Navas *et al.*, 2012). L-Arg acts as the donor for NO, and with increasing L-Arg concentrations, the rate limiting enzyme - inducible nitric oxide synthase (iNOS) is activated to synthesize large amounts from L-Arg. This pathway is called the L-Arg-NO pathway (Becker *et al.*, 2009)(Shan *et al.*, 2013). In recent years, extensive researches have been conducted on L-Arg-NO pathway and NO in the field of biology and clinical medicine. Extrinsic L-Arg can promote the increase in NO concentrations in the body through the L-Arg-NO pathway to protect myocardial cells (Yang *et al.*, 2013) and lung tissues (Antosova,& Strapkova., 2013), to protect against liver damage (Carnovale, & Ronco., 2012), to prevent acute nephrotoxicity (Mahran *et al.*, 2011), among other effects.

The available experimental evidences highlight contrasting pro- and anti-tumor effects of iNOS expression, which appear to be reconciled by consideration of the concentrations of NO involved, the temporo-spatial mode of NO action, intracellular targets, cellular redox state and the timing of an apoptotic stimulus. Several clinical and experimental studies indicate that the presence of NO in tumor microenvironment is detrimental to tumor cell survival and metastasis (Singh,& Gupta., 2011)(Tate *et al.*, 2012) (Frederiksen *et al.*, 2007) (Raber *et al.*, 2012) (Bonavida, & Baritaki., 2011)(Baritaki *et al.*, 2010). In contrast, numerous reports suggest that NO can have tumor-promoting effects (Hiraku *et al.*, 2010)(Yang *et al.*, 2013) (Islam *et al.*, 2012)(Kafousi *et al.*, 2012) (Safarinejad *et al.*, 2013)(Korde Choudhari *et al.*, 2012). However, the relationship between the

L-Arg-NO pathway and tumor growth has not yet been clearly elucidated. This study aimed to explore the effects of L-Arg on cell proliferation, iNOS expression and cell cycle in human colon carcinoma cell line LS174.

2.Material and Methods

Materials

The human colon carcinoma cell line LS174 was provided by the Molecular Biology Experiment Center of Xi'an Jiaotong University. L-Arg, MTT, DMSO, trypsin and Annexin V/PI kits were all purchased from U.S. Sigma, Inc. NO kits were purchased from Nanjing Jiancheng Bioengineering. Mouse anti-human monoclonal antibody against iNOS was purchased from U.S. Santa Cruz Biotechnology, Inc. SP-9000 kits were purchased from U.S. Zymed Corporation.

Methods

Cell culture

The human colon carcinoma cell line LS174 was cultured in DMEM culture medium and was grown in monolayer subculture inside a 37℃ cell incubator with 5% CO_2 and saturated humidity. Logarithmic growth phase cells were obtained for experiments. Cells in the experimental groups were treated with L-Arg at different concentrations (0.125, 0.5, 2, 8, or 32 mmol/L), and the control group was maintained in equal volume of culture solution.

Observation by light microscope and electron microscope

After LS174 cells were treated with L-Arg at different concentrations for 48 h, cellular morphology was observed under an inverted optical microscope and photographed. LS174 cells were collected in centrifuge tubes, fixed at 4℃ in 2.5% glutaraldehyde, washed 3 times with PBS (pH 7.2), fixed again at 4℃ with 1% osmic acid, and washed again with PBS (pH 7.2). LS174 cells were subsequently dehydrated with gradient ethanol, embedded in epoxy resin and cut into 60-nm ultra-thin sections. The sections were, double-stained with uranyl acetate and lead citrate, observed under a transmission electron microscope (TEM) and photographed.

Detection of the effect of L-Arg on LS174 cell growth (MTT assay)

LS174 cells were mixed in a 1×10^5/ml cell suspension and then seeded in 96-well plates and cultured for 24 h. After the culture solution was removed, 200 μl of complete culture solution with different concentrations of L-Arg was added to every well in the trial group, and only an equal volume of culture solution was added in control group. At 24 h, 48 h and 72 h, one plate was removed from the incubator, and 20 μl MTT was added to every well, and the plate was returned to the incubator for 4 h. DMSO was added to dissolve the MTT reduction products completely. The OD value of every well was measured at 490 nm with an ELISA instrument as a measure of cell viability.

