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SHORT COMMUNICATIONS

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HIGH FORMATION OF SUPEROXIDE ANION AND NITRIC OXIDE, AND MATRIX METALLOPROTEINASES ACTIVITY IN VASCULAR WALL OF RECTAL CARCINOMA VESSELS

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Aim: **To study the relationship between the level of generation of reactive oxygen species (ROS) and nitric oxide (NO) and activity of matrix metalloproteinases (MMPs) MMP-2 and MMP-9 in the vessels isolated from rectal tumors and *Arteria rectalis superior*. *Methods:* EPR at the room temperature and 77 °K, Spin Traps technology and zymography in polyacrylamide gels were applied. *Results:* In the vessels isolated from rectal tumors and *Arteria rectalis superior* high levels of ROS, $NO^{\cdot}$ and formation of complexes of $NO^{\cdot}$ with FeS-proteins at the sites of electron-transporting chain of mitochondria have been detected. High activities of MMP-2 and MMP-9 in vascular wall were also observed. The direct positive correlation between the rate of $NO^{\cdot}$ generation and formation of complexes of $NO^{\cdot}$ with FeS-proteins as well as between ROS and NO formation and MMPs activities have been revealed. *Conclusion:* Altered oxidative equilibrium in mitochondria of cells in vascular wall promotes formation of cell hypoxia and its autocatalytic potentiation accompanied with activation of MMPs.**

Key Words: **vascular wall, radical oxygen species, nitric oxide, matrix metalloproteinases-2 and -9.**

It is known that ROS and NO may activate directly and/or via signaling molecules the expression of a number of genes controlling the function of the vessels both at normal and pathologic states [1]. The angiogenesis is tightly linked to the development of tumor and malignant progression, in particular metastasis. It is clearly shown that tumor invasion as well as metastasis is accompanied with intra- and extravasation, and destruction of extracellular matrix by matrix metalloproteinases (MMPs), in particular, MMP-2 and MMP-9 [2, 3]. There is evidence that degradation and reorganization of the extracellular matrix are key events in vascular remodeling [4]. It was determined that both pro-MMP-2 and pro–MMP-9 secreted from human vascular smooth muscle cells are activated by ROS [5]. The activations of MMP-2 and MMP-9 in human gastric cancer by ROS as well as NO were observed [6]. Taking into account the above mentioned data this study was aimed to detect the levels of generation of ROS and NO and activities of MMP-2 and MMP-9 in vessels isolated from rectal tumor and adjacent normal tissue.

Twenty seven patients undergoing surgical resection of histologically proven rectal cancer ($T_3N_1M_0$, grade G2) were included into the trial. The patients were not treated previously. All patients underwent potentially curative resections. The vessels were obtained by means of preparation of freshly harvested tumor tissue with anatomical tools under magnification that allows to yield at least 100 mg of vascular materials. In addition, *a. rectalis superior* feeding the tumor node in the rectum was obtained by the resection of superampullar part of rectum on the distance of 4–5 cm from tumor boundary. The weight of obtained vascular specimen was approximately 500 mg. Moreover, the branches of *a. gastroepiploica sinistra* (at least 100 mg) were obtained by the operation of patients with gastric ulcer (n = 7). Local ethics committee approval was obtained and all patients gave informed consent.

Obtained vascular tissues were snap frozen in liquid nitrogen at the time of operation and stored at –70 °C. The superoxide radical-anions formation was assessed by the method of EPR with the use of 1-hydroxy-2,2,6,6-tetramethyl-4-oxopiperidin (Russia) and Spin Traps technology. The rates of superoxide radical-anions generation were calculated in nmol/g fresh tissue per min. The level of NO was determined using diethyldithiocarbomate spin trap (Sigma, USA) and EPR technology. The generation of NO was registered during 5 min followed by cessation of NO generation by liquid nitrogen (77 K). The levels of NO were calculated in nmol/g fresh tissue.

Activities of MMP-2 and MMP-9 were determined by zymography in 12% polyacrylamide gel with the addition of 0.1% of gelatin as substrate and using MMP-2 and MMP-9 standards (Sigma, USA) [7].

The results were statistically evaluated by computer programs Statistics 6.0 and Exel 2003 using parametric and correlation analysis.

In the vessels isolated from tumor tissue and in *a. rectalis superior*, there have been revealed elevated levels of generation rate of superoxide radical-anions compared to those in branches of a. gastroepiploica sinistra (Fig. 1). Both in the vascular tissue isolated from tumors and *a. rectalis superior*, activation of $NO^{\cdot}$ synthesis by NO-synthase systems, in particular, mNOS has been revealed. In the tumor vessels, $NO^{\cdot}$ levels were at the range of 3.75 nmol/g of fresh tissue, whilst in *a. rectalis superior* $NO^{\cdot}$ level yielded 3.01 nmol/g of fresh tissue (Fig. 2). These indices are significantly higher than those in *a. gastroepiploica sinistra* (Fig. 2). It has to be mentioned that significant

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Abbreviations used: NO – nitric oxide; ROS - radical oxygen species.

differences between the rate of superoxide radical-anions generation and NO levels in vessels feeding the region of gastric wall with ulcer lesion and those in vessels feeding the tumor tissue were determined. The specificity of increased formation of reactive oxygen and nitrogen species in tumor vessels is required to be confirmed in the special investigation with the extended spectra of vascular specimens.

