Nutrition 26 (2010) 818–822

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Effects of different arginine concentrations on angiogenic protein production induced by HeLa cells

Szu-Yuan Chou M.D. [a], Chun-Sen Hsu M.D. [a,*], Ming-Yi Hsu M.D. [a], So-Jung Liang M.D. [a], Chiu-Li Yeh Ph.D. [b], Sung-Ling Yeh Ph.D. [c,*]

[a] Department of Obstetrics and Gynecology, Taipei Medical University–Associated Wan Fang Hospital, Taipei, Taiwan
[b] Institute of Nutrition and Health Sciences, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan
[c] School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 6 February 2009
Accepted 9 August 2009

Keywords:
Arginine
HeLa cells
Endothelial cells
Angiogenic protein
CD51/CD61
Inducible nitric oxide

ABSTRACT

Objectives: This in vitro study investigated the effects of different arginine (Arg) concentrations on angiogenic protein expressions of HeLa cells and endothelial cells (ECs) after stimulation. In addition, an inducible nitric oxide (iNO) synthase inhibitor (1400 W) was used to investigate the possible role of iNO in angiogenesis.
Methods: Endothelial cells and HeLa cells were treated with different concentrations of Arg and 1400 W: Arg 0, 50, 100, and 1000 μmol/L; Arg 100 μmol/L + 1400 W 10 μmol/L; and Arg 1000 μmol/L + 1400 W 10 μmol/L for 24 h. Then, ECs and HeLa cells were cocultured for 2 h, and the supernatant in the transwell was collected for analysis of angiogenic protein secreted. The expression of CD51/CD61 by ECs was also analyzed.
Results: The productions of vascular endothelial growth factor, basic fibroblast growth factor, prostaglandin E_2, and matrix metalloproteinase-2 were higher with Arg 100 and 1000 μmol/L than with Arg 0 and 50 μmol/L Arg, and this was consistent with the expression of CD51/CD61 by ECs. Inhibition of iNO production resulted in lower angiogenic protein expressions comparable with groups with low Arg administration.
Conclusion: The findings of this study suggest that Arg administration at levels similar to or higher than physiologic concentrations enhance the production of angiogenic protein and iNO may partly play a role in promoting angiogenesis in the presence of HeLa cells.

Introduction

Arginine (Arg) is a non-essential amino acid for healthy adults with plasma concentrations of ~100 μmol/L [1]. Arg has been shown to possess numerous useful physiologic properties. Previous reports have shown that supplemental dietary Arg accelerates wound healing, enhances immune function, and improves survival in injured conditions [2–4]. Also, Arg is fundamental for the function of T lymphocytes [5,6]. Studies in tumor-bearing mice and patients with cancer have indicated that increased metabolism of L-Arg inhibits the T-lymphocyte response [7,8]. Several animal tumor models and many clinical trials have demonstrated that immunotherapy in subjects with advanced tumors failed to achieve a therapeutic response as a result of loss of the T-cell response [9]. Arg supplementation is important in restoring T-cell antitumor function. Arg is often used in immunonutrition regimens. A previous study has shown that immunoenhancing diets containing Arg decrease the infection rate in the postoperative period for patients with head, neck, and esophageal cancers [10]. However, a report by Park et al. [11] described an increase in tumor proliferation markers in patients with breast cancer treated with dietary Arg supplements. Previous studies also have shown that Arg deprivation arrests the growth of cells and causes the death of malignant cells [12,13].

Arginine is the substrate of nitric oxide synthase (NOS) and a precursor of nitric oxide (NO). NO can act as a signal transducer and cellular messenger in homeostasis and host defense.

This study was supported by research grant 95TMU-WFH-06 from Taipei Medical University–Associated Wan Fang Hospital, Taipei, Taiwan.
* Corresponding author. C.-S. Hsu: Tel.: 8862-2930-7930, ext. 2501; S.-L. Yeh: Tel.: +8862-2736-1661, ext. 6547; fax: +8862-2737-3112.
E-mail addresses: morritw@yahoo.com.tw (C.-S. Hsu), sangling@tmu.edu.tw (S.-L. Yeh).