Detection of NO content in the cell culture solution by nitrate reductase

Supernatants collected from the cell culture solutions from the above MTT experiment were centrifuged at 2500 rpm for 5 min. Other steps were performed according to the operational process in the specification of kits. Colorimetric analysis was performed using a 722-type spectrophotometer, and absorbance values were read at 550 nm to calculate the NO content (μmol/L) in the samples. The formula used to determine NO content is as follows: (determination tube absorbance − blank tube absorbance) / (standard pipe absorbance − blank tube absorbance) × 100.

Detection of cell cycle by flow cytometry (FCM)

Cells were routinely collected after being treated with L-Arg at different concentrations for 48 h and trypsinized, centrifuged at 300g 5min and fixed in 75%, 4℃ pre-cooled ethanol overnight. The following day, the cells were centrifuged at 300g for 5min, and the supernatant was removed. RNase was added, and staining was performed with PI. The flow cytometer was employed for detection, and the data were analyzed by computer and printed.

Detection of iNOS proteins with western blot

Cells were routinely collected after being treated with L-Arg at different concentrations for 48 h and lysed. Proteins were extracted from the sample, separated by SDS-PAGE and electrotransferred to PVDF membranes. Dried skim milk (5%) was used for blocking, and primary antibodies (β-actin, iNOS antibodies) were added. After the membrane was rinsed, the secondary antibody was added, and DAB color development was performed. Images were stored in a computer, and western blot results were analyzed with Quantitative One software to calculate the integral density of the bands. The β-actin band was used to normalize the expression of iNOS.

Immunohistochemical staining of iNOS proteins

Cells were routinely collected after being treated with L-Arg at different concentrations for 48 h and seeded onto cover slips. The SP method was employed for immunohistochemical staining of iNOS, according to the manufacturer's instructions. Positive granules were brown. iNOS was distributed in the cell membrane and cytoplasm, and the cell nucleus was unstained. High-power fields (HPFs) were randomly selected as observation zones. The obtained image information was analyzed by a Leica computer image analysis system, and gray level was employed to express the intensity of positively stained cells in observation zone; the higher the protein content, the darker the staining, the darker the computer image and the smaller the gray-level series, and vice versa. The average gray level of each group was obtained

according to different group conditions to indirectly reflect the expression of iNOS proteins**.**

Statistical analysis

Data were expressed as the mean ± standard deviation ($\bar{\chi}$±s), SPSS 13.0 for Windows was used for single-factor ANOVA with α=0.05.

3. Results and Discussion

The effect of different concentrations of L-Arg on LS174 cell morphology Changes in cell size and shape

LS174 cells were colony-centered, and the center cells were round and grew to the periphery. Peripheral cells were polygonal or spindle-shaped. The nuclei were large, round and oval-shaped, the cytoplasm was translucent and intercellular connections were tight. When treated with low doses (0.125 mmol/L) of L-Arg, the cell density increased, intercellular connections tightened, and cell extensions persisted. However, when the cells were treated with high concentrations of L-Arg (0.5, 2, 8, or 32 mmol/L), the cytoplasm became less translucent, intercellular gaps increased, and cellular extensions were gradually lost. These changes became more evident with increasing concentrations (Fig-1).

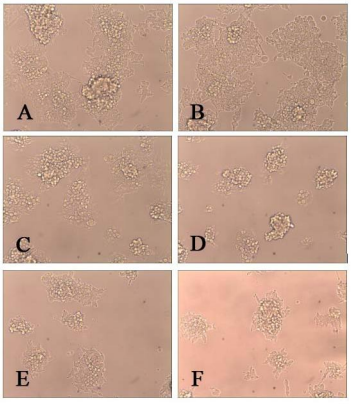

Fig-1 The size and shape of LS174 cells after treated with different concentration of L-Arg (Original magnification: ×100). LS174 cells were treated with L-Arg: untreated as control (A), 0.125 mM group (B), 0. 5 mM group (C), 2 mM group (D), 8 mM group (E) and 32 mM group (F) for 48 h.

