Exp Oncol 2019
41, 2, 210–215

Experimental ONCOLOGY

ROLE OF NITRIC OXIDE IN PATHOGENESIS OF TUMOR GROWTH AND ITS POSSIBLE APPLICATION IN CANCER TREATMENT

V.V. Holotiuk, A.Y. Kryzhanivska, I.K. Churpiy, B.B. Tataryn, D.Ya. Ivasiutyn*
SHEI "Ivano-Frankivsk National Medical University", Ivano-Frankivsk 76018, Ukraine

In this review, the role of nitric oxide (NO) in the pathogenesis of the tumor growth and possibilities of its application in the treatment of cancer patients are analyzed. NO is one of the most important mediators of physiological processes being involved in the regulation of practically all body functions in health and disease. The role of NO in the development of many pathological conditions has been extensively studied and debated in recent years. Today it is clear that NO in relation to malignant tumors may exhibit a dual activity — can stimulate tumor growth and cause an opposite antitumor effect. Effects of NO are mostly dependent on its concentration. At low concentrations, NO could inhibit apoptosis and cause mutations that potentially lead to the formation of malignant growth loci. However, a high concentration of NO appears to be detrimental to malignant cells, in particular under conditions of simultaneous exposure to ionizing radiation. In humans, the inducible NO synthase (iNOS, type II) is the most powerful form of NO synthases (NOS) and has the ability to synthesize large amounts of NO for a long time and exert a protective function. iNOS is expressed in macrophages, monocytes, neutrophils, fibroblasts, hepatocytes, and other cell types. In tissue of malignant tumors, the macrophagal iNOS is the main form. Experimental data provide an evidence that activated macrophages and leukocytes, which are the part of peritumorous inflammatory infiltrate, can provide radiosensitization of tumors by direct synthesis of NO and indirectly — through the secretion of cytokines stimulating iNOS activity in cancer cells. Such approach could be useful for the development of new schemes and methods of anticancer therapy based on the activation of endogenous NO biosynthesis pathways.
***Key Words*: antitumor therapy, malignant tumors, nitric oxide, radiosensitization.**

A gaseous chemical messenger — nitric oxide (NO) is one of the most important mediators of physiological processes in human body. NO is involved in the regulation of practically all body functions in health and disease [1, 2]. The role of NO in the development of many pathological conditions has been widely studied and debated in recent years. NO is defined by the scientists as a trigger factor in many pathophysiological mechanisms, being a regulatory molecule, damaging one, or *vice versa* a protective agent [1–3].

Similarly, NO affects cancer development, and the redox-sensitive NO molecules play a key role in redox regulation of proliferation of cancer cells [4, 5]. NO also may have a huge impact on other aspects of tumor biology, including angiogenesis and metastasizing [6].

Moreover, recent studies provide the theoretical and experimental rationale for NO application with the purpose of chemo- and radiosensitization of malignant tumors, including rectal cancer, prostate cancer, etc. [5, 7–10]. Thus, in-depth study of NO properties in biological systems under the conditions of malignancy will be useful for developing new therapeutic approaches and personalization of cancer treatment.

Submitted: May 26, 2018.
*Correspondence: E-mail: golotiuk1@ukr.net
Abbreviations used: CRC – colorectal cancer; eNOS – endothelial nitric oxide synthase; ERK – extracellular signal-regulated kinase; iNOS – inducible nitric oxide synthase; mtNOS – mitochondrial nitric oxide synthase; NO – nitric oxide; NOS – nitric oxide synthase; nNOS – neuronal nitric oxide synthase; PI3K – phosphoinositide 3-kinases; RNS – reactive nitrogen species; ROS – reactive oxygen species; SR – superoxide radicals.

BIOCHEMICAL PROPERTIES OF NO

In biological systems, NO is unstable and highly reactive lipophilic compound. The small size and lack of charge cause the high penetration of the NO molecule through the cell membranes and subcellular organelles (NO diffusion rate is the highest among all the molecules of the body). In addition, S-nitrosothiols can transfer NO between cells via binding with SH-groups of proteins. That's why NO has the ability to affect not only the cells wherein NO is produced, but also the nearby cells [1, 2].

