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BIOENERGETIC STATUS AND HYPOXIA IN LEWIS LUNG CARCINOMA ASSESSED BY ^{31}P NMR SPECTROSCOPY: CORRELATION WITH TUMOR PROGRESSION

L. Bubnovskaya, V. Mikhailenko, A. Kovelskaya, S. Osinsky*
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Kyiv, Ukraine

Aim: **To evaluate the hypoxia level and some indices of Lewis lung carcinoma energy metabolism by means of ^{31}P NMR spectroscopy in perchloric acid (PCA) tissue extracts during growth of primary tumor and metastasis.** ***Materials and Methods:*** **C57Bl/6 mice-bearing Lewis lung carcinoma were used in this study. Tumor energy metabolism was studied by ^{31}P NMR spectroscopy and the metabolic NMR ratios were used as parameters for metabolic status and hypoxia level.** ***Results:*** **It was shown that growth of primary tumor is accompanied with increase of Pi/PCr, Pi/βNTP and PME/βNTP ratios that reflect drop of tumor energy status and oxygenation level in tumor tissue. These changes in relevant metabolic ratios correlate with enlargement of primary tumor volume ($r = 0.87$, $p = 0.0045$; $r = 0.90$, $p = 0.0012$; $r = 0.764$, $p = 0.05$, respectively) as well as with the number of lung metastases ($r_s = 0.761$, $p = 0.028$; $r_s = 0.86$, $p = 0.0049$; $r_s = 0.77$, $p = 0.040$, respectively).** ***Conclusion:*** **In present study it was shown that ^{31}P NMR spectroscopy of PCA tumor tissue extracts may be used as reliable method for the assessment of the level of oxygenation as well as changes in energy metabolism in the experimental tumors. It may be helpful to evaluate the energy status of human tumors by investigation both of biopsy and surgical specimens. Hypoxia and hypoxia-associated metabolic events in primary tumor are linked with malignant progression, in particular metastasis.**
Key Words: **NMR spectroscopy, bioenergetic, hypoxia, metastasis, Lewis lung carcinoma.**

In the majority of malignant tumors uncontrolled microvascularity as well as low oxygen tension are the most peculiar features of tumor microenvironment that play a crucial role in malignant progression. It has been confirmed by experimental and clinical studies, that such "abnormal" microenvironment can promote the development of metastatic disease [1, 2]. In various solid tumors hypoxia status may provide a physiological pressure in tumors selecting for metastatic cell phenotypes [3].

Hypoxia has been studied extensively and assessed by a number of different techniques that are direct or indirect. The measurement of tumor oxygenation using Eppendorf oxygen-sensitive needle electrodes is often considered as the "gold standard" but it is not that easy to use [4]. Moreover, it does not always give "true" hypoxia values and is limited to accessible tumors that are suitable for electrode insertion.

Indirect measurements, where a reporter of oxygen level is the endpoint, are usually inverse, i.e. provide positive signal in the absence of oxygen. Indirect hypoxia markers can be endogenous or exogenous [5]. A registration of the distribution of hypoxia at the microregional level is obtained by immunohistochemical detection of exogenous or endogenous hypoxia markers. Endogenous hypoxia markers are genes or gene products that are specifically up-regulated under hypoxic conditions. The well-known angiogenic factor VEGF, glucose transporters Glut-1 and Glut-3, carbonic anhydrase-9 (CA9) as well intrinsic marker as HIF-1α are the most promising. They have the potential advantage that patients would only require a biopsy for their detection. However at present it appears that the endogenous markers are not solely regulated by tissue oxygenation [6]. These markers may have prognostic potential but their value for selection of local treatment strategies for individual patients may be limited and the majority of these assays are not ready for clinical application.

Exogenous hypoxia markers are drugs or chemicals that, after administration, specifically accumulate and/or are reduced under hypoxia conditions. Clinically relevant hypoxia-detecting exogenous markers are the 2-nitroimidazole agents, in particular pimonidazole [5]. A disadvantage of exogenous markers is that they require intravenous administration before surgical biopsy taking. They are seen very attractive but application to clinical material appears still questionable [6].