Ultrastructural changes

Normal LS174 cells are large and round with an intact envelope, and microvilli and pseudopodium-like protrusions on the surface. The nuclear volume is large, with a high proportion of karyoplasm, rich nuclear chromatin and a large nucleolus. After LS174 cells were treated with low concentrations L-Arg (0.125 mmol/L) for 48 h, the cells exhibited no significant difference from the control group with respect to ultrastructural morphology. However, when LS174 cells were treated with L-Arg at high concentrations (0.5, 2, 8, or 32 mmol/L), the cells became smaller, the microvilli on cell surface decreased in number or disappeared, the intracellular vacuole increased, the cytoplasmic electron density decreased, and the nuclear chromatin of some cells aggregated peripherally, condensed and localized close to the nuclear membrane. With increasing concentrations, apoptosis was more evident, and apoptotic bodies were observed (Fig-2).

The effect of L-Arg at different concentrations on LS174 cell growth

MTT analysis revealed that the absorbance values 24 h, 48 h and 72 h after treatment with 0.125 mmol/L L-Arg increased significantly compared with the control group (P<0.05); However, when the cells were treated with 0.5 mmol/L and 2 mmol/L L-Arg, the detected absorbance values decreased with respect to the control group, and the difference between the groups was more significant at 72 h(P<0.05, P<0.01) than at 24 h or 48 h. At L-Arg concentrations of 8 mmol/L and 32 mmol/L, the detected absorbance values decreased greatly and were significantly different from those of the control group (P<0.05, P<0.01) (Table 1).

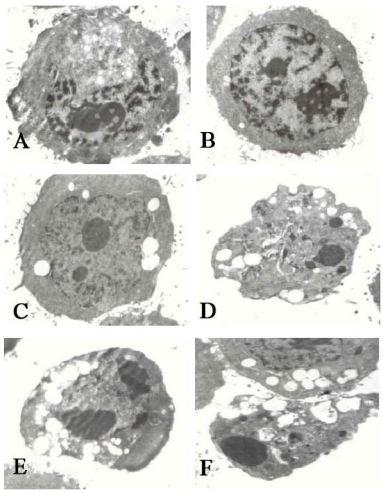

Fig-2 Ultrastructual changes of LS174 cells after treated with different concentration of L-Arg (Original magnification: ×5000). LS174 cells were treated with L-Arg: untreated as control (A), 0.125 mM group (B), 0. 5 mM group (C), 2 mM group (D), 8 mM group (E)and 32 mM group (F) for 48 h.

The effect of different concentrations of L-Arg on NO secretion in LS174 cells

The detected concentration of NO in cell culture supernatant of LS174 cells tended to increase with increasing L-Arg concentrations, but there was no statistically significant difference (P>0.05) in the NO concentration compared with the control group at low concentrations of L-Arg (0.125 mmol/L). However, the NO concentrations after treatment with high

concentrations of L-Arg (0.5, 2, 8, or 32 mmol/L) were significantly higher than that of the control group and the group treated with low-dose L-Arg (0.125 mmol/L) (P<0.05, P<0.01) (Table 2).

The effect of different concentrations of L-Arg on LS174 cell cycle

Flow cytometry analysis revealed that low concentrations (0.125 mmol/L) of L-Arg had little effect on LS174 cell cycle analysis. However, when the cells were treated with high concentrations of L-Arg (0.5, 2, 8, or 32 mmol/L), the proportion of G_0/G_1 phase cells declined while the proportion of S-phase cells increased (P<0.05, P<0.01) (Table 3, Fig-3).

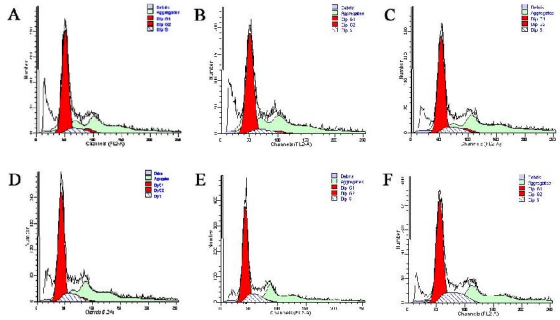

Fig-3 Effect of different concentration of L-Arg on cell cycle in LS174 cells. LS174 cells were treated with L-Arg: untreated as control (A), 0.125 mM group (B), 0. 5 mM group (C), 2 mM group (D), 8 mM group (E) and 32 mM group (F) for 48 h.