The range of physiological activity of NO is extremely wide. Through cGMP-dependent mechanism, NO reduces the tone of smooth muscles of blood vessels, regulating peripheral resistance, blood pressure and affecting the redistribution of blood [1, 5]. NO prevents monocyte chemotaxis, inhibits adhesion of leukocytes to the endothelium, prevents platelet adhesion and aggregation. Of particular interest is the ability of NO to affect the synthesis of a number of important proteins and enzymes — both at the transcription and translation level, including stress proteins, antioxidant proteins, ferritin, transferrin receptor proteins. NO may affect the activity of guanylate cyclase, ribonucleotide reductase, cyclooxygenase-2, caspase-1, 2, 3, 4, 8 and 9, p53 protein, Ras, Bcl-2, 8-oxoguaninglycosylase, components of the respiratory chain of mitochondria and glycolysis, transcription factor NF-kB, cytochrome P-450, ion channels, and others by the chemical posttranslational modifications, mostly S-nitrosylation [1, 5, 11–13].

In humans, NO is produced with involvement of three isoforms of the enzyme NO synthase (NOS): neuronal (nNOS, type I), endothelial (eNOS, type III) and indu-

cible (iNOS, type II) [5]. The first two NOS isoforms are constitutive and calcium and calmodulin dependent. They are inefficient and represented in tumor tissue in trace amounts [5]. Instead, iNOS, having the ability to synthesize large amounts of NO for a long time, is much more powerful, functions independently of the calcium concentration in the cell and is usually expressed in response to cell activation by cytokines or bacterial antigens [1, 14]. Within a short period, iNOS provides for at least 40-fold increase in NO concentration depending on the strength of stimulating factor. NO under the action of iNOS is synthesized by two-stage reaction of hydroxylation of citrulline cycle from L-arginine (arginine — arginine-succinate — citrulline), resulting in generation of NO radical and L-citrulline [5, 15].

There are some data showing direct formation of nitrite anion NO_2^-, nitrate anion NO_3^- or $ONOO^-$ in the NOS-reaction. This indicates the ability of NOS to produce reactive oxygen species (ROS) and NO. Therefore, NOS should be considered as a complex enzyme that synthesizes highly reactive compounds depending on the different functional state of the cell [3]. iNOS can be detected in macrophages, monocytes, neutrophils, fibroblasts, hepatocytes, and other cell types [16].

It should be noted that NO, unlike many other mediators of inflammation, does not require enzymatic modification. It can interact with other molecules, taking a direct part in the activation of free radical processes and reactive nitrogen species (RNS) — biologically active toxic products of the reaction of NO with molecular oxygen O_2, superoxide radicals (SR) or hydrogen peroxide H_2O_2 [1, 15, 17]. The main highly toxic reactive forms of NO, which during their excessive synthesis *in vivo* cause formation of nitro-oxidative stress are dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$), respectively, which is often formed in the reaction of NO with SR [18]:

$$O_2^- + NO \rightarrow ONOO^-$$

Peroxynitrite exhibits well-defined properties of oxidant and nitrosating agent with a short half-life in biological systems, which can lead to the inactivation of superoxide dismutase, lipid peroxidation and single-stranded DNA breaks [3]. In turn, peroxynitrite can cause further ROS formation, including the reaction of interaction of peroxynitrite with the proton, which can be a major source of hydroxyl radicals, because it does not require the participation of metals of variable valences:

$$2ONOO^- + 2H + \rightarrow ONOOH + HO^{\cdot} + NO_2$$

The toxicity resulting from excessive synthesis of NO or NO released from its exogenous donors is largely determined by the high affinity of NO for the "free" iron followed by inhibition of electron-transport chain enzymes of mitochondria, glutathione reductase, ribonucleotide reductase, xanthine oxidase and other metal enzymes [3, 19].

