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ORIGINAL ARTICLE

Nitric oxide synthesis modulation - a possible diagnostic and therapeutic target in colorectal cancer

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Summary

Purpose: *Considering the contradictory literature data about the role of nitric oxide (NO) in colon carcinogenesis, the purpose of this study was to examine the changes of L-arginine metabolites in colon cancer and surrounding tissue as possible molecular markers of tumor behavior after surgery and the possibility of NO synthesis modulation in new individualized therapeutic strategies.*

Methods: *The study encompassed 50 patients who underwent surgery for colorectal cancer (CRC). The three tissue specimens were taken by surgery (tumor, adjacent and healthy tissue) and the concentrations of NO_2+NO_3, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) were determined in the tissue specimens.*

Results: *The results proved higher NO_2+NO_3 concentrations in adjacent tissue compared to the tumor, implicating high angiogenic potential of the tumor-surrounding tissue, which could have clinical importance in the assessment of the probability of tumor local recurrence and metastasis. Increased ADMA concentrations in tumor tissue associated with low NO levels, could lead to new therapeutic strategies directed to the use of inhibitors of NO synthesis as ideal candidates for molecular therapy of CRC. ADMA concentration in adjacent tissue was an independent predictor of distant metastasis.*

Conclusions: *The obtained results suggest that determination of the examined biomarkers in CRC and adjacent tissue samples could give useful information about tumor proliferative and angiogenic potential, which in turn could enable individualization of therapy and the choice of proper adjuvant therapy in patients with CRC.*

Key words: *ADMA, arginine, colorectal cancer, metastases, nitric oxide, SDMA*

Introduction

With approximately 500,000 cases per year [1], CRC is one of the leading causes of cancer morbidity and mortality in the world [2]. A significant proportion of patients with stages I, II and III disease could be treated by surgery alone or in combination with chemotherapy, and present high survival rates compared to those with stage IV [2,3]. Even if the primary treatment is surgical, adjuvant therapy is applied routinely in patients with high risk for tumor recurrence or in those who have metastatic disease at the time of diagnosis. It is of essential importance to identify those subgroups of patients that could benefit from adjuvant chemotherapy, and, at the same time, to avoid the potential toxic effects of excessive treatment and the unnecessary financial burden on the budget of the health system [4-6]. The improvement of the efficacy of postoperative

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adjuvant chemotherapy in early-stage CRC should not be based only on the discovery of new drugs, but also on the possibility of individualized treatment based on tumor molecular characteristics. Therefore, in clinical practice, the key objective is the discovery of prognostic markers which could accurately predict the clinical and biological behavior of the tumor.

NO, the gaseous intra- and intercellular signaling molecule, controls a broad spectrum of biological functions in human body, including regulation of vascular tone, gastrointestinal motility, programmed cell death, proliferation and neurotransmission [7]. It is synthesized from the semi-essential amino acid L-arginine in reaction with NADPH and oxygen catalyzed by the enzyme nitric oxide synthase (NOS). Constitutively expressed, Ca^{2+}-dependent isoforms of NOS, nNOS (NOS-1, expressed in nervous tissue) and eNOS (NOS-3, endothelial enzyme) produce small amounts of NO in response to physiological stimuli. The induction of expression of iNOS (NOS-2, inducible and Ca^{2+}-independent) is stimulated by cytokines and some microroganism products. Once induced, iNOS can produce NO for a long time and in high concentrations, thus multiplying the organism exposure to its damaging effects [8-12]. In such situations, directly or mediated by its derivatives, NO nonselectively destroys tissues, causing mitochondrial dysfunction, protein tyrosine nitration, thiol group nitrosylation, electrolyte disbalance, etc. [11].

NO is a lipophilic molecule which easily diffuses across cell membrane, thus exerting its effects on surrounding cells from the site of synthesis [12,13]. The importance of NO is determined by its interaction with target molecules, available at the moment, forming a variety of metabolites which mediate its roles [14]. The metabolic pluripotency of NO and the possibility of its metabolites' further reactions, as well its influence on other metabolic pathways explain the wide spectrum of its biological effects, both physiological and pathological, and, frequently, quite paradoxical.

Arginine availability for NO synthesis in biological systems is regulated by its cellular entry mediated by transport systems on cell membrane, but, also by the activities of other metabolic pathways it is involved in. It has been also suggested that biosynthetic pathways of L-arginine are coordinated in the sense of arginine level modulation, related also to the synthesis of NO [15].