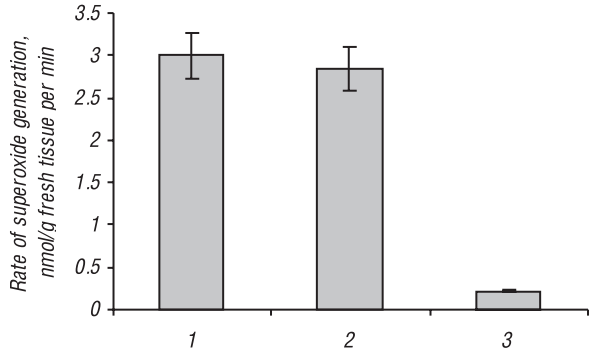

Fig. 1. The rate of superoxide radical-anions generation in the vessels isolated from tumors (*1*) and *A. rectalis superior* (*2*) compared with such in vessels isolated from stomach wall in patient with gastric ulcer (*3*)

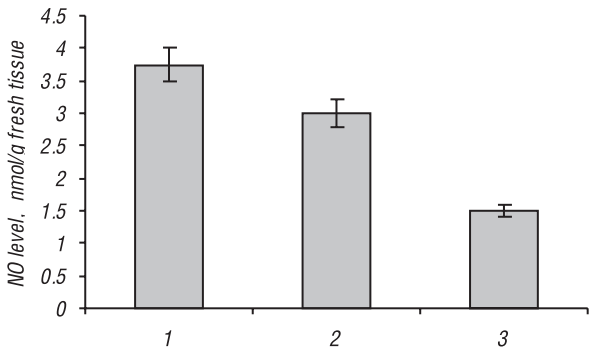

Fig. 2. The levels of NO in the vessels isolated from tumors (*1*) and *Arteria rectalis superior* (*2*) compared with such in vessels isolated from stomach wall in patient with gastric ulcer (*3*)

Intensity of EPR signal in the region of the factor of spectroscopic splitting, g = 2.03, on the EPR spectra of the vessels isolated from tumors differentiated from such obtained with *a. rectalis superior* (Fig. 3).

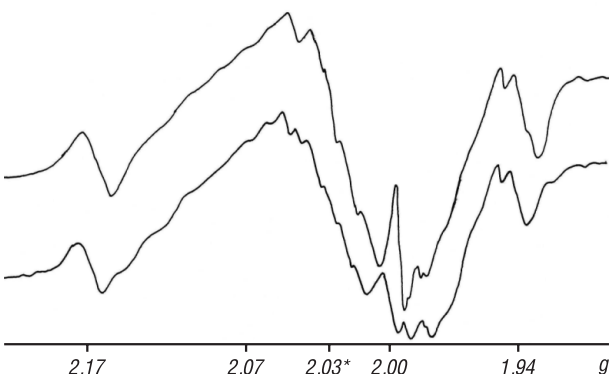

Fig. 3. EPR spectra of vessels isolated from tumor of rectum (*1*), and *Arteria rectalis superior* (*2*)
*g = 2.03 characterizes the level of generated $NO^{\cdot}$-FeS-proteins complexes in mitochondrial membranes.

As we have reported earlier [8], the intensity of this signal responds to the level of NO-FeS-protein complexes generated upon interaction of $NO^{\cdot}$ with FeS-proteins of respiratory chain of mitochondrial membranes, in particular with the proteins from N and S clusters in NADPH-ubiquinone-oxidoreductase and succinatede hydrogenase, respectively. In the tumoral vessels, high content of NO-FeS-protein complexes (1.8 ± 0.11 a. u) compared to such (0.58 ± 0.05 a. u) in *a. rectalis superior*) was revealed (Fig. 4). One should note that in normal state such complexes are generated at physiologic concentrations (0.15 ± 0.01 a. u) for regulation of the rate of electron flow through respiratory chain of mitochondria. High levels of the complexes of NO with FeS-proteins in mitochondria are considered as indexes of oxidative reactivity of the cells further expressed by NO effects in the formation of cell hypoxia and tumor progression. Mechanism of such disturbance upon chemical carcinogens and during tumor growth was described in our earlier investigations [6, 9].

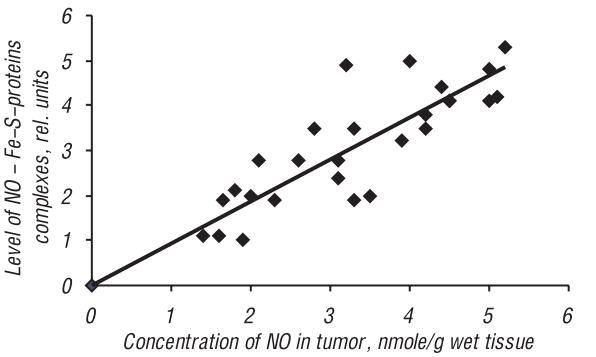

Fig. 4. Dependence between the level of generation of $NO^{\cdot}$-FeS-proteins complexes at the sites of respiratory chain of mitochondria of cellular elements of the tumoral vessels and concentration of $NO^{\cdot}$ synthesized in the vascular wall of rectal tumor vessels

Also, we have revealed the direct positive relation between generation of $NO^{\cdot}$ complexes with FeS-proteins at the sites of respiratory chain of mitochondria in the vessels of rectal tumors and concentration of $NO^{\cdot}$ produced in the tumor ($r = 0.84$; $p < 0.01$).