0899-9007/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2009.08.004

Previous studies have shown that NO plays a critical role in antipathogenic and tumoricidal responses of the immune system [14,15]. However, NO has also been implicated as a deleterious agent in various pathophysiologic conditions including cancer [16,17]. Some studies have shown that tumors expressing NOS are more aggressive than their counterparts without NOS expression [16,17]. The Arg–NO-mediated modulatory effect on various cancers remains controversial.

Cancer was the leading cause of death in Taiwan in 2008. Cervical cancer was ranked eighth in mortality among all patients with cancer [18]. Because plasma Arg concentrations are decreased and protein–energy malnutrition is often present in patients with cancer [19], an Arg-supplemented formula may be recommended for patients with cancer. However, human gynecologic cancers are malignant tumors with high levels of NOS activity [20]. A study by Wheatley et al. [21] also found that Arg deprivation reduced DNA synthesis and protracted the cell cycle in HeLa cells. We speculated that Arg supplementation might promote the progression of cervical cancer. Therefore, we designed this in vitro study using a transwell apparatus to investigate the interactions between endothelial cells (ECs) and a cervical cancer cell line (HeLa). ECs and HeLa cells were cocultured at different Arg concentrations (0, 50, 100, and 1000 μmol/L). Several angiogenic factors including CD51/CD61 (αvβ3), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinase (MMP) produced by cancer cells and ECs were analyzed. N(-[3-(aminomethyl)benzyl]acetamidine) (1400 W) is a highly selective inhibitor for inducible NOS (iNOS) [22]. In this study, we used 1400 W to investigate the possible role of inducible NO (iNO) in angiogenic protein production.

Materials and methods

Cell culture

Human umbilical vein endothelial cells were isolated from an umbilical cord vein according to the method of Jaffe et al. [23]. The umbilical vein was cannulated, washed with phosphate buffered saline (PBS; Sigma Chemical Co., St. Louis, MO, USA) and perfused with PBS containing 0.1% collagenase for 10 min at 37 °C in 5% CO_2. ECs were collected and established as a primary culture in medium-199 (Gibco Life Technologies, Rockville, MD, USA) containing 20% fetal bovine serum (FBS; Gibco Life Technologies), 20 mM $NaHCO_3$, 25 mM HEPES, antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL), heparin sodium 10 IU/mL, and endothelial cell growth supplement 15 mg/L (Gibco Life Technologies) at 37 °C in an environment with 5% CO_2. Cells were serially passaged two to three times for the experimental assay. ECs (1×10^5 cells/well) from second subcultures were grown on fibronectin-coated inserts (3-μm pore size, 6.4 mm; Becton Dickson, Franklin Lakes, NJ, USA) until the monolayer was confluent. HeLa cells were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan), and cultured in L-15 medium supplemented with 10% FBS and a penicillin/streptomycin mixture. Adherent monolayer cultures were maintained at 37 °C in 5% CO_2. Cells were routinely trypsinized (0.05% trypsin/ethylenediaminetetra-acetic acid) and subcultured in flasks. ECs and HeLa cells were incubated in medium-199 (with 20% heat-inactivated FBS) with different concentrations of Arg and 1400 W: Arg 0 μmol/L, Arg 50 μmol/L, Arg 100 μmol/L, Arg 1000 μmol/L, Arg 100 μmol/L + 1400 W 10 μmol/L, and Arg 1000 μmol/L + 1400 W 10 μmol/L for 24 h. The viability of human umbilical vein endothelial cells and HeLa cells after incubation was greater than 95% as confirmed by trypan blue staining.

Measurements of MMP-2, MMP-9, VEGF, bFGF, and PGE_2 in the supernatant

The ECs were placed in the upper chamber and HeLa cells in the lower chamber of a transwell apparatus unit (Becton Dickson). After the EC–HeLa cell interaction had proceeded for 2 h, solutions in the upper chambers of the transwells were collected and centrifuged at $125 \times g$ for 10 min for further analysis. Concentrations of MMP-2, MMP-9, VEGF, and bFGF were measured using commercially available sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). PGE_2 concentrations were measured by competitive enzyme-linked immunosorbent assay. Acetylcholinesterase covalently coupled to PGE_2 was used as the enzymatic tracer (R&D Systems). Procedures followed the manufacturer's instructions.