Another technique to assess tissue oxygenation is nuclear magnetic resonance spectroscopy (MRS) that has been used to study aspects of tumor metabolism both *in vitro* and *in vivo* [7–10]. In spite of the MRS method does not provide a direct measure of oxygenation and is considered as indirect tool for assessment of hypoxia extent, it bases on examination of endogenous signals. The advantage of MRS technique is the possibility to evaluate some other metabolic processes in tumor tissue simultaneously and to receive full chemical analysis of all cell phosphate-containing compounds.

Our study was aimed to employ the NMR spectroscopy in perchloric extracts of tumor tissue as reliable method to evaluate the level of tumor oxygenation and control its changes as a function of time after tumor transplantation, and also to determine relevant ^{31}P NMR metabolic parameters closely connected with tumor hypoxia and metastasis.

MATERIALS AND METHODS

Experimental animals. All studies were conducted with strain IEPOR bred mice C57Bl/6 (R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine) weighting 22–25 g and bearing Lewis lung carcinoma (3LL) transplanted intramuscularly (0.5 x

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*Correspondence: E-mail: osion@onconet.kiev.ua
Abbreviations used: 3LL – Lewis lung carcinoma, NMR – nuclear magnetic resonance.

10^6 cells/mice) into right flank. The principles and method of transplantation were conventional. Animals were kept in Makrolon cages bedded with dust free wood granules, and had free access to a standard diet and tap water. All experiments had been approved by the regional animal ethic committee.

On the 12th day after tumor transplantation (tumor volume was approximately 150–200 mm^3) experiment was started and then samples were examined on every other day up to 24th day (in some cases up to 27th day) when tumor volume reached 1.7–2.3 $см^3$ and metastasis accrued. The tumor with a large volume was used as a model for tumor with high level of hypoxia. Taking into account that large tumors have necrotic areas in the center of tumor node we excised the central necrotic mass before tissue processing. Tumors of the same small volumes from three to five animals were combined to obtain one spectrum. From 5 to 7 samples were prepared for each time point. Simultaneously samples of muscle tissue were prepared at the same manner. Total number of mice used in this investigation was 195.

Mice were anaesthetized with sodium pentobarbital (45 mg/kg, i. p.) and were kept at normal body core temperature. It was shown that such condition was absolutely necessary to obtain reproducible spectra. When the body core temperature was controlled the anesthesia itself was found to have insignificant effects, if any at all, on the NMR spectra [11].

NMR protocol. ^{31}P NMR spectra (121,5 MHz) of perchloric acid (PCA) tissue extracts were acquired using a high-resolution Mercury-300BB Spectrometer (Varian, США), equipped by Sparc station 4, and a probe of 5 mm inner diameter.

Data were obtained under fully relaxed conditions, proton decoupling and a spectrum width of 10,000 Hz, 16K data points, a 90° pulse applied with 8 s recycling delay. As a standard methylenediphosphonic acid, trisodium salt (Sigma, USA) was used. Under these condition the resonance of phosphocreatine was set at 0.00 p. pm. Areas of the spectral signals were measured by the integration mode of the spectrometer.

Perchloric acid (PCA) extraction. For PCA tissue extraction both tumor and hip muscle were excised and immediately frozen in liquid nitrogen. The frozen tissues were pulverized, mixed with cold 1.2 N PCA and PCA-tissue suspension was allowed to thaw up to 10 min. Then chilled distilled deionized water was added and kept on ice with continuous stirring 20 min prior to centrifugation (9,000 rpm) to remove cellular debris. Supernatant was adjusted to pH 7.5–8.0 with 5 N KOH. Insoluble $KClO_4$ was removed by centrifugation. To remove divalent ions Chelex 100 (10 mg/5 ml) (Sigma, USA) was added to supernatant. Samples were clarified by filtration, lyophilized to dryness and kept at (–20 °C). Before NMR recording the lyophilized samples were dissolved in 1.0 ml of D_2O, centrifuged again and transferred into the NMR tubes.

The assignment of resonances of the compounds on ^{31}P NMR spectra of 3LL tumor tissue were as follows: PME — phosphomonoesters, mainly phosphoethanolamine (PE) and phosphocholine (PC), and including sugar phosphates (e. g. glycolytic intermediates and adenosinemonophosphate — AMP); Pi; PDE — phosphodiesters, mainly glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE); PCr — phosphocreatine; γ-NTP and β -NTP — nucleoside triphosphate and diphosphate; α-NTP and α-NDP together with various other compounds such as NADPH/NADP+ and NADH/NAD+; DPDE — diphosphodiesters, possibly uridin diphosphate glucose (UDPG); and β-NTP are in accordance with literature data. The β-phosphate resonance of NTP was used to monitor NTP levels [7].