Accumulated data prove that NO may affect directly the regulation of mitochondrial energy functions [20, 21]. At high concentrations NO, competing with molecules of oxygen, can reversibly bind with iron heme a3 of cytochrome c-oxidase of IV complex — terminal electronic transport acceptor of mitochondrial chain. As a result, transport of electrons by the complexes of the respiratory chain, which normally provides the proton extrusion from the mitochondrial matrix and creates a potential gradient in the inner membrane of mitochondria, is disrupted. Besides reducing membrane potential, the above mechanism results in excessive formation of free radicals and peroxide compounds — products of incomplete reduction of oxygen, including SR and peroxynitrite [20, 21]. Thus, the mitochondria become a source of free radicals in cells synthesizing a large amount of SR, peroxide and peroxynitrite, which after getting into the cytosol, create the background for the formation of oxidative and nitro-oxidative stress [20, 21]. In addition, it is known that mitochondria have their own ability to synthesize NO. Constitutively expressed mitochondrial NOS (mtNOS) — α-isoform of nNOS identified on the inner mitochondrial membrane is a physiological inhibitor of functional activity of organelles through binding of NO with cytochrome c-oxidase [22, 23].

NO AND TUMOR GROWTH

NO plays dual role in cancer biology since it can stimulate tumor growth as well as provide the opposite anti-tumor effect. It modulates various tumor events including angiogenesis, apoptosis, cell cycle, infiltration and metastasing. Effects of NO in the tumor mostly depend on its concentration [1, 5, 15, 17, 18, 24]. At low concentrations, such as in the setting of chronic inflammation, NO has the ability to inhibit apoptosis, while NO and RNS, interacting with DNA during long time, cause mutations that potentially lead to the formation of malignant growth loci. Thus, NO is involved in the genotoxic damage that may occur as a direct modification of DNA, or inhibition of reparation of nucleic acids. The inflammation-related mechanisms of carcinogenesis mediated by NO and $ONOO^-$ also include post-translational modification of proteins and activation of signaling pathways stimulating cell proliferation, neovascularisation, and dissemination of tumor cells [3, 16, 17, 25].

It was shown that NO contributes to the genomic instability in combination with ionizing radiation and that the action of NO and ascorbic acid on blood lymphocytes of healthy persons enhances chromosome instability of cells, resulting in two-fold increase of chromatid aberrations [26, 27].

The cytoprotective action of NO in low concentrations is largely caused by its ability to inhibit the caspase activity via S-nitrosating of cysteine residues in the catalytic centers [1, 12, 13]. The efficiency of S-nitrosating processes in normoxic conditions is determined by the intracellular level of nonheme iron (Fe^{2+}), which after interaction with NO forms dinitrosyl complexes, in which NO is in the oxidized state (NO^+) and can join into S-nitrosating processes [13]. There is an alternative view that NO limits the activity of caspases not due to S-nitrosating but affecting certain stages of processing [16].

Macrophagal iNOS is the main form in the tissue of malignant tumors. NO as a product of iNOS activity, is an important mediator of various interactions between tumor and macrophages as a component of microenvironment. On the other hand, among immune cells that directly interact with the tumor tissue, macrophages are the most numerous [28]. In particular, they have the ability to infiltrate deep perinecrotic and hypoxic zone of tumors, accumulate there, in some cases, up to 50% of tumor volume [29].

A small amount of NO produced in tumors by M_2-macrophages in the hypoxia setting, is capable through the stabilization of transcription factors HIF family and activation of ERK1/2 (extracellular signal-regulated kinase) and PI3K (phosphoinositide 3-kinases) signaling pathways to stimulate the synthesis of pro-angiogenic factors, including matrix metalloproteinases, tumor growth factor-β, vascular endothelial growth factor, fibroblast growth factor-2, platelet growth factor, insulin-like growth factor-2, interleukin-8 [28, 30]. It should be noted that the expression of the major pro-angiogenic molecular factors HIF-1 or HIF-2 sharply increases in hypoxic conditions (5% oxygen) and almost disappears upon sufficient oxygenation of tissues [17]. In addition, HIF-1 activates processes of glycolysis in hypoxic tissues, increases erythropoietin production and stimulates mechanisms of metastasizing [3, 30, 31].