In the vessels isolated from tumor tissues and in *a. rectalis superior* high levels of MMP-2 and MMP-9 activities were observed (Fig. 5), especially of the last one that is considered characteristic for vascular endothelium and play a pivotal role in the processes related to the formation and function of vessels both at normal and pathological states. It is known that physiologic state of the vessels is characterized by very low (up to zero) activities of MMP-2 and MMP-9. Upon malignant progression activities of MMP-2 and MMP-9 are increased making the conditions for intra- and extravasation of tumor cells by the destruction of extracellular matrix that can results in the promotion of tumor invasion and metastasis [2, 3].

In the vessels of tumor and *a. rectalis superior* the direct positive correlations between the levels of ROS and $NO^{\cdot}$ generation and activities of MMP-2 and MMP-9 have been revealed ($r = 0.38$; $r = 0.42$; $r = 0.47$; $r = 0.63$; $p < 0.05$, respectively) that characterizes the regulatory influence of ROS and $NO^{\cdot}$ toward gelatinases via activation of latent forms of the enzymes [6].

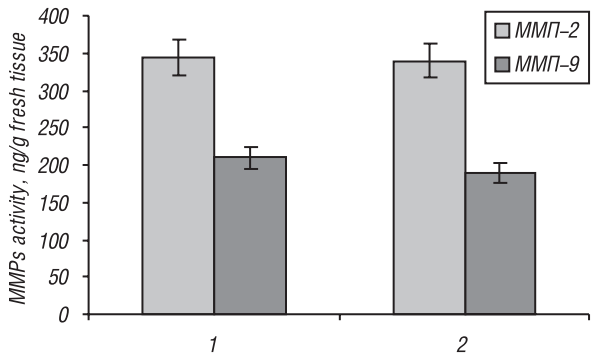

Fig. 5. Activities of matrix metalloproteinases in the vessels isolated from tumor (*1*) and in *a. rectialis superior* (*2*)

It is known that superoxide radical-anions produced by endothelial and vascular smooth muscle cells may activate specific signal transduction pathways and coordinate interrelated pathologic reactions in the vascular wall [10]. There are the data showing that the process of extravasation is potentiated simultaneously with an increase of $NO^{\cdot}$ level generated by human HT1080 fibrosarcoma cells [11].

In conclusion, taking into account the above mentioned data and the results of other authors [8, 12, 13] it may be supposed that high levels of ROS and NO generated in the vascular wall participate in the formation of cellular hypoxia and activation of matrix metalloproteinases in the cellular elements of tumor vessels. These events may contribute to the metastatic process simultaneously with those observed in tumor cells.

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ВЫСОКИЙ УРОВЕНЬ ОБРАЗОВАНИЯ СУПЕРОКСИДНОГО РАДИКАЛ-АНИОНА И ОКСИДА АЗОТА И АКТИВНОСТИ МАТРИКСНЫХ МЕТАЛЛОПРОТЕИНАЗ В СТЕНКЕ СОСУДОВ ОПУХОЛЕЙ ПРЯМОЙ КИШКИ

Цель: **изучить взаимоотношение между уровнями образования активных форм кислорода (АФК) и оксида азота (ОА) и активности матриксных металлопротеиназ (MMPs) MMP-2 и MMP-9 в сосудах, изолированных из опухолевой ткани прямой кишки, и в *Arteria rectalis superior*. *Методы:* ЭПР при комнатной температуре и при 77 °K, технология спиновых ловушек, зимография в полиакриламидном геле. *Результаты:* в сосудах, изолированных из опухолевой ткани прямой кишки, и в *Arteria rectalis superior* обнаружены высокие уровни образования АФК, $NO^{\cdot}$ и комплексов $NO^{\cdot}$ с FeS-белками в участках электронтранспортной цепи митохондрий. Определена также высокая активность MMP-2 и MMP-9 в стенке сосудов, находящихся в опухолевом узле. Обнаружена прямая положительная корреляция между величиной образования $NO^{\cdot}$ и образованием комплексов $NO^{\cdot}$ с FeS-белками, а также между образованием АФК и NO и активностью MMPs. *Выводы:* измененное окислительное равновесие в митохондриях клеток стенки сосудов, находящихся в опухолевой ткани, усиливает формирование в них клеточной гипоксии, ее аутокаталитическое потенцирование, что сопровождается активацией в этих клетках MMPs.**

Ключевые слова: **сосудистая стенка, активные формы кислорода, оксид азота, матриксные металлопротеиназы-2 и -9.**