Methylated arginines, NG-monomethyl-L-arginine, NG,NG-dimethyl-L-arginine (ADMA) and NG,N'G-dimethyl-L-arginine (SDMA) are synthesized by the methylation of L-arginine residues in proteins [16]. All three arginine derivatives are present in circulation. They are released upon proteolysis of posttranslationally methylated arginine residues in proteins [17]. Arginine methylation is regulated by the family of specific protein arginine methyltransferases (PRMT) type I (PRMT1, 3, 4, 6 and 8) and type II (PRMT5,7 i FBXO11) (Figure 1) [16]. PRMT1 synthesizes ADMA, while SDMA is synthesized by the action of PRMT2 [18,19].

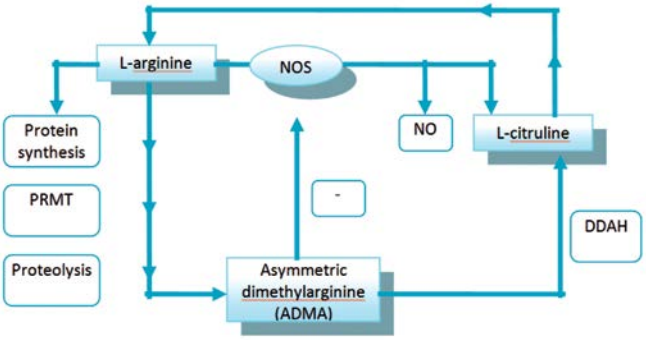

Figure 1. Methylated arginines synthesis and degradation.

ADMA is competitive NOS inhibitor. It has been proved that high ADMA concentrations (between 2 and 10 micromol/L) significantly inhibit NO production, so ADMA could be an important factor in the modulation of NO biological effects. Valance et al. [20] confirmed that high ADMA concentrations have an important role in pathological processes in which pathogenetic mechanisms were mediated by NO. ADMA is degraded under the influence of dimethylarginine dimethylaminohydrolase (DDAH) followed by the formation of dimethylamine and L-citruline [21].

The other methylarginine, SDMA, is not NOS inhibitor, but it can compete with L-arginine for cationic amino acid membrane transporter for intracellular arginine intake from extracellular space. Thus, SDMA indirectly influences NO synthesis, decreasing arginine availability for NOS catalyzed reaction [22].

The main causes for ADMA accumulation are increased protein methylation and metabolism, decreased DDAH activity and reduced urinary ADMA excretion [23]. Most frequently, ADMA concentration increase, with consequent NO increase, occurs due to the diminished activity of DDAH in inflammation and oxidative stress [24], which could be of importance in carcinogenesis.

Considering the dual role of NO in both phys-

iological and pathological processes, it is not surprising that there is an increasing number of investigations of NO role in the pathogenesis of different diseases, including CRC, in the last decade [25-27]. Unfortunately, the results of these studies are controversial and the mechanisms of its actions in disease have not been defined yet.

Methods

Patient and tumor specimen preparation

The study encompassed 50 patients hospitalized at the Department of Colorectal Surgery of Clinic of Surgery, Clinical Centre Nis. All of them underwent colon resection for CRC adenocarcinoma. Three tissue specimens were taken for study (tumor tissue specimen, specimen of tumor surrounding tissue/adjacent tissue and normal colonic mucosa specimen (healthy tissue more than 10 cm from tumor border - at the incision margin).

Immediately after surgery, the tissue specimens were washed in cold saline and kept at -20°C. Then, the tissues were cut into small pieces and homogenized using Ultra Turray IKA T18 basic homogenizer with teflon pestle (IKA-Werke GmbH & Co.KG, Staufen, Germany). The homogenates were frozen at -20°C until biochemical analyses were performed.

Biochemical analyses

The biochemical part of the research was done at the Research Centre for Biomedicine and Institute for Biochemistry, Faculty of Medicine, University of Nis. In the 3 tissue specimens the following parameters were determined: nitrite+nitrate (NO_2+NO_3) concentrations, as the measure of NO production, and ADMA and SDMA concentrations.

NO_2+NO_3 determination in tissue homogenates

In the presence of oxygen NO is rapidly oxidized to stable nitrites and nitrates products. Therefore, the concentration of NO_2+NO_3 has been used as the marker of NOS activity and endogenous NO production. Tissue NO_2+NO_3 concentration was determined using method of Navaro-Gonzalvez et al. [28], based on Griess reaction.

ADMA and SDMA concentrations determination in tissue homogenates

ADMA and SDMA concentrations were determined by HPLC methodology according to Pi et al. (2000) on HPLC apparatus (Agilend Technologies, Santa Clara, California, USA) with fluorimetric detection, modified according to our conditions [29].

Protein determination in tissue homogenates

Tissue proteins were determined according to the method of Lowry et al. [30].