Measurements of CD51/CD61 expression by ECs

The EC surface expressions of CD51/CD61 were measured after the EC–HeLa cell interaction had proceeded for 4 h in the transwell coculture system. After removing the supernatant, ECs were washed twice with PBS, and the pellets were incubated with iced medium containing 2 mM ethylenediaminetetra-acetic acid to detach adherent ECs. Then the pellets were incubated with 100 μL of medium-199 (FBS free, containing 2 mM ethylenediaminetetra-acetic acid) for a further 30 min at 4 °C with the addition of fluorescein isothiocyanate–conjugated mouse anti-human CD51 and phycoerythrin-conjugated mouse anti-human CD61 (Serotec, Oxford, UK). The suspension was collected into a tube and resuspended in 500 μL of PBS (containing 0.3 mL of 350 mM formaldehyde). The percentages of CD51/CD61 expressed on ECs were analyzed by flow cytometry (Coulter, Miami, FL, USA). Fluorescence data were collected and the results are presented as a percentage of CD51/CD61-presenting cells in 5×10^3 ECs.

Tube formation assay

The tube formation assay was performed as previously described [24]. Twenty-four–well plates were coated with 100 μL of Matrigel per well and incubated at 37 °C for 30 min to promote jelling and then seeded with ECs (1×10^4 cells). After being cultured with various concentrations of Arg and 1400 W as described earlier and stimulated with supernatant of HeLa cells for 18 h at 37 °C, the formation of capillaries by ECs was observed using a microscope (400×). Three random measurements of each of four wells at each culture condition were made. Each experiment was repeated at least three times.

Statistical analysis

Data are expressed as the mean ± standard deviation. Results are representative of three independent experiments. Differences among groups were analyzed by analysis of variance using Duncan's test. $P < 0.05$ was considered statistically significant.

Results

MMP-2, MMP-9, VEGF, bFGF, and PGE_2 concentrations in the supernatant

Concentrations of VEGF, and bFGF were higher with Arg 100 and 1000 μmol/L than with Arg 0 and 50 μmol/L. PGE_2 levels were higher with Arg 1000 μmol/L than in the other groups. MMP-2 concentrations were higher in the Arg 100 and 1000 μmol/L groups than in the other groups. There were no differences in MMP-9 concentrations among all groups. Increments in MMP-2, VEGF, bFGF, and PGE_2 levels were abolished when 1400 W was added (Table 1).

CD51/CD61 expression by ECs

The CD51/CD61 expression by ECs was higher with Arg 100 and 1000 μmol/L when stimulated with HeLa cells than with Arg 0 and 50 μmol/L. There were no differences in percentages of CD51/CD61-expressing cells among the Arg 0 and 50 μmol/L and the groups with 1400 W administration (Fig. 1).

Tube formation of ECs

Cells incubated with Arg 0 and 50 μmol/L had large numbers of unorganized cells (Fig. 2A,B), whereas the Arg 100 and 1000 μmol/L groups formed more organized tubes than did the Arg 0 and 50 μmol/L groups (Fig. 2C,D). The enhanced EC differentiation in the Arg 100 and 1000 μmol/L groups was abrogated when 1400 W was administered (Fig. 2E,F).

Table 1
Effects of various concentrations of Arg and 1400 W on VEGF, bFGF, PGE_2, MMP-2, and MMP-9 concentrations in supernatant*

	VEGF (pg/mL)	bFGF (pg/mL)	PGE_2 (pg/mL)	MMP-2 (ng/mL)	MMP-9 (ng/mL)
Arg 0 μmol/L	105.5 ± 2.1	246.2 ± 70.1	671.3 ± 22.8	306.7 ± 3.2	8.89 ± 0.3
Arg 50 μmol/L	123.7 ± 3.4	301.4 ± 60.2	699.7 ± 28.6	312.2 ± 2.6	8.94 ± 0.4
Arg 100 μmol/L	140.3 ± 7.6†	373.1 ± 31.6†	702.2 ± 32.6	321.0 ± 2.5†	9.01 ± 0.4
Arg 1000 μmol/L	166.7 ± 5.6†	419.2 ± 25.4†	732.5 ± 26.8†	331.5 ± 1.8†	9.16 ± 0.8
Arg 100 μmol/L + 1400 W	110.0 ± 4.2	206.3 ± 51.6	563.3 ± 32.6	298.5 ± 2.0	8.81 ± 0.6
Arg 1000 μmol/L + 1400 W	115.3 ± 3.2	184.2 ± 23.1	545.1 ± 30.8	293.0 ± 2.2	8.96 ± 0.8

1400 W, N(-[3-(aminomethyl)benzyl]acetamidine); Arg, arginine; bFGF, basic fibroblast growth factor; MMP, matrix metalloproteinase; PGE_2, prostaglandin E_2; VEGF, vascular endothelial growth factor

* Results are representative of triplicate measurements. Data are presented as mean ± SD.