High level expression of HIF-1 in solid tumors is associated with radioresistance of tumors and poor prognosis [30, 32]. It was found that the therapy aimed at eliminating hypoxia and/or reducing the activity of HIF-1 in the tumor, can increase the chemo- and radiosensitivity of tumors, thus improving prognosis [30]. Experimental study of pathogenic changes in tumors during radiotherapy showed that iNOS of tumor-associated macrophages is the main but not single source of NO in tumor tissue. Besides this, under the influence of ionizing radiation the number of iNOS-positive macrophages increases. Low concentrations of NO in well-oxygenated areas of tumors, including colorectal cancer (CRC), stabilize HIF structure, causing its accumulation [28, 33]. On the other hand, NO can promote the release of RNS, which in turn will lead to hydroxylation and degradation of HIF-1 in the hypoxic areas of chemo- and radioresistant carcinomas by binding to cytochrome c-oxidase of mitochondria [30, 34]. NO donors were tested with positive effect as intermediaries able to inhibit HIF-1 in experimental conditions [32].

In contrast to effects of small amounts of NO, at sufficiently high concentrations, NO and ROS (> 400 pM) stimulate apoptosis in malignant cells in addition to direct damaging effects through activation of free radical oxidation reactions [7, 10, 20]. Cell death is mediated by enhancing the expression of p53 and tumor necrosis factor-α; inhibition of transcription factor NF-kB; reduced expression of Bcl-2 family proteins; caspase activation; and direct DNA damage [1, 28, 32]. In addition, under certain conditions, NO and its derivatives, including peroxynitrite, could induce apoptosis via mitochondrial signaling pathway by blocking cytochrome c-oxidase and oxidating thiols of mitochondrial membrane [20, 35, 36].

In the blood samples of patients with colon cancer, formation of hemoglobin-NO complexes was demonstrated, and their level in patients with metastatic forms of the disease significantly exceeded that in patients with resectable forms of cancer. It has been proven that the molecular marker of NO-Hb in the blood of cancer patients can be used as an indicator of the impairment of its transport and other functions, which leads to decreasing oxygen supply in peripheral tissues in cancer patients [37].

NO DONORS IN CANCER TREATMENT

It was proved that the maximum amount of NO, which may be produced within tumors, is not sufficiently high to kill cancer cells, but is sufficient for their sensitization to the cytotoxic effect of chemotherapy or ionizing radiation [32, 35]. The results of several studies provide theoretical and experimental rationale for the use of NO for the purpose of chemo- or radiosensitization of malignant tumors [7, 10, 38]. There are two main approaches to increase the concentration of NO in tumor: stimulation of NO production in the tumor by increasing iNOS expression or administration of NO-donors such as organic nitrates, S-nitrozotiols, N-nitrosamines, nitrozimines, metal-NO complexes, or combinations of non-steroid anti-inflammatory drugs with NO, which can release NO in biological environments spontaneously or following enzymatic biotransformation in the tissues [5, 11, 18, 32, 35, 39].

In vivo and *in vitro* investigations provide the evidence that activated macrophages and leukocytes, which are part of peritumorous inflammatory infiltrates, can radiosensitize tumors by direct NO synthesis and indirectly — through the secretion of cytokines which have ability to stimulate iNOS expression in cancer cells [32, 39]. The experiments demonstrated tumor-associated macrophage activation by cytokines (interleukin-1β, interferon-γ) and lipid A providing the enhanced production of endogenous NO [39]. Such achievement of radiosensitizing effect compared with the use of exogenous NO donors, which are administered systemically, becomes possible with extracellular concentrations of NO by 10–30 times lower [39]. Due to the severe toxic side effects (severe hypotension, neurotoxic effects, accumulation of toxic metabolites, including cyanides) after systemic administration of the therapeutically effective doses of NO donors *in vivo* [40], for clinical use it seems more reasonable to increase NO concentration directly in the tumor via activation of iNOS activity [5, 38, 39].

iNOS expression in tumors is activated by cytokines and lipopolysaccharides on the transcriptional level. In addition, interleukin-1β and interferon-γ accelerate intake of L-arginine by cells and increase the activity of arginine succinate liase that catalyzes resynthesis of L-arginine from L-citrulline [32, 35].