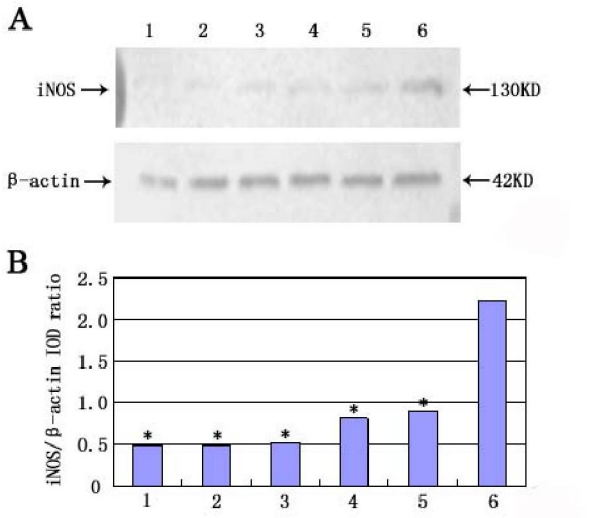

Fig-4 Expression of iNOS after treated with different concentrations of L-Arg for 48 h detected by Western blot. Fig.4A: untreated as control (1), 0.125 mM group (2), 0. 5 mM group (3), 2 mM group (4), 8 mM group (5)and 32 mM group (6) for 48 h. Fig.4B: statistics graph for Western blot. Compared with L-Arg group of 32mM, *P<0.05.

Detection of iNOS proteins by western blot

The gray-scale analysis by Quantitive One software revealed that with increasing concentrations of L-Arg, the expression of iNOS increased with respect to the control group. Higher the concentration of L-Arg resulted in correspondingly larger effects (Fig-4).

Detection of iNOS proteins by immunohistochemical staining

Forty-eight hours after LS174 cells were treated with various concentrations of L-Arg (0, 0.125, 0.5, 2, 8, or 32 mmol/L), the iNOS protein expressions levels were 181.00 ± 5.20, 180.80 ± 3.77, 180.47 ± 5.06, 172.93 ± 5.04, 171.39 ± 3.94 and 162.64 ± 2.47, respectively, and there were significant differences between the control group and the cells treated with high concentrations of L-Arg (2, 8, or 32 mmol/L) (P<0.05).

4.Discussion

Nitric oxide synthase (NOS) generates NO from L- Arg, and the body regulates NO primarily through the regulation of different NOS family members (Dzugkoev *et al*.,2013) (Rochette *et al*.,2013) (Movsesyan *et al*.,2012). NOS exists three isozymes: neuron NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). The first two are collectively known as constitutive NOS (cNOS), which is Ca^{2+}-dependent, is mainly found in nerve and endothelial cells. cNOS can produce a small amount of short-acting NO as a neurotransmitter, regulating regional blood flow and mediating a series of physiological functions (Hebeda *et al*.,2011). iNOS is a Ca^{2+}-independent NOS and is found in almost all tissues and cells, although it is most abundant in macrophages and vascular smooth muscle cells. Under normal physiological conditions, cells will not express iNOS; iNOS expression activated under pathological conditions (inflammation, tumor and so on) to catalyze the synthesis of a large amount of NO that will participate in the immune process, tumor development, tumor progression, etc (Raposo *et al*.,2013)(Belgorosky *et al*.,2013) (Singh,& Gupta.,2011). The results of this study demonstrated that increasing L-Arg concentrations result in increased NO secretion by LS174 cells, suggesting that L-Arg concentrations and NO generation are closely related. Furthermore, as L-Arg concentrations increase in the culture solution, the expression of iNOS gradually increases, which suggests that L-Arg could increase the secretion of NO by increasing the expression of iNOS; that is, L-Arg could improve the generation of NO by inducing the expression of iNOS.

Tumor development is a multi-stage process involving many factors. Studies so far have demonstrated that NO plays a role in the incidence,