† Significantly differs from the Arg 0- and 50-μmol/L groups and groups with 1400 W.

Discussion

Supplemental Arg has been demonstrated to improve the immunologic response. The clinical relevance of the effects has been documented in several animal experiments and human studies [2,4,25,26]. To understand whether Arg concentrations may have an effect on progression of cervical cancer cells, we treated ECs and HeLa cells with different Arg concentrations, including low (50 μmol/L), approximately physiologic (100 μmol/L), and high (1000 μmol/L) Arg levels in an in vitro study to observe the EC–HeLa cell interactions.

Angiogenesis is a prerequisite for tumor growth and metastasis. Vascular EC proliferation, migration, and capillary formation are stimulated by angiogenic growth factors [27]. VEGF is a mitogen specific for ECs. Levels of VEGF have been positively associated with angiogenesis [28]. Basic FGF acts synergistically with VEGF in stimulating capillary growth [29]. PGE_2 is a product of arachidonic acid metabolism and may contribute to physiologic neovascularization and tumor-associated angiogenesis [30]. One study has shown that VEGF and bFGF mRNA expressions are induced by PGE_2 in a rat retinal glial cell line [31]. MMPs are a family of zinc endopeptidases capable of degrading essentially cell matrix components. MMP-2 and MMP-9 degrade components of basement membranes and are believed to be crucial for the invasion by malignant tumors [32]. In this study we measured the angiogenic proteins, which were produced by ECs and HeLa cells. Angiogenic proteins are involved in remodeling during tissue morphogenesis and wound healing in physiologic and pathologic conditions. Although ECs produced angiogenic proteins, these proteins were predominantly secreted by tumor cells under the present experimental condition. We found that all of these proteins, except MMP-9, were higher with Arg 100 and 1000 μmol/L than those with Arg 0 and 50 μmol/L. These findings parallel that of the effect of Arg on EC CD51/CD61 expressions. Integrin CD51/CD61 is a glycoprotein membrane receptor. ECs exposed to growth factors or those undergoing angiogenesis in tumors express high levels of CD51/CD61 [33]. Previous studies have suggested that CD51/CD61 may serve as a useful diagnostic or prognostic indicator of tumors [34]. The results observed in this study suggest that low Arg levels result in lower angiogenic protein expressions, whereas normal or higher than physiologic Arg levels promote angiogenesis. Angiogenesis involves basement membrane degradation and EC attachment, migration, and proliferation. In this study, we used Matrigel as a basement membrane matrix on which ECs underwent capillary-like tube formation. We found that ECs treated with Arg 100 and 1000 μmol/L formed organized tubes, whereas those treated with Arg 0 and 50 μmol/L did not, indicating that normal or higher than physiologic Arg treatment enhanced EC differentiation in vitro.

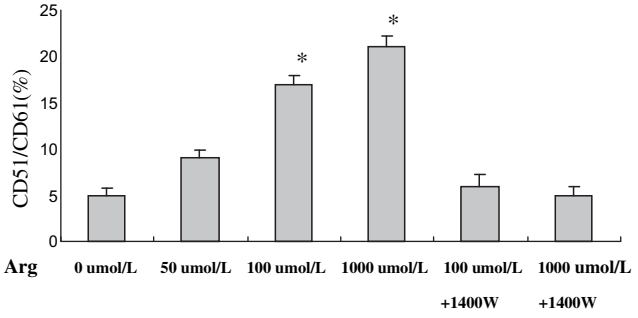

Fig. 1. Expression of CD51/CD61 by endothelial cells induced by HeLa cells. * Significantly differs from the Arg 0 and 50 μmol/L groups and the groups with 1400 W. 1400 W, N(-[3-(aminomethyl)benzyl]acetamidine); Arg, arginine.