In contrast, there are a number of mechanisms that ensure the maintenance of low NO concentrations in tumor. Among the most effective are: synthesis of arginase-1 by the tumor-associated macrophages; capture of NO by erythrocytes and nitrosylation of glutathione and hemoglobin; inactivation of iNOS activity by hypoxic microenvironment [15].

There are 5 main factors that affect the rate of NOS-dependent synthesis NO: 1) the rate of transcription of genes responsible for synthesis of NOS; 2) mRNA-NOS maturation; export of mRNA-NOS into the cell loci of protein synthesis; 3) the content of NADPH, FAD, FMN and protoporphyrin IX in the cells that synthesize NO; 4) enzyme activity of synthesized NOS molecules and their chemical stability; 5) the concentration of L-arginine inside cells that synthesize NO.

Factors 1 and 2 affect the number of synthesized NOS molecules, but not their enzymatic activity. Factors 3, 4 and 5 are post-translational. Lowering pH in cytoplasm does not affect the number of molecules of mRNA-NOS and synthesis of NOS molecules in the cell but prevents NADPH participation in iNOS-dependent NO synthesis. On the one hand, tissue hypoxia slows down iNOS-dependent synthesis of NO from L-arginine and oxygen molecules because O_2 is one of the components of iNOS-dependent NO synthesis reaction. Excess of O_2 molecules (for example, with hyperbaric oxygenation) reduces the content of NO by the oxidation of NO to nitrites and nitrates [3, 15].

The studies of the mechanisms of synergistic action of radiotherapy and NO on cancer cells *in vivo* and *in vitro* demonstrated the importance of p53 activation [10, 38, 41]. It was established that in cancer cells p53 is activated due to the damage of DNA molecules by peroxynitrite [42]. In addition, it was found that sensitization to radiotherapy due to NO is largely implemented through the mechanism of S-nitrosylation of transcription factors or regulators of apoptosis [32]. The high expression of iNOS in human CRC cell lines enhances radiation-induced apoptosis through the caspase-dependent mechanism [43]. In the experiment on the model of metabolically-induced hypoxia it was shown that NO radiosensitizing effect in hypoxic cells is far more pronounced compared to the cells in normoxic conditions, while NO influence simulated the effect of oxygen [39]. Vasodilating effects of NO improving oxygenation of tissues may be another probable mechanism contributing to the increase of radiosensitivity in hypoxic areas of tumors [44].

The intravenous administration of NO biological synthesis precursor — L-arginine hydrochloride before sessions of radiotherapy in patients with rectal cancer promotes the synthesis of endogenous NO by iNOS expressed in neutrophils and activated (M1) macrophages in tumor tissue [8, 9]. Moreover, M1 macrophages, which are present in peritumorous inflammatory infiltrates, can provide radiosensitization of tumors through the secretion of proinflammatory cytokines, thus consequently increasing the expression and functional activity of iNOS in cancer cells [32, 39].

Another way to increase NO concentration in the tumor involves the modulation of polyamine metabolism. It was proved that reducing arginase activity by norarginin (inhibitor of arginase) creates favorable conditions for NOS [45].

The prognostic significance of iNOS expression in tumors is still unclear. Some authors indicate a high level of iNOS expression in colon cancer [46, 47]. A positive correlation between iNOS levels, vascular endothelial growth factor expression and the density of the microvascular vessels of the malignant tumors was found, besides iNOS expression was higher in metastatic tumors [48]. Gochman *et al*. [46] emphasize the relationship of iNOS expression in tumor with mechanisms of CRC metastasizing basedon the positive correlation between iNOS in the tumor tissue and the level of matrix metalloproteinase-2 in the intestinal wall [46].

Regarding the intratumoral distribution of iNOS, the majority of researchers point to the prevalence of iNOS expression in the stroma of tumors including macrophages [47, 49–51]. Some of researchers did not found iNOS expression in the cells of tumor parenchyma [49]. In addition, there are some data on relatively higher iNOS expression in the tumor parenchyma cells [46, 52]. However, these differences may be due to different methods used for preparation and staining of the samples [28].