development and metastasis of tumors, but NO functions vary widely. Some believed that NO inhibits tumor growth (Singh, & Gupta., 2011)(Tate *et al*., 2012) (Frederiksen *et al*., 2007)(Raber *et al*., 2012) (Bonavida, & Baritaki., 2011)(Baritaki *et al*., 2010), whereas others hold that NO promotes tumor growth (Hiraku *et al*., 2010)(Yang *et al*., 2013)(Islam *et al*., 2012) (Kafousi *et al*., 2012)(Safarinejad *et al*., 2013) (Korde Choudhari *et al*., 2012). This contradiction with respect to the NO effect on tumor growth has become a hot spot in NO study. According to the results of this experiment, when 0.125 mmol/L L-Arg was added to the cell culture, LS174 cells grew at a high density and exhibited tight intracellular connections. Additionally, the color of the culture solution changed quickly, which suggested that the cells exhibited vigorous growth and metabolism and that L-Arg at low concentrations could promote the growth of LS174 cells. However, when high concentrations of L-Arg (0.5, 2, 8, or 32 mmol/L) were added, the cytoplasm became less translucent, the intercellular gap increased, and cell extensions were gradually lost. These changes became more evident with increasing concentrations, suggesting that L-Arg at high concentrations could inhibit the growth of LS174 cells. Meanwhile, according to the MTT analysis, compared with the control group, absorbance significantly increased when the cells were treated with L-Arg was at low concentrations (0.125 mmol/L) but decreased significantly when the cells were treated with L-Arg was at high concentrations (0.5, 2, 8, or 32 mmol/L), which further confirmed that L-Arg at low concentrations could promote the growth of LS174 cells and that L-Arg at high concentrations could inhibit the growth of LS174 cells.

Cancer is a type of cell cycle disease and a disease of the gradual destruction of cell cycle regulation; cell cycle dysregulation is the 'final common path' to the pathogenesis of all tumors. The complete cell cycle includes the DNA pre-synthesis phase (G_1 phase), the DNA synthesis phase (S-phase), the DNA post-synthesis phase (G_2 phase) and the mitotic phase (M phase). Cells in the dormant phase (G_0 phase) have withdrawn from the cell cycle and can re-enter the cell cycle upon stimulation by certain stimuli; these cells can also be a source of tumor recurrence. Four specific cell cycle check-points regulate cell cycle progression: G_1-S phase check-point, S-phase check-point, G_2 phase check-point and M phase check-point (Evan, & Vousden., 2001) (Massague., 2004)(Alabsi *et al.*, 2012). Our results suggest that high concentrations by which of L-Arg can inhibit LS174 cell growth. To explore the mechanism by which high concentrations of L-Arg inhibit LS174 cell growth, flow cytometry was performed to detect the effect of high concentrations L-Arg (0.5, 2, 8, or 32 mmol/L) on the cell cycle of LS174 cells. The results demonstrated that with increasing L-Arg concentrations increase the S phase cell population while decreasing G_0-G_1 phase cell population. The increasing proportion of S-phase cells reflects the high proportion of proliferative cells and overall vigorous cell growth. High concentration of L-Arg inhibited tumor cell growth while also increasing the population of S-phase, demonstrating that the cells were arrested in S-phase. The S-phase inhibition of the LS174 cells induced apoptosis because high concentrations of L-Arg inhibited DNA synthesis and arrested cells in S-phase. Sarkar et al (1997) have reported that as a NO donor, S-nitroso-N- acetyl-DL penicillin-amine (SNAP) can inhibit the growth of vascular smooth muscle cells. The author indicated that SNAP could inhibit vascular smooth muscle cells at concentrations of 0.03-0.1 mmol/L, and cell counts revealed that 0.1 mmol/L SNAP increased the S-phase population by 50%. This report was consistent with our experimental results.

L-Arg can induce the expression of iNOS, resulting in an increased production of nitric oxide (NO). Low concentrations of L-Arg can promote the growth of LS174 cells, whereas high concentrations can inhibit cell growth and proliferation. High concentrations of L-Arg induced S-phase arrest.

Table 1 Effect of different concentration of L-Arg on cell growth in LS174 cells

L-Arg Concentration	24h	48h	72h
Control	0.643±0.019	1.235±0.021	2.219±0.026
0.125mM	0.702±0.022*	1.323±0.028*	2.325±0.022*
0.5 mM	0.658±0.018	1.176±0.019	1.527±0.027*
2 mM	0.663±0.027	1.166±0.017	1.404±0.028**
8 mM	0.515±0.022*	0.924±0.021*	1.361±0.019**
32 mM	0.433±0.023*	0.636±0.024*	0.952±0.025**

Compared with control group, * $P<0.05$； ** $P<0.01$

Table 2 Effect of different concentration of L-Arg on excretion of NO in LS174 cells（μmol/L）