Results

NO_2+NO_3 concentration in tumor, adjacent and healthy colon tissue homogenates

NO_2+NO_3 concentration in colon cancer tissue (58.608±22.789 nmol/mg prot) was statistically significantly lower (p<0.001) in comparison to healthy tissue (81.556±38.182). In the adjacent tissue, NO_2+NO_3 concentration (85.100±37.972) was significantly (p<0.001) higher compared with tumor tissue (Figure 2).

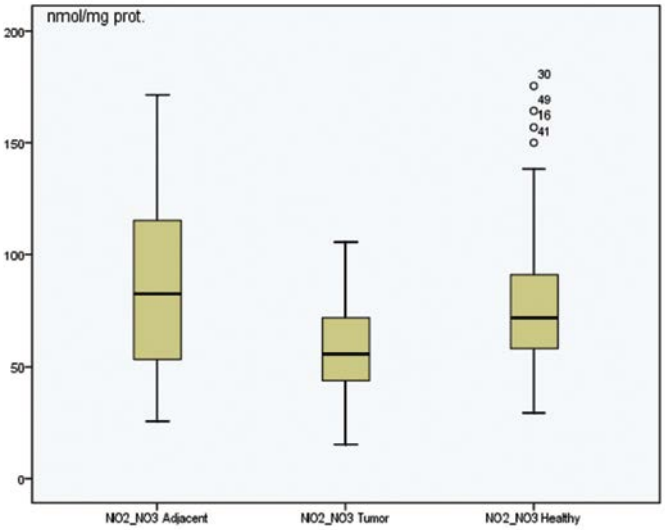

Figure 2. NO2+NO3 concentrations in tumor, adjacent and healthy colon tissue. Vertical boxplot. The boundaries of the box are Tukey's hinges. The median is identified by a line inside the box. The length of the box is the interquartile range (IQR) computed from Tukey's hinges. Values more than 1.5 IQR but less than 3 IQR from the end of the box are labeled as outliers (o).

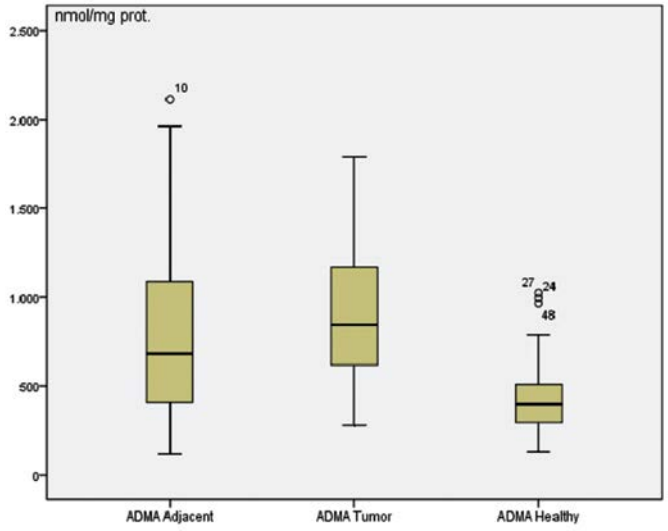

Figure 3. ADMA concentrations in tumor, adjacent and healthy colon tissue. Vertical boxplot. The boundaries of the box are Tukey's hinges. The median is identified by a line inside the box. The length of the box is the interquartile range (IQR) computed from Tukey's hinges. Values more than 1.5 IQR but less than 3 IQR from the end of the box are labeled as outliers (o).

ADMA and SDMA concentration in tumor, adjacent and healthy colon tissue homogenates

ADMA concentration in colon cancer tissue (870.0±370.1 nmol/mg prot) was statistically significantly higher (p<0.001) compared to the healthy tissue (446.5±249.7 nmol/mg prot). The concentration of ADMA in the tumor surrounding tissue (709.6±465.6 nmol/mg prot) was similar compared to the tumor tissue, but it was also significantly increased compared to healthy tissue (p<0.001) (Figure 3).