Several in vitro studies have used exogenous NO to investigate the effect of NO on the progression of tumor cells. In this study, we administered Arg to evaluate the effect of Arg-derived NO on angiogenic protein secretion. In addition, 1400 W, an iNOS inhibitor, was used to evaluate the possible role of iNO in regulating angiogenesis and tube formation. Our results showed that, concomitant with the administration of the iNOS inhibitor, the adverse effects of increasing angiogenic protein expressions and the increased tube formation observed with physiologic and high Arg administrations were abolished. These findings provide evidence for a modulatory role of iNO in EC–HeLa cell interactions. A study by Ma et al. [35] found that L-Arg administration suppressed colorectal tumorigenesis, and this effect was thought to be related to the increased serum NO concentration. In contrast to the antitumor roles of NO, NO has been proposed to be an important mediator of tumor growth [16,17,36]. Discrepancies among studies may have resulted from the environmental exposure of tissues to the concentrations and duration of NO itself, the types of cells, or the potentiation of other genotoxic agents [37]. Cervical cancers are tumors that express NOS [21]. Previous studies have shown that the activities of some angiogenic factors are upregulated by NO [38]. In addition, NO increases the production of PGE_2, which may in turn increase the leakiness of a tumor's vasculature. Enhanced permeability may promote angiogenesis, thus facilitating further tumor growth [39]. Our data support the description that NO may play a critical role in the progression of HeLa cells.

In summary, this in vitro study showed that a low Arg concentration resulted in lower VEGF, bFGF, MMP-2, and PGE_2 secretions and reduced the CD51/CD61-expressing EC percentage when stimulated with HeLa cells. Arg administration at levels similar to or higher than physiologic concentrations increased angiogenic protein production. Also, ECs undergoing capillary-like tube formation were more pronounced. Inactivation of iNO

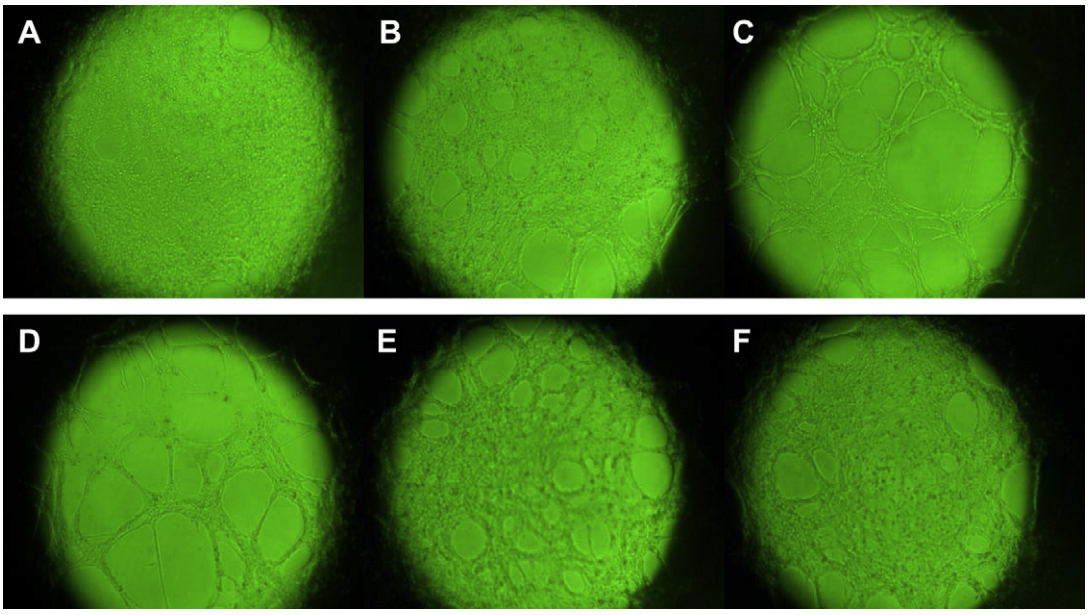

Fig. 2. Effect of various concentrations of arginine and N(-[3-(aminomethyl)benzyl]acetamidine) (an inducible nitric oxide synthase inhibitor) on the formation of capillaries by human umbilical vein endothelial cells. (A) Arginine 0 μmol/L; (B) arginine 50 μmol/L; (C) arginine 100 μmol/L; (D) arginine 1000 μmol/L; (E) arginine 100 μmol/L + N(-[3-(aminomethyl)benzyl]acetamidine) 10 μmol/L; (F) arginine 1000 μmol/L + N(-[3-(aminomethyl)benzyl]acetamidine) 10 μmol/L.

resulted in lower angiogenic protein expressions, a finding that indicates that iNO at least partly plays a role in promoting angiogenesis in the presence of HeLa cells. These results imply that Arg supplementation in cervical cancer should be carefully evaluated.