The relationship between low level of iNOS and poor prognosis in CRC patients was reported in some studies [47, 49, 51], while other authors demonstrated an association between low survival rates of patients and high expression levels of iNOS in tumor tissue [46, 52]. In addition, high iNOS expression was related to hematogenous and lymphogenous metastasis [53, 54], low differentiation grade of tumors [55], increased depth of tumor invasion [55], vascular invasion and development of obstructive forms of CRC, which is known to be more common in tumors with infiltrative type of growth [54].

Recently, it was found that the expression of iNOS in tumor does not always correspond to NO production level, which obviously can affect the results of clinical studies on the prognostic value of iNOS expression in cancer patients [28].

CONCLUSION

Numerous studies demonstrated the functional dualism of NO in carcinogenesis and tumor progression. A wide range of biological effects of NO could be useful for the development of new schemes and methods of anticancer therapy based on the activation of endogenous NO biosynthesis pathways. To date, it has been proven *in vivo* and *in vitro* that NO, being a biologically active compound, can be successfully used as a therapeutic agent in cancer treatment.

REFERENCES

1. Blaise GA, Gauvin D, Gangal M, *et al.* Nitric oxide, cell signaling and cell death. Toxicology 2005; **208**: 177–92.

2. Mashyna SJu, Vanin AF, Serezhenkov VA. Identification and evaluation of NO depot in the body of the conscious rat. Bull Exp Biol 2003; **316**: 32–6 (in Russian).

3. Burlaka AP, Sidorik EP. Redox-dependent signal molecules in the mechanisms of tumor process. Naukova Dumka, 2014. 255 p. (in Russian).

4. Bonavida B. Nitric oxide and cancer: pathogenesis and therapy. Springer, 2015. 308 p.

5. Huerta S, Chilka S, Bonavida B. Nitric oxide donors: novel cancer therapeutics. Int J Oncol 2008; **33**: 909–27.

6. Cheng H, Wang L, Mollica M, *et al.* Nitric oxide in cancer metastasis. Cancer Lett 2014; **353**: 1–7.

7. Donia M, Maksimovic-Ivanic D, Mijatovic S, *et al.* *In vitro* and *in vivo* anticancer action of saquinavir-NO, a novel nitric oxide-derivative of the protease inhibitor saquinavir, on hormone resistant prostate cancer cells. Cell Cycle 2011; **10**: 492–9.

8. Golotiuk VV. Peculiarities of the distribution and dynamics of Bax apoptotic marker expression in patients with rectal cancer under the influence of neoadjuvant chemoradiotherapy with the use of nitrogen oxide precursor. Patologija 2015; **2**: 71–4 (in Ukrainian).

9. Golotiuk VV, Bagrij MM. Features of the distribution and dynamics of inducible nitric oxide synthase expression in patients with rectal cancer under the influence of neoadjuvant chemoradiation therapy and their relationship to the effectiveness of radiomodification with the use L-arginine. Morfologija 2016; **10**: 99–103 (in Ukrainian).

10. Mojic M, Mijatovic S, Maksimovic-Ivanic D, *et al.* Therapeutic potential of nitric oxide-modified drugs in colon cancer cells. Mol Pharmacol 2012; **82**: 700–10.

11. Aranda E, Lopez-Pedrera C, De La Haba-Rodriguez JR, *et al.* Nitric oxide and cancer: the emerging role of S nitrosylation. Curr Mol Med 2012; **12**: 50–67.

12. Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. Trends Mol Med 2009; **15**: 391–404.

13. Hess DT, Stamler JS. Regulation by S-nitrosylation of protein post-translational modification. J Biol Chem 2011; **287**: 4411–8.

14. Osborne NN. A hypothesis to suggest that light is a risk factor in glaucoma and mitochondrial optic neuropathies. Br J Ophthalmol 2006; **2**: 237–41.

15. Lechner M, Lirk P, Rieder J. Inducible nitric oxide synthase in tumor biology: the two sides of the same coin. Semin Cancer Biol 2005; **15**: 277–89.