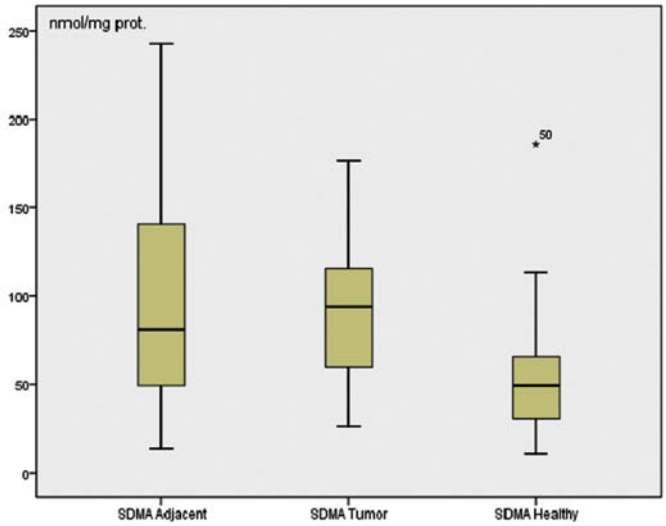

Figure 4. SDMA concentrations in tumor, adjacent and healthy colon tissue. Vertical boxplot. The boundaries of the box are Tukey's hinges. The median is identified by a line inside the box. The length of the box is the interquartile range (IQR) computed from Tukey's hinges. Values more than three IQR from the end of a box are labeled as extreme, denoted with an asterisk (*). Values more than 1.5 IQR but less than 3 IQR from the end of the box are labeled as outliers (o).

SDMA concentrations in colon cancer tissue (89.22±38.90 nmol/mg prot) were significantly increased (p<0.001) in comparison with the healthy tissue values (53.11±33.32 nmol/mg prot). In adjacent tissue, SDMA concentration (90.62±57.26 nmol/mg prot) was similar to that in tumor tissue, but also significantly higher that the value in healthy tissue (p<0.001) (Figure 4).

NO_2+NO_3, ADMA and SDMA association analyses

Correlation analysis revealed a trend for positive association between NO_2+NO_3 and ADMA values in tumor, adjacent and healthy colon tissue (Figure 5).

A similar trend of positive association could also be seen between NO_2+NO_3 and SDMA values in tumor, adjacent and healthy colon tissue (Figure 6).

The predictive significance of ADMA and SDMA concentrations in tumor, adjacent and healthy tissue for the occurrence of metastases during 5-year period was analyzed using binary regression analysis (backward logistic regression model), and the results are presented in Table 1.

In the used model (Backward LR), ADMA concentration in adjacent tissue proved to be the only independent predictor for occurrence of metastases (OR 1.003, 95%CI 1.000-1.006, p<0.05), while ADMA concentration increase for one unit in the surrounding tissue increased the risk for metastasis by 0.3%.

Discussion

Cellular NO concentration could be controlled by regulating the L-arginine availability for its synthesis, NOS activity, as well as the levels of ADMA and SDMA, the methylated arginine derivatives. Protein arginine methylation (R-methylation), the form of protein posttranslational modification, and ADMA and SDMA biological and pathophysiological functions are poorly understood despite the numerous data about the important role of PRMT enzymes family in the regulation of transcription [31-33]. But, it soon became clear that ADMA and SDMA could influence NO production, so their determination could be of importance in understanding the mechanisms of NO synthesis control and the possibility of nitrosative stress modulation in numerous pathological states, including carcinogenesis.

The first literature data about ADMA in human cancers appeared in 2010. Yoshimatsu et al. [34] were the first to prove serum ADMA concentration increase in patients with different kinds of cancers. In the same study, they found over-expression of PRMT1 and PRMT6 isoenzymes in cancer tissue samples.

Literature data about ADMA levels in colon cancer patients are scarce. In the only published study, the authors have proved increased serum ADMA concentration in patients with colon cancer in comparison with healthy subjects [35]. The same authors studied ADMA influence on cell proliferation and apoptosis in *in vitro* LoVo colon cancer cell line culture and proved that the treatment of cancer cells with ADMA diminished cell death caused by doxorubicin, but had no impact on normal fibroblast viability. ADMA suppresses Fas (APO-1/CD95)/JNK activation in signal pathway activated by doxorubicin.

The increase of ADMA occurs most probably as a consequence of the increased PRMT activity.