References

[1] de Jonge WJ, Marescau B, D'Hooge R, De Deyn PP, Hallemeesch MM, Deutz NE, et al. Overexpression of arginase alters circulating and tissue amino acids and guanidino compounds and affects neuromotor behavior in mice. J Nutr 2001;131:2732–40.
[2] Barbul A, Lazarou SA, Efron DT, Wasserkrug HL, Efron G. Arginine enhances wound healing and lymphocyte immune responses in humans. Surgery 1990;108:331–6.
[3] Yeh CL, Lee CH, Chen SC, Hou YC, Yeh SL. Effects of arginine-containing total parenteral nutrition on N balance and phagocytic activity in rats undergoing a partial gastrectomy. Br J Nutr 2005;93:267–72.
[4] Gianotti L, Alexander JW, Pyles T, Fukushima R. Arginine-supplemented diets improve survival in gut-derived sepsis and peritonitis by modulating bacterial clearance. The role of nitric oxide. Ann Surg 1993;217:644–53.
[5] Evoy D, Lieberman MD, Fahey III TJ, Daly JM. Immunonutrition: the role of arginine. Nutrition 1998;14:611–7.
[6] De-Souza DA, Greene LJ. Pharmacological nutrition after burn injury. J Nutr 1998;128:797–803.
[7] Kirk SJ, Regan MC, Wasserkrug HL, Sodeyama M, Barbul A. Arginine enhances T-cell responses in athymic nude mice. JPEN 1992;16:429–32.
[8] Ochoa JB, Bernard AC, Mistry SK, Morris Jr SM, Figert PL, Maley ME, et al. Trauma increases extrahepatic arginase activity. Surgery 2000;127:419–26.
[9] Gattinoni L, Powell Jr DJ, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol 2006;6:383–93.
[10] Casas-Rodera P, Gomez-Candela C, Benitez S, Mateo R, Armero M, Castillo R, et al. Immunoenhanced enteral nutrition formulas in head and neck cancer surgery: a prospective, randomized clinical trial. Nutr Hosp 2008;23:105–10.
[11] Park KG, Heys SD, Blessing K, Kelly P, McNurlan MA, Eremin O, et al. Stimulation of human breast cancers by dietary L-arginine. Clin Sci (Lond) 1992;82:413–7.
[12] Philip R, Campbell E, Wheatley DN. Arginine deprivation, growth inhibition and tumor cell death: 2. enzymatic degradation of arginine in normal and malignant cell cultures. Br J Cancer 2003;88:613–23.
[13] Wheatley DN, Philip R, Campbell E. Arginine deprivation, and tumor cell death: arginase and its inhibition. Mol Cell Biochem 2003;244:177–85.
[14] Fukumura D, Yonei Y, Kurose I, Saito H, Ohishi T, Higuchi H, et al. Role in nitric oxide in Kupffer cell–mediated hepatoma cell cytotoxicity in vitro and ex vivo. Hepatology 1996;24:141–9.
[15] Cifone MG, Festuccia C, Cironi L, Cavallo G, Chessa MA, Pensa V, et al. Induction of the nitric oxide–synthesizing pathway in fresh and interleukin 2–cultured rat natural killer cells. Cell Immunol 1994;157:181–94.
[16] Mordan LJ, Burnett TS, Zhang LX, Tom J, Cooney RV. Inhibitors of endogenous nitrogen oxide formation block the promotion of neoplastic transformation in C3H 10T1/2 fibroblasts. Carcinogenesis 1993;14:1555–9.
[17] Gottke M, Chadee K. Exogenous nitric oxide stimulates mucin secretion from LS174T colonic adenocarcinoma cells. Inflamm Res 1996;45:209–12.
[18] Department of Health, Taiwan. Top 10 leading causes of death in Taiwan; 2009. Available at: www.doh.gov.tw.
[19] Vissers YL, Dejong CH, Luiking YC, Fearon KC, von Meyenfeldt MF, Deutz NE. Plasma arginine concentrations are reduced in cancer patients: evidence for arginine deficiency? Am J Clin Nutr 2005;81:1142–6.
[20] Thomsen LL, Lawton FG, Knowles RG, Beesley JE, Riveros-Moreno V, Moncada S. Nitric oxide synthase activity in human gynecological cancer. Cancer Res 1994;54:1352–4.
[21] Wheatley DN, Scott L, Lamb J, Smith S. Single amino acid (arginine) restriction: growth and death of cultured HeLa and human diploid fibroblasts. Cell Physiol Biochem 2000;10:37–55.
[22] Thomsen LL, Scott JM, Topley P, Knowles RG, Keerie AJ, Frend AJ. Selective inhibition of inducible nitric oxide synthase inhibits tumor growth in vivo: studies with 1400W, a novel inhibitor. Cancer Res 1997;57:3300–4.
[23] Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest 1973;52:2745–56.
[24] Malinda KM, Sidhu GS, Banaudha KK, Gaddipati JP, Maheshwari RK, Goldstein AL, et al. Thymosin alpha 1 stimulates endothelial cell migration, angiogenesis, and wound healing. J Immunol 1998;160:1001–6.
[25] Efron D, Barbul A. Role of arginine in immunonutrition. J Gastroenterol 2000;35(suppl 12):20–3.
[26] Braga M, Gianotti L, Vignali A, Carlo VD. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. Surgery 2002;132:805–14.
[27] Rose DP, Connolly JM. Regulation of tumor angiogenesis by dietary fatty acids and eicosanoids. Nutr Cancer 2000;37:119–27.
[28] Dobbs SP, Hewett PW, Johnson IR, Carmichael J, Murray JC. Angiogenesis is associated with vascular endothelial growth factor expression in cervical intraepithelial neoplasia. Br J Cancer 1997;76:1410–5.
[29] Asahara T, Bauters C, Zheng LP, Takeshita S, Bunting S, Ferrara N, et al. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. Circulation 1995;92(suppl): II365–71.
[30] Messina EJ, Weiner R, Kaley G. Prostaglandins and local circulatory control. Fed Proc 1976;35:2367–75.
[31] Cheng T, Cao W, Wen R, Steinberg RH, LaVail MM. Prostaglandin E_2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat Muller cells. Invest Ophthalmol Vis Sci 1998;39:581–91.