Statistical analysis. All data are presented as the mean ± SE,using a two-tailed test with $p < 0.05$ for significance. For correlation analysis Pearson r and Spearman' rho coefficients were used.

RESULTS

The results of experiments show clear evidence that during growth 3LL tumors showed characteristic changes in the phosphate compound resonance intensities, i. e. a progressive loss of energy-rich compounds PCr and βNTP, with increasing Pi and PME that are probably caused partly by increase in metabolically active hypoxic cell fraction (Figure). Although the bioenergetic status of the aerobic cells in tumors at any time is very close to a steady state this near-steady state may change gradually during tumor growth due to cooperative biochemical interactions between the aerobic and the metabolically depleted hypoxic cells and this could also have contributed significantly to the volume dependence of the ^{31}P NMR spectral parameters [11]. At small tumor volume (150–250 mm^3) there was seen full profile of ^{31}P NMR spectra of tumor tissue but as tumor volume enlarged up to 1.2–2.3 cm^3 (20–24th days after tumor transplantation) it was registered gradual significant decline both of PCr and βNTP signals almost up to immeasurable values (23 and 15% of investigated tumor samples, respectively) what accompanied with increases of PME and Pi signals by a factor of 1.3 ($p = 0.046$) and 2.2 ($p = 0.037$) respectively.

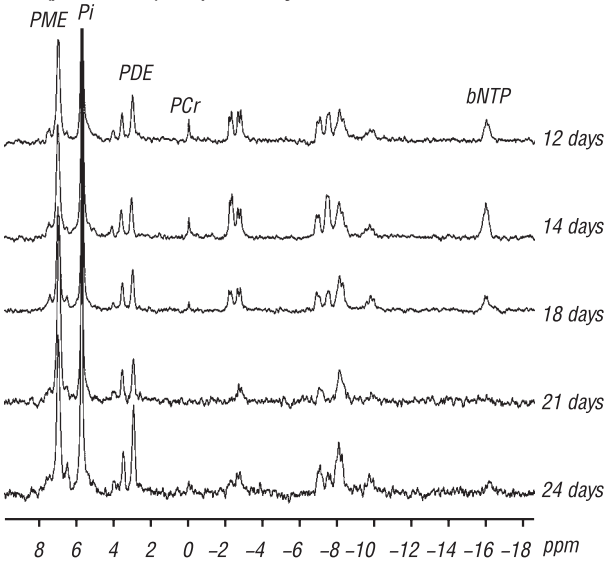

Figure. ^{31}P NMR spectra of 3LL tumor perchloric acid extracts with peak assignments and chemical shifts at different time intervals (days) after tumor transplantation

Increases in intracellular PME pool were hypothesized to be associated with enhanced cell membrane synthesis, cellular growth and cell nutritional state [12].

The PDE peak mainly corresponds to GPC and GPE both membrane phospholipid decomposition products. Therefore the PME/PDE ratio may reflect membrane phospholipid turnover and repair processes which can take place in the recovering tumor cells [13–15]. Since no significant changes in this ratio was seen with tumor volume increase it was concluded that no substantial variation in the balance of membrane building blocks and degradation products occurred during tumor growth (mean PME/PDE ratio = 2.4 ± 0.21).

The bioenergetic status parameters have been derived from NMR measurements, the most common and probably most reliable of which are the Pi/PCr, Pi/βNTP, PME/βNTP rtatios [8]. These ratios are all measures of tumor metabolic and physiological state. It is known that there is a strong relationship between tumor bioenergetic status and oxygenation. The increases of these ratios reflect metabolic decline and rise of hypoxia level because there is cellular metabolic adaptation to the reduction of oxygen delivery.

From the very beginning of experiment and up to 24 day of tumor growth the Pi/PCr and Pi/βNTP ratios increased by a factor of 2.8 and 2.4, respectively, that testifies the substantial growth of the tumor hypoxia level and decline of tumor bioenergetic status (Table 1).