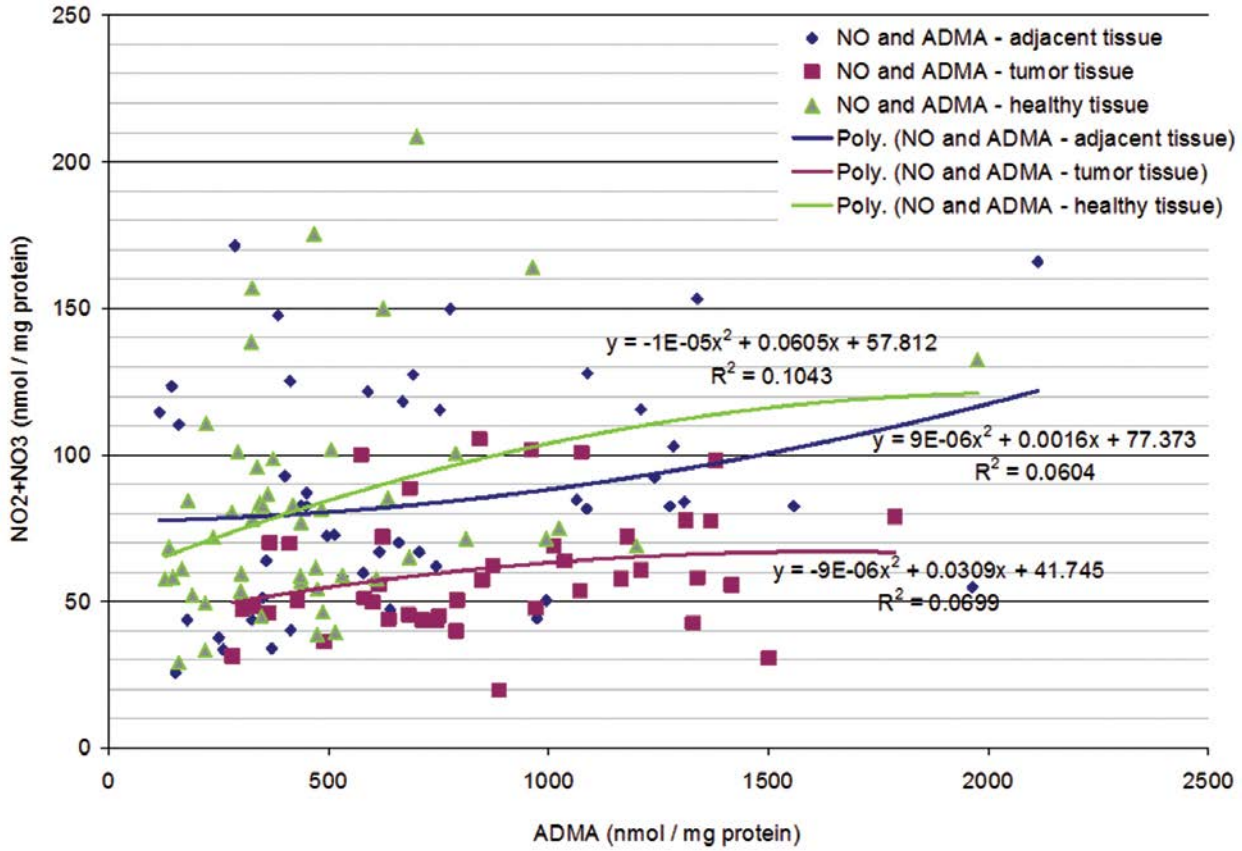

Figure 5. Association between NO_2+NO_3 and ADMA values.