[32] Vihinen P, Kahari VM. Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer 2002;99:157–66.
[33] Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994;264:569–71.
[34] Sipkins DA, Cheresh DA, Kazemi MR, Nevin LM, Bednarski MD, Li KC. Detection of tumor angiogenesis in vivo by alphaVbeta3-targeted magnetic resonance imaging. Nat Med 1998;4:623–6.
[35] Ma Q, Wang Y, Gao X, Ma Z, Song Z. L-arginine reduces cell proliferation and ornithine decarboxylase activity in patients with colorectal adenoma and adenocarcinoma. Clin Cancer Res 2007;13:7407–12.
[36] Jenkins DC, Charles IG, Thomsen LL, Moss DW, Holmes LS, Baylis SA, et al. Roles of nitric oxide in tumor growth. Proc Natl Acad Sci U S A 1995;92:4392–6.
[37] Wink DA, Vodovotz Y, Laval J, Laval F, Dewhirst MW, Mitchell JB. The multifaceted roles of nitric oxide in cancer. Carcinogenesis 1998;19:711–21.
[38] Montrucchio G, Lupia E, de Martino A, Battaglia E, Arese M, Tizzani A, et al. Nitric oxide mediates angiogenesis induced in vivo by platelet-activating factor and tumor necrosis factor-alpha. Am J Pathol 1997;151:557–63.
[39] Maeda H, Noguchi Y, Sato K, Akaike T. Enhanced vascular permeability in solid tumor is mediated by nitric oxide and inhibited by both new nitric oxide scavenger and nitric oxide synthase inhibitor. Jpn J Cancer Res 1994;85:331–4.