16. Grimm EA, Sikora AG, Ekmekcioglu S. Molecular pathways: inflammation-associated nitric-oxide production as a cancer-supporting redox mechanism and a potential therapeutic target. Clin Cancer Res 2013; **19**: 5557–63.

17. Wink DA, Hines HB, Cheng RY, *et al.* Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol 2011; **89**: 873–91.

18. Choudhari SK, Chaudhary M, Bagde S, *et al.* Nitric oxide and cancer: a review. World J Surg Oncol 2013; **11**: 18–27.

19. Malakhov VO, Zavgorodnyaya GM, Licko VS, *et al*. The problem of nitric oxide in neurology. Sumy State Pedagogical University by A.S. Makarenko, 2009. 241 p. (in Russian).

20. Brown GC. Nitric oxide and mitochondria. Front Biosci 2007; **12**: 1024–33.

21. Erusalimsky JD, Moncada S. Nitric oxide and mitochondrial signaling: from physiology to pathophysiology. Arterioscler Thromb Vasc Biol 2007; **27**: 2524–31.

22. Giulivi C. Mitochondria as generators and targets of nitric oxide. Novartis Found Symp 2007; **287**: 92–104.

23. Lacza Z, Pankotai E, Csordás A, *et al.* Mitochondrial NO and reactive nitrogen species production: does mtNOS exist? Nitric Oxide 2006; **14**: 162–8.

24. Weigert A, Brune B. Nitric oxide, apoptosis and macrophage polarization during tumor progression. Nitric Oxide 2008; **19**: 95–102.

25. Burlaka AP, Vovk AV, Burlaka AA, *et al.* Rectal cancer: redox state of venous blood and tissues of blood vessels from electron paramagnetic resonance and its correlation with the five-year survival. Biomed Res Int 2018; **2018**: 4848652.

26. Domina EA, Mikhailenko VM, Pylypchuk EP. Influence of nitrogen oxide on formation of chromosomic instability in lymphocytes of blood human in conditions of co-mutagen modification. Science Rise 2015; **2/1**: 7–10 (in Ukrainian).

27. Mikhailenko VM, Diomina EA, Muzalov II, *et al.* Nitric oxide coordinates development of genomic instability in realization of combined effect with ionizing radiation. Exp Oncol 2013; **35**: 58–64.

28. Rahat MA, Hemmerlein B. Macrophage-tumor cell interactions regulate the function of nitric oxide. Front Physiol 2013; **4**: 144–5.

29. Mantovani A, Allavena P, Sica A, *et al.* Cancer-related inflammation. Nature 2008; **454**: 436–44.

30. Moeller BJ, Dewhirst MW. HIF-1 and tumour radiosensitivity. Br J Cancer 2006; **95**: 1–5.

31. Shinojima T, Oya M, Takayanagi A, *et al.* Renal cancer cells lacking hypoxia inducible factor (HIF)-1alpha expression maintain vascular endothelial growth factor expression through HIF-2alpha. Carcinogenesis 2007; **28**: 529–36.

32. Yasuda H. Solid tumor physiology and hypoxia-induced chemo/radio-resistance: Novel strategy for cancer therapy: nitric oxide donor as a therapeutic enhancer. Nitric Oxide 2008; **19**: 205–16.

33. Chowdhury R, Godoy LC, Thiantanawat A, *et al.* Nitric oxide produced endogenously is responsible for hypoxia-induced HIF-1alpha stabilization in colon carcinoma cells. Chem Res Toxicol 2012; **25**: 2194–202.

34. Fitzpatrick B, Mehibel M, Cowen RL, *et al.* INOS as a therapeutic target for treatment of human tumors. Nitric Oxide 2008; **19**: 217–24.

35. Jeannin JF, Leon L, Cortier M, *et al.* Nitric oxide-induced resistance or sensitization to death in tumor cells. Nitric Oxide 2008; **19**: 158–63.

36. Leon L, Jeannin JF, Bettaieb A. Post-translational modifications induced by nitric oxide (NO): implication in cancer cells apoptosis. Nitric Oxide 2008; **19**: 77–83.