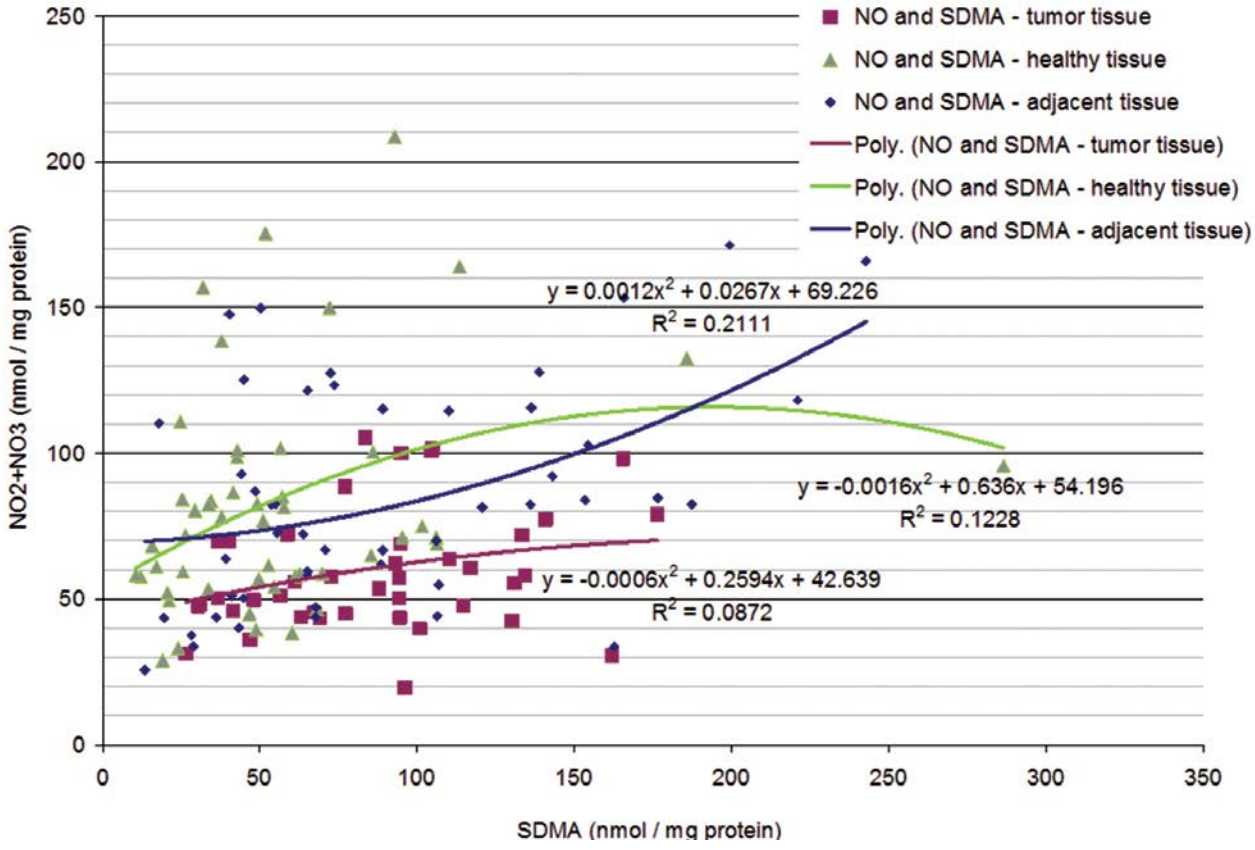

Figure 6. Association between NO_2+NO_3 and SDMA values.

Table 1. The predictive significance of ADMA and SDMA concentrations for metastasis appearance in binary regression model

		B	*SE*	*df*	*Sig*	*Exp(B)*	*95% CI or EXP(B)*	
							Lower	*Upper*
Step 1[a]	ADMA_O	0.03	0.05	1.00	0.56	1.03	0.94	1.13
	SDMA_O	-0.16	0.29	1.00	0.59	0.86	0.49	1.50
	ADMA_T	0.00	0.01	1.00	0.96	1.00	0.98	1.02
	SDMA_T	-0.03	0.11	1.00	0.76	0.97	0.79	1.20
	ADMA_Z	0.05	0.10	1.00	0.59	1.06	0.87	1.29
	SDMA_Z	-0.54	1ž.02	1.00	0.60	0.58	0.08	4.34
	Constant	-11.21	15.85	1.00	0.48	0.00		
Step 6[a]	ADMA_O	0.00	0.00	1.00	0.03	1.00	1.00	1.01
	Constant	-5.88	2.17	1.00	0.01	0.00		

[a]Variable(s) entered on step 1: ADMA_O. SDMA_O. ADMA_T. SDMA_T. ADMA_Z. SDMA_Z; Model: backward LR

It seems that these enzymes have a critical role in the regulation of cancer cell lines growth, since it has been reported that PRMT1 or PRMT6 knockdown suppresses cancer cells' growth. Northern blot analyses showed that PRMT6 expression was very low in normal tissues, pointing out that PRMT1 and PRMT6 inhibitors could be ideal candidates for molecular targeted cancer therapy and focusing the interest on investigations on the possibilities of PRMT inhibitors use in cancer therapy [36-38] in the last few years.

Meanwhile, it is necessary to mention that in the conditions of inflammation and oxidative stress there is reduced activity of DDAH (ADMA degradation enzyme), which could be one of the possible explanations for the increased ADMA concentration in tumor tissue found in our study [39,40]. Moreover, Aggarwal et al. [39] proved that, in inflammation, the targeted DDAH over-expression could significantly inhibit ADMA accumulation, suggesting the possibility of using DDAH activity stimulation in adjuvant therapy of the pathological process formed on the basis of inflammatory background.

Also, in chronic hypoxia, present in cancer tissue, there is disbalance between vasodilation and vasoconstriction towards favoring the second one, and which is due to increased ROS production, eNOS inhibition and reduced NO synthesis. Kumarasamy et al. [41] found PRMT1 responsible for this disbalance related to increased ADMA production and the consequent eNOS inhibition. This hypothesis could also offer explanation for the low NO concentrations found in tumor tissue of the patients in the present study.

The other methylated arginine, SDMA, is not NOS inhibitor, but it has been reported that it has an impact on the intensity of NO production, since it competes with L-arginine for the same plasma membrane transporter [42,43], thus influencing L-arginine intracellular concentration and its availability for NO synthesis. Besides, SDMA metabolism is related to the inflammatory process. Scheper et al. [44] showed that, in inflammation, SDMA activated NF-κB, leading to increased TNF-α and IL-6 production. So, it is considered that SDMA proiflammatory effect, together with its impact on increased free radical production [45] and indirect influence on NOS represent the potential mechanisms of SDMA effects in colon carcinogenesis.