L-Arg Concentration	24h	48h	72h
Control	10.14±0.98	10.27±0.97	11.00±0.96
0.125mM	11.07±1.07	11.58±1.11	12.56±1.15
0.5mM	14.58±1.10*	15.26±1.21*	15.98±1.23*
2 mM	15.46±1.24*	16.32±1.25*	17.03±1.24*
8 mM	24.32±1.68**	25.03±1.71**	26.12±1.82**
32 mM	26.08±1.83**	27.12±1.89**	28.92±1.94**

Compared with control group, *$P<0.05$ **$P<0.01$

Table 3 Effect of different concentration of L-Arg on cell cycle in LS174 cells for 48 hours（%）

Group	G_0/G_1	S	G_2/M
Control group	81.56±2.35	11.30±1.15	7.14±1.05
0.125mM	83.92±2.21	10.35±0.96	5.73±1.85
0.5mM	78.55±2.09	14.27±1.39*	7.18±1.29
2 mM	77.45±3.03	16.51±1.36*	6.04±1.45
8 mM	76.50±1.78*	19.43±1.51*	4.07±1.33*
32mM	71.32±3.05*	25.95±1.82**	2.73±1.56*

Compared with control group, * $P<0.05$； ** $P<0.01$

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References

1. Alabsi AM, Ali R, Ali AM, Al-Dubai SA, Harun H, Abu Kasim NH, et al. Apoptosis induction, cell cycle arrest and in vitro anticancer activity of gonothalamin in a cancer cell lines. Asian Pac J Cancer Prev 2012,13(10):5131-5136.
2. Antosova M, Strapkova A. L-arginine supplementation and experimental airway hyperreactivity. Adv Exp Med Biol 2013, 756:81-89.
3. Baritaki S, Huerta-Yepez S, Sahakyan A, Karagiannides I, Bakirtzi K, Jazirehi A, et al. Mechanisms of nitric oxide-mediated inhibition of EMT in cancer: inhibition of the metastasis-inducer Snail and induction of the metastasis-suppressor RKIP. Cell Cycle 2010, 9(24):4931-4940.
4. Becker T, Mevius I, De Vries DK, Schaapherder AF, Zu Vilsendorf AM, Klempnauer J. The L-arginine/NO pathway in end-stage liver disease and during orthotopic liver and kidney transplantation: biological and analytical ramifications. Nitric Oxide 2009, 20(1):61-67.
5. Belgorosky D, Langle Y, Cormick BP, Colombo L, Sandes E, Eiján AM. Inhibition of nitric oxide is a good therapeutic target for bladder tumors that express iNOS. Nitric Oxide 2013, 36C:11-18.
6. Bonavida B, Baritaki S. Dual role of NO donors in the reversal of tumor cell resistance and EMT: Downregulation of the NF-κB/Snail/YY1/RKIP circuitry. Nitric Oxide 2011, 24(1):1-7.
7. Carnovale CE, Ronco MT. Role of nitric oxide in liver regeneration. Ann Hepatol 2012, 11(5):636-647.
8. Dzugkoev SG, Metel'skaya VA, Dzugkoeva FS. Effects of Endogenous Regulators of Endothelial NO Synthase on Nitric Oxide Homeostasis and Blood Serum Lipoproteins during Experimental Diabetes Mellitus. Bull Exp Biol Med 2013, 156(2):205-208.
9. Evan GI, Vousden KH. Proliferation, Cell cycle and apoptosis in cancer. Nature 2001, 411 (6835): 342-348.
10. Frederiksen LJ, Sullivan R, Maxwell LR, Macdonald-Goodfellow SK, Adams MA, Bennett BM, et al. Chemosensitization of cancer in vitro and in vivo by nitric oxide signaling. Clin Cancer Res 2007, 13(7):2199-2206.
11. García-Navas R, Munder M, Mollinedo F. Depletion of L-arginine induces autophagy as a cytoprotective response to endoplasmic reticulum stress in human T lymphocytes. Autophagy 2012, 8(11):1557-1576.
12. Hebeda CB, Teixeira SA, Tamura EK, Muscará MN, de Mello SB, Markus RP, et al. Nitric oxide modulates lipopolysaccharide-induced endothelial platelet endothelial cell adhesion molecule expression via interleukin-10. Clin Exp Immunol 2011, 165(2):172-179.
13. Hiraku Y, Kawanishi S, Ichinose T, Murata M. The role of iNOS-mediated DNA damage in infection- and asbestos-induced carcinogenesis. Ann N Y Acad Sci 2010,1203:15-22.
14. Islam MS, Matsumoto M, Hidaka R, Miyoshi N, Yasuda N. Expression of NOS and VEGF in feline mammary tumours and their correlation with angiogenesis. Vet J 2012, 192(3):338-344.
15. Kafousi M, Vrekoussis T, Tsentelierou E, Pavlakis K, Navrozoglou I, Dousias V, et al. Immunohistochemical study of the angiogenetic network of VEGF, HIF1α, VEGFR-2 and endothelial nitric oxide synthase (eNOS) in human breast cancer. Pathol Oncol Res 2012, 18(1):33-41.
16. Korde Choudhari S, Sridharan G, Gadbail A, Poornima V. Nitric oxide and oral cancer: a review. Oral Oncol 2012, 48(6):475-483.
17. Mahran YF, Khalifa AE, El-Demerdash E. A comparative study of protective mechanisms of glycine and L-arginine against cisplatin-induced nephrotoxicityin rat renal cortical slices. Drug Discov Ther 2011,5(1):32-40.
18. Massague J. G1 Cell-cycle control and cancer. Nature 2004, 432(7015):298-306.
19. Movsesyan HA, Alchujyan NKh, Movsesyan NH, Guevorkian AG,Hairapetyan HL, Barsegyan KA, et al. Nitrergic response to Clostridium perfringens infection in the rat brain regions: effect of red light irradiation. Cent Nerv Syst Agents Med Chem 2012,12(2):146-152.
20. Raber P, Ochoa AC, Rodríguez PC. Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell suppression and therapeutic perspectives. Immunol Invest 2012,41(6-7):614-634.
21. Raposo C, Nunes AK, Luna RL, Araújo SM, da Cruz-Höfling MA, Peixoto CA. Sildenafil (Viagra) protective effects on neuroinflammation: the role of iNOS/NO system in an inflammatory demyelination model. Mediators Inflamm 2013,2013:321460.
22. Rochette L, Lorin J, Zeller M, Guilland JC, Lorgis L, Cottin Y,et al.Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? Pharmacol Ther 2013,140(3):239-257.

23. Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Effects of the T-786C, G894T, and Intron 4 VNTR (4a/b) polymorphisms of the endothelial nitric oxide synthase gene on the risk of prostate cancer. Urol Oncol 2013,31(7):1132-1140.
24. Sarkar R, Gordon D, Stanley JC, Webb RC. Cell cycle effects of nitric oxide on vascular smooth muscle cells . Am J physiol 1997,272(4pt2): H1810-1818.
25. Shan L, Wang B, Gao G, Cao W, Zhang Y. L-Arginine supplementation improves antioxidant defenses through L-arginine/nitric oxide pathways in exercised rats. J Appl Physiol 2013,115(8):1146-1155.
26. Singh S, Gupta AK. Nitric oxide: role in tumour biology and iNOS/NO-based anticancer therapies. Cancer Chemother Pharmacol 2011,67(6):1211-1224.
27. Tate DJ Jr, Patterson JR, Velasco-Gonzalez C, Carroll EN, Trinh J, Edwards D, et al. Interferon-gamma-induced nitric oxide inhibits the proliferation of murine renal cell carcinoma cells. J Biol Sci 2012,8(8):1109-1120.
28. Ying Lu, Weimin Wang, Junchen Wang, et al. Overexpression of Arginine Transporter CAT-1 Is Associated with Accumulation of L-Arginine and Cell Growth in Human Colorectal Cancer Tissue. PloS one 2013,8(9):e73866.
29. Yang J, Gonon AT, Sjöquist PO, Lundberg JO, Pernow J .Arginase regulates red blood cell nitric oxide synthase and export of cardioprotective nitric oxide bioactivity. Proc Natl Acad Sci USA 2013,110(37):15049-15054.
30. Yang Z, Misner B, Ji H, Poulos TL, Silverman RB, Meyskens FL, et al. Targeting nitric oxide (NO) signaling with nNOS inhibitors as a novel strategy for the therapy and prevention of human melanoma. Antioxid Redox Signal 2013,10; 19(5):433-447.

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