37. Burlaka AP, Vovk AV, Ganusevych II, *et al.* Superoxide- and NO-dependent mechanisms of formation of metastatic microenvironment distant sites of metastasis in patients with colorectal cancer. Oncology 2017; **19**: 64–70 (in Ukrainian).

38. Singh S, Gupta AK. Mini review nitric oxide: role in tumour biology and iNOS/NO-based anticancer therapies. Cancer Chemother Pharmacol 2011; **67**: 1211–24.

39. De Ridder M, Verovski VN, Darville MI. Macrophages enhance the radiosensitizing activity of lipid A: a novel role for immune cells in tumor cell radioresponse. Int J Radiat Oncol Biol Phys 2004; **60**: 598–606.

40. Lee DH, Pfeifer GP. Mutagenesis induced by the nitric oxide donor sodium nitroprusside in mouse cells. Mutagenesis 2007; **22**: 63–7.

41. Cook T, Wang Z, Alber S. Nitric oxide and ionizing radiation synergistically promote apoptosis and growth inhibition of cancer by activating p53. Cancer Res 2004; **64**: 8015–21.

42. Schneiderhan N, Budde A, Zhang Y, *et al.* Nitric oxide induces phosphorylation of p53 and impairs nuclear export. Oncogene 2003; **22**: 2857–68.

43. Chung P, Cook T, Liu K, *et al.* Overexpression of the human inducible nitric oxide synthase gene enhances radia-

tion-induced apoptosis in colorectal cancer cells via a caspase-dependent mechanism. Nitric Oxide 2003; **8**: 119–26.

44. Jordan BF, Misson PD, Demeure R, *et al.* Changes in tumor oxygenation/perfusion induced by the no donor, isosorbide dinitrate, in comparison with carbogen: monitoring by EPR and MRI. Int J Radiat Oncol Biol Phys 2000; **48**: 565–70.

45. Yanish YuV, Artamonova AB, Zaletok SP. Impact of arginine and polyamines metabolism modulators on the arginaze activity and nitrogen oxides production by cells of Ehrlich carcinoma *in vivo*. Clin Oncol 2016; **2**: 1–3 (in Ukrainian).

46. Gochman E, Mahajna J, Shenzer P, *et al.* The expression of iNOS and nitrotyrosine in colitis and colon cancer in humans. Acta Histochem 2012; **114**: 827–35.

47. Ropponen KM, Kellokoski JK, Lipponen PK, *et al.* Expression of inducible nitric oxide synthase in colorectal cancer and its association with prognosis. Scand J Gastroenterol 2000; **11**: 1204–11.

48. Cianchi F, Cortesini C, Fantappie O, *et al.* Inducible nitric oxide synthase expression in human colorectal cancer: correlation with tumor angiogenesis. Am J Pathol 2003; **162**: 793–801.

49. Moochhala S, Chhatwal VJ, Chan ST, *et al.* Nitric oxide synthase activity and expression in human colorectal cancer. Carcinogenesis 1996; **17**: 1171–4.

50. Ambs S, Bennett WP, Merriam WG, *et al.* Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer. J Natl Cancer Inst 1999; **9**: 86–8.

51. Ohta T, Takahashi M, Ochiai A. Increased protein expression of both inducible nitricoxide synthase and cyclooxygenase-2 in human colon cancers. Cancer Lett 2006; **239**: 246–53.

52. Zafirellis K, Zachaki A, Agrogiannis G, *et al.* Inducible nitric oxide synthase expression and its prognostic significance in colorectal cancer. APMIS 2010; **118**: 115–24.

53. Lagares-Garcia JA, Moore RA, Collier B, *et al.* Nitric oxide synthase as a marker in colorectal carcinoma. Am Surg 2001; **67**: 709–13.

54. Nozoe T, Yasuda M, Honda M, *et al.* Immunohistochemical expression of cytokine induced nitric oxide synthase in colorectal carcinoma. Oncol Rep 2002; **9**: 521–4.

55. Yu Qin. The dynamic roles of angiopoietins in tumor angiogenesis. Future Oncol 2005; **1**: 475–84.