The proved high ADMA levels in tumor tissue and the tumor surrounding tissue in our study, could point out ADMA antiapoptotic effects, which would characterized ADMA as a prognostic marker for tumor invasion potential. Our hypothesis has been proved by the association analyses of ADMA values and the clinical outcome of our patients, which revealed that ADMA could be an independent predictor of metastasis.

Taking into account the established role of NO in different tumors' transformation into angiogenic phenotype in the process of neovasculization [46], the results of this study showed lower NO concentrations in tumor tissue compared to the adjacent tissue, pointing out high angiogenic potential which could have clinical importance in the assessment of the probability of tumor local recurrence. Increased ADMA and SDMA concentrations in tumor tissue associated with low NO levels, could make the basis for new therapeutic strategies targeting NO synthesis (the use of NO synthesis inhibitors as ideal candidates for molecular therapy of CRC). ADMA concentration in adjacent tissue was proved to be an independent predictor of distant metastasis. The obtained results suggest that determination of the examined biomarkers

in CRC tissue samples, after surgery, could give useful information about tumor proliferative and angiogenic potential, which could enable individualization of therapy and the choice of proper adjuvant therapy.

In our future prospective research on L-arginine metabolism in colon carcinogenesis with higher number of patients, we'll focus on the precise definition of all the molecular players (including polyamines, methylated arginines, etc.) involved in the complex network of signal transmission related to proliferative and angiogenic potential of colon cancer cells supported by associated analyses with the clinical correlates of the disease.

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Conflict of interests

The authors declare no confict of interests.

References

1. O'Connell JB, Maggard M, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J Natl Cancer Inst 2004;96:1420-1425.
2. van der Voort van Zijp J, Hoekstra HJ, Basson MD. Evolving management of colorectal cancer. World J Gastroenterol 2008;14:3956-3967.
3. Civelek B, Aksoy S, Sendur KA et al. Adjuvant chemotherapy outcomes in patients over 65 years with early stage colorectal carcinoma. JBUON 2014;19:906-912.
4. Midgley RS, Yanagisawa Y, Kerr DJ. Evolution of nonsurgical therapy for colorectal cancer. Natl Clin Pract Gastroenterol Hepatol 2009;6:108-120.
5. Sobin LH, Fleming ID. TNM classification of malignant tumors (5th Edn). Cancer 1997;80:1803-1804.
6. Seretis C, Mankotia R, Goonetilleke K, Rawstorne E. Quality assessment of decision-making in colorectal cancer multidisciplinary meetings. JBUON 2014;19:913-916.
7. Guix FX, Uribesalgo I, Coma M, Munoz FJ. The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 2005;76:126-152.
8. Jaffrey SR, Erdjument-Bromage H, Ferris CD, Tempst P, Snyder SH. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol 2001;3:193-197.
9. Kone BC, Kuncewicz T, Zhang W, Yu ZY. Protein interactions with nitric oxide synthases: controlling the right time, the right place, and the right amount of nitric oxide. Am J Physiol Renal Physiol 2003;285:178-190.
10. Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. Nitric Oxide 2010;23:75-93.
11. Knott AB, Bossy-Wetzel E. Nitric oxide in health and disease of the nervous system. Antioxid Redox Signal 2009;11:541-553.
12. Gotoh T, Mori M. Arginase II downregulates nitric oxide production and prevents NO-mediated apoptosis in murine macrophage-derived RAW 264.7 cells. J Cell Biol 1999;144:1-8.
13. Abraham M, Gola J, Cometto-Muniz E, Cain W. The solvation properties of nitric oxide. J Chem Soc Perkin Trans 2000;2:2067-2070.
14. Elfering SL, Sarkela TM, Giulivi C. Biochemistry of mitochondrial nitric-oxide synthase. J Biol Chem 2002;277:38079-38086.
15. Gaston BM, Carver J, Doctor A, Palmer LA. S-Nitrosylation signaling in cell biology. Mol Interv 2003;3:253-263.
16. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. Mol Cell 2009;33:1-13.
17. Zoccalia C, Kielstein JT. Asymmetric dimethylarginine: a new player in the pathogenesis of renal disease? Cur Opin Neph Hypert 2006;15:314-320.
18. Gary JD, Clarke S. RNA and protein interactions modulated by protein arginine methylation. Prog Nucleic Acid Res Mol Biol 1998;61:65-131.
19. Scott HS, Antonarakis SE, Lalioti MD, Rossier C, Silver PA, Henry MF. Identification and characterization of two putative human arginine methyltransferases (HRMT1L1 and HRMT1L2). Genomics 1998;48:330-340.
20. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of NO synthesis in chronic renal failure. Lancet 1992;339:572-575.
21. Cam TL, Tran J, Leiper M, Vallance P. The DDAH/ADMA/NOS pathway Atherosclerosis 2003; 4 (Suppl):33-40.
22. Closs EI, Basha FZ, Habermeier A, Förstermann U. Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. Nitric Oxide 1997;1:65-73.
23. Zoccali C, Mallamaci F, Tripepi G. Asymmetric dimethylarginine (ADMA) as a cardiovascular risk factor in end-stage renal disease (ESRD). Eur J Clin Phar-

macol 2006;62:131-135.