Table 1. Relevant metabolic ^{31}P NMR ratios in Lewis lung carcinoma at certain intervals after tumor transplantation

Ratio	Time after tumor transplantation (day)			
	12–14 (180–460)*	16–118 (640–960)*	20–122 (1170–1700)*	24–27 (1900–2300)*
Pi/PCr	6.2 ± 1.0	10.2 ± 0.8	17.5 ± 1.5	23.5 ± 2.7
Pi/βNTP	3.5 ± 0.6	4.8 ± 1.2	8.5 ± 0.4	15.0 ± 1.85
PME/βNTP	2.8 ± 0.3	3.9 ± 0.7	6.1 ± 1.1	9.9 ± 1.3
PME/Pi	0.87 ± 0.06	0.91 ± 0.03	0.67 ± 0.16	0.49 ± 0.07

*Primary tumor volume in parenthesis (mm^3).

Take into account that signals of PCr as well as βNTP derived from tissue of large tumor are not often seen on many spectra it is not possible to assess the state of hypoxia level in all tumor samples that were examined by means of these ratios. Therefore in the absence of a detectable PCr signal in the spectrum the ratio of PME/βNTP can be used as an alternative reliable ^{31}P NMR energy parameter that also elevates upon deterioration of tissue energy status and therefore may reflects the metabolic hypoxia level. During tumor growth twofold increase of this ratio was found up to 24th day of tumor growth compare with the initial value at the beginning of experiment. Changes in the Pi/βNTP as well as in the PME/βNTP paralleled the increase the Pi/PCr.

In the situation when both PCr and βNTP signals are not detactable in a spectrum that is the most characteristic feature of advanced tumor the ratio PME/Pi is very useful for tumor tissue spectrum analysis. It is very sensitive to changes in tissue oxygenation and there is highly significant linear correlation ($p < 0.001$) between the mean tissue pO_2 value and the PME/Pi ratio [7]. In small tumors (the 12th day after tumor transplantation) with perhaps still adequate O_2 availability the PME/Pi ratio was approximately 0.87 ± 0.06. During the further tumor growth with deteriorating oxygenation status, the PME/Pi ratio became smaller and smaller from the very beginning of the experiment with an average 32% drop up to 24th day.

Relationships between ^{31}P NMR ratios and primary tumor size as well as the number of lung metastases are presented in Table 2. All correlations have been provided using data obtained from the beginning of experiment up to 24 day after tumor transplantation.

Table 2. Correlations between tumor metabolic ^{31}P NMR ratios and both primary tumor size and number of lung metastases

	Pi/PCr	Pi/βNTP	PME/βNTP	Primary tumor volume (mm^3)
Primary tumor volume (mm^3)	r = 0.87, p = 0.0045	r = 0.90, p = 0.0012	r = 0.764, p = 0.05	
Number of lung metastases	r_s = 0.761, p = 0.028	r_s = 0.86, p = 0.0049	r_s = 0.77, p = 0.040	r_s = 0.915, p = 0.001

Some of these correlations were not seen consistently in a very late stage of tumor growth (24–27th day after tumor transplantation) that may be explained by the more substantial score of necrosis in advanced tumor. The attention should be paid to the changes observed in most of tumor tissue samples 16–18th day after transplantation. Decline of such ratios as Pi/βNTP and PME/βNTP (by a factor of 1.4 in both cases) was registered (not reflected in Table 1) in spite of continuous enlargement of tumor size, that may be explained by some enhancement of tumor energy status. Perhaps during this term after tumor transplantation there is a key point connected with the beginning of active metastasis and further maintenance of this process needs additional energy [16]. To reveal such metabolic processes is very difficult and needs special investigation.

^{31}P NMR spectra of muscle of untreated animals represent spectra of normal tissue of tumor-bearing animals and simultaneous they served to control the procedure of PCA extraction for each tumor tissue sample as they represent "classic" ^{31}P NMR spectra. Spectra of muscle and tumor tissues are significantly different. There is a sharp high peak of PCr to compare with Pi, rather high βNTP resonance and no detectable PDE signals on the ^{31}P NMR spectra of muscle in comparison with spectra of tumor tissue.

The Pi/PCr, Pi/βNTP and PME/βNTP ratios were significantly lower in muscle in comparison with those in tumor. We did not observe any naturally occurring fluctuations in oxygenation level in muscle from the beginning of investigation up to day 24th. Then these ratios gradually increased by a factor of 1.25, 1.3 and 1.15 respectively up to the 31th day (only for muscle). Simultaneously, the PME/Pi ratio decreased by a factor of 1.2. Such non significant but still observed worse oxygenation in muscle may be possibly explained by physiological pressure of advanced tumor on a late stage of its growth.

DISCUSSION

One of the most typical feature of solid tumor, as it is well known, is the high level of hypoxia, and this phenomenon reflects one of the fundamental differences between malignant tumor and normal tissues [1, 2]. Recent results of majority of experimental and

clinical studies designated hypoxia as a particular powerful factor that impact on tumor progression, especially metastasis [17–19]. The hypoxic status of various solid tumors is related to a poor prognosis, for example, clinical studies of head and neck tumors, cervix cancer and soft tissue sarcoma demonstrated correlations between high level of tumor hypoxia and poor overall survival [5, 14, 20].

The point of view is discussed that selection of patients based on estimation of tumor hypoxia is relevant for the optimal use of anticancer therapy, in particular radiation [21]. Taking into account the above mentioned it is possible to suggest expediently exploitation the data about tumor hypoxia level in the monitoring of patients. Various methods have been developed for assessing tumor hypoxia in tumor models and in patients [4, 5]. A number of such methods are currently applied in clinical practice, in particular measurement of tissue oxygen pressure by polarographic oxygen electrode, immunohistochemical detection of expression of pimonidazole metabolites, CA9, Glut1, HIF-1α and other hypoxia-associated substances, but examine more specific and clinically informative methods to assess tumor hypoxia remains actual. It has to be noted that most methods allow assessment of tumor hypoxia only at single time point and only few ones can be used for serial measurements of tumor hypoxia [22]. They include various oxygen sensing devices and hypoxia imaging techniques, including magnetic resonance imaging (MRI). NMR spectroscopy is very informative method that has been applied comparatively long ago to evaluate tumor energy metabolism and oxygenation [9, 10, 12]. Previous studies have indicated a relation between tumor oxygenation and metabolic status. Okunieff et al. [23] noted that the increase in the hypoxic fraction occurs in parallel to the decrease in high-energy phosphates. Rofstad et al. [11] observed a relationship between high energy phosphates and tumor oxygenation. Vaupel et al. [7] determined a significant correlation between the partial pressure of oxygen (measured by oxygen electrode) and NMR-measured metabolic ratios, and concluded that NMR spectroscopy could be used for detection of the changes in tumor energy status induced by changes in tumor oxygenation. In the study of Fu et al. [8] significant correlations between hypoxic fraction and some metabolite ratios assesses by ^{31}P NMR spectroscopy were detected. The changes in ^{31}P NMR signal ratios such as Pi/PCr, Pi/βNTP, Pi/(PCr+βNTP), or related forms, have been reported in numerous studies suggesting that spectral examination of tumors may provide a method to measure chronic oxygenation status [24–26].

Most studies for assessment of tumor bioenergetic status as well as oxygenation are performed with NMR spectroscopy in vivo but it has to be noted that this technique is limited by anatomical accessibility of tumors. At the same time NMR spectroscopy of PCA tissue extracts can be used to examine biopsy and surgical specimens. By this it appears the possibility to perform the original chemical analysis of tumor tissue by means of MRS and detect both semi-quantitatively and quantitatively the number of relevant metabolic indexes of the given tissue. It is important that multiple tissue samples from the same region may be also analyzed histologically, immuhistochemically and biochemically to receive the full biological profile of studied tumors.

We tried to evaluate the level of tumor hypoxia by means of NMR spectroscopy of PCA tissue extracts of experimental tumors in order to propose to use this technique for hypoxia level assessment of human malignancies. The obtained results may be assessed as a clear evidence of close relationship between tumor energy status and tumor growth, between relevant metabolic parameters and metastasis. Our data confirmed that ^{31}P NMR spectroscopy of PCA tumor tissue extracts is very informative for assessment the hypoxia levels in tumor specimens and may be extended for clinical application to evaluate the state of tumor energy metabolism within biopsy as well as surgical speciment. Naturally, the comparative analysis of hypoxia parameters received with NMR spectroscopy of PCA tumor tissue extracts with those obtained with oxygen pressure measurement and/or immunohistochemical detection of endo- and exogenous hypoxic markers expression seem relevant. Moreover, it will be informative to compare parameters of tumor energy status obtained with NMR spectroscopy of PCA tumor tissue extracts and MRI that could allow to extend the application of this technology in clinical use. Our preliminary results obtained in clinical study have shown that the PME/Pi ratio in gastric cancer is correlated with expression of HIF-1α in tumor tissue and overall survival of patients [27].

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REFERENCES:

1. **Le Q-T, Denko NC, Giaccia AJ.** Hypoxic gene expression and metastasis. Cancer Met Rev 2004; **23**: 293–310.

2. **Rofstad EK, Galappathi K, Mathiesen B, Ruud E-BM.** Fluctuating and diffusion-limited hypoxia in hypoxia-induced metastasis. Clin Cancer Res 2007; **13**: 1971–8.

3. **Ciaccia AJ, Koumenis C, Denko N.** The influence of tumor hypoxia on malignant progression. In: P Vaupel, D Kelleher (eds), Tumor Hypoxia: pathophysiology, clinical significance and therapeutic perspectives. Stuttgart, Wissenschaftliche Verlagsgesellschaft: 1999: 115–24.

4. **Eriksen JG, Horsman MR.** Tumor hypoxia – a characteristic feature with a complex molecular background. Radiother Oncol 2006; **81**: 119–21.

5. **Evans SM, Koch CJ.** Prognostic significance of tumor oxygenation in humans. Cancer Lett 2003; **195**: 1–16.

6. **Bussink J, Kaanders JHAM, van der Kogel AJ.** Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. Radiother Oncol 2003; **67**: 3–15.

7. **Vaupel P, Okunieff P, Kallinowski F, Neuringer LJ.** Correlations between ^{31}P-NMR spectroscopy and tissue O_2 tension measurements in a murine fibrosarcoma. Radiat Res 1989; **120**: 477–93.

8. **Fu KK, Wendland MF, Iver SB, Lam KN, Engeseth H, James TL.** Correlations between in vivo ^{31}P NMR spectroscopy

measurements, tumor size, hypoxic fraction and cell survival after radiotherapy. Int J Radiat Oncol Biol Phys 1990; **18**: 1341–50.

9. **Steen RG.** Characterization of tumor hypoxia by ^{31}P MR Spectroscopy. Amer J Roentgenol 1991; **157**: 243–8.

10. **Evolhoch J L, Gillies RJ, Karczmar GS, Koutcher JA, Maxwell RJ, Nalciouglu O, Raghunand N, Ronen SM, Ross BD, Swartz HM.** Application of Magnetic Resonance in model systems: cancer therapeutics. Neoplasia 2000; **2**: 152–65.

11. **Rofstad EK, DeMuth P, Fenton BM, Sutherland RM.** ^{31}P magnetic resonance spectroscopy studies of tumor energy metabolism and its relationship to intracapillary oxyhemoglobin saturation status and tumor hypoxia. Cancer Res 1988; **48**: 5440–6.

12. **Podo F.** Tumor phospholipid metabolism. NMR Biomed 1999; **12**: 413–39.

13. **Merchant TE, Kasimos JN, Vroom T, de Bree E, Iwata JL, de Graaf PW, Glonek T.** Malignant breast tumor phospholipid profiles using 31P magnetic resonance. Cancer Lett 2002; **176**: 159–67.

14. **Dewhirst MW, Poulson JM, Yu D, Sanders L, Lora-Michiels M, Vujaskovic Z, Jones EL, Samulski TV, Powers BE, Brizel DM, Prosnitz LR, Charles HC.** Relation between pO_2, ^{31}P magnetic resonance spectroscopy parameters and treatment outcome in patients with high-grade soft tissue sarcomas treated with thermoradiotherapy. Int J Radiat Oncol Biol Phys 2005; **61**: 480–91.

15. **Vaupel P, Okunieff P, Neuringer LJ.** *In vivo* ^{31}P-NMR spectroscopy of murine tumors before and after localized hyperthermia. Int J Hyperthermia 1990; **6**: 15–31.

16. **Osinsky SP, Ganusevich II, Bubnovskaya LN, Valkovskaya NV, Kovelskaya AV, Sergienko TK, Zimina SV.** Hypoxia level and matrix metalloproteinases-2 and -9 activity in Lewis lung carcinoma: correlation with metastasis. Exp Oncol 2005; **27**: 202–5.

17. **Zhou J, Schmidt T, Schnitzer S, Bruene B.** Tumor hypoxia and cancer progression. Cancer Lett 2006; **237**: 10–21.

18. **Rofstad EK, Galappathi K, Mathiesen B, Ruud E-BM.** Fluctuating and diffusion-limited hypoxia in hypoxia-induced metastasis. Clin Cancer Res 2007; **13**: 1971–8.

19. **Chaudary N, Hill RP.** Hypoxia and metastasis. Clin Cancer Res 2007; **13**: 1947–9.

20. **Wouters BG, Weppler SA, Koritzinsky M, Landuyt W, Nuyts S, Theys J, Chiu RK, Lambin P.** Hypoxia as a target for combined modality treatments. Eur J Cancer 2002; **38**: 240–57.

21. **Lukacova S, Overgaard J, Alsner J, Horsman MR.** Strain and tumor specific variations in the effect of hypoxia on osteopontin levels in experimental models. Radiother Oncol 2006; **80**: 165–71.

22. **Nelson DW, Cao H, Zhu Y, Sunar-Redder B, Choi CYH, Faix JD, Brown JM, Koong AC, Giaccia AJ, Le Q-T.** A noninvasive approach for assessing tumor hypoxia in xenografts: developing a urinary marker for hypoxia. Cancer Res 2005; **65**: 6151–8.

23. **Okunieff PG, Koutcher JA, Gerweck L, McFarland E, Hitzig B, Urano M, Bradly T, Neuringer L, Suit HD.** Tumor size dependent changes in a murine fibrosarcoma: use of *in vivo* ^{31}P NMR for non-invasive evaluation of tumor metabolic status. Int J Radiat Oncol Biol Phys 1986; **12**: 793–9.

24. **Toxer GM, Griffiths JR.** The contribution made by cell death and oxygenation to ^{31}P MRS observation of tumor energy metabolism. NMR Biomed 1992; **5**: 279–89.

25. **Vaupel P, Hoeckel M.** Oxygenation of human tumors. In: Blood perfusion and microenvironment of human tumors. Molls M, Vaupel P, eds. Berlin-Heidelberg, Springer Verlag 1998, pp. 63–72.

26. **Olsen DR, Rofstad EK.** Monitoring of tumor reoxygenation following irradiation by ^{31}P magnetic resonance spectroscopy: an experimental study of human melanoma xenografts. Radiother Oncol 1999; **52**: 261–7.

27. **Osinsky S, Gluzman D, Olijnichenko G, Bubnovskaya L, Ganusevich I, Osinsky D, Merentsev S, Gumenjuk L.** Hypoxia-regulated proteins in gastric cancer: correlation with disseminated tumor cells and clinical outcome. Eur J Cancer Suppl 2006; **4**: 49 (P60).

БИОЭНЕРГЕТИЧЕСКИЙ СТАТУС И ГИПОКСИЯ В КАРЦИНОМЕ ЛЕГКОГО ЛЬЮИС, ОПРЕДЕЛЕННЫЕ С ПОМОЩЬЮ ^{31}P ЯМР СПЕКТРОСКОПИИ: КОРРЕЛЯЦИЯ С ОПУХОЛЕВОЙ ПРОГРЕССИЕЙ

Цель: **определить уровень гипоксии и некоторые показатели энергетического метаболизма карциномы легкого Льюис с помощью ^{31}P ЯМР-спектроскопии перхлорных (PCA) экстрактов ткани в процессе роста первичной опухоли и ее метастазировании.** ***Материалы и методы:*** **использовали в исследовании мышей C57Bl/6 с перевивной карциномой легкого Льюис. Энергетический метаболизм опухоли изучали с помощью ЯМР спектроскопии, отношения фосфорсодержащих метаболитов использовали в качестве параметров метаболического статуса и уровня гипоксии.** ***