24. Spittle MA, Hoenich NA, Handelman GJ, Adhikarla R, Homel P, Levin NW. Inflammation and Atherosclerosis in End-Stage Renal Disease. Am J Kidney Dis 2001;38:1408-1413.
25. Martınez MC, Andriantsitohaina R. Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. Antioxid Redox Signal 2009;11:669-702.
26. Reiter T. NO chemistry: a diversity of targets in the cell. Redox Rep 2006;11:194-206.
27. Pacher P, Schulz R, Liaudet L, Szabo C. Nitrosative stress and pharmacological modulation of heart failure. Trends Pharmacol Sci 2005;26:302-310.
28. Navaro-Gonzalvez JA, Garcia-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin Chem 1998;44:679-681.
29. Pi J, Kumagai Y, Sun G, Shimojo N. Improved method for simultaneous determination of L-arginine and its mono- and dimethylated metabolites in biological samples by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000;742:199-203.
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265-275.
31. Mallappa C, Hu YJ, Shamulailatpam P, Tae S, Sif S, Imbalzano AN. The expression of myogenic microRNAs indirectly requires protein arginine methyltransferase (Prmt)5 but directly requires Prmt4. Nucleic Acids Res 2011;39:1243-1255.
32. Lee YH, Stallcup MR. Minireview: protein arginine methylation of nonhistone proteins in transcriptional regulation. Mol Endocrinol 2009;23:425-433.
33. Argentini M, Tora L. PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function. Exp Cell Res 2009;315:1273-1286.
34. Yoshimatsu M, Toyokawa G, Hayami S et al. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. Int J Cancer 2011;128:562-573.
35. Li H, Zhou Y, Zhao A et al. Asymmetric dimethylarginine attenuates serum starvation-induced apoptosis via suppression of the Fas (APO-1/CD95)/JNK (SAPK) pathway. Cell Death Disease 2013;4:e830; doi:10.1038/cddis.2013.345.
36. Ragno R, Simeoni S, Castellano S et al. Small molecule inhibitors of histone arginine methyltransferases: homology modeling, molecular docking, binding mode analysis, and biological evaluations. J Med Chem 2007;50:1241-1253.
37. Spannhoff A, Heinke R, Bauer I et al. Target-based approach to inhibitors of histone arginine methyltransferases. J Med Chem 2007;50:2319-2325.
38. Cheng D, Yadav N, King RW, Swanson MS, Weinstein EJ, Bedford MT. Small molecule regulators of protein arginine methyltransferases. J Biol Chem 2004;279:23892-23899.
39. Aggarwal S, Gross CM, Kumar S et al. Dimethylarginine dimethylaminohydrolase II overexpression attenuates LPS-mediated lung leak in acute lung injury. Am J Respir Cell Mol Biol 2014;50:614-625.
40. Pope AJ, Druhan L, Guzman JE et al. Role of DDAH-1 in lipid peroxidation product-mediated inhibition of endothelial NO generation. Am J Physiol Cell Physiol 2007;293:C1679-1686.
41. Kumarasamy C, Singh G, Raman P, Mala K. Effect of protein arginine methyltransferase-1 inhibition on hypoxia-induced vasoconstriction. Med Hypotheses 2015;85:740-743.
42. Chinje E, Stanford IJ. The role of nitric oxide in growth of solid tumors: a balancing act. Essays Biochem 1997;32:6-72.
43. Leiper J, Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. Cardiovasc Res 1999;43:542-548.
44. Schepers E, Barreto DV, Liabeuf S et al. Symmetric dimethylarginine as a proinflammatory agent in chronic kidney disease. European Uremic Toxin Work Group (EUTox). Clin J Am Soc Nephrol 2011;6:2374-2383.
45. Schepers E, Glorieux G, Dhondt A, Leybaert L, Vanholder R. Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. Nephrol Dial Transplant 2009;24:1429-1435.
46. Erdamar S, Bagci P, Oz B, Dirikan A. Correlation of endothelial nitric oxide synthase and vascular endothelial growth factor expression with malignancy in patients with astrocytic tumors. JBUON 2006;14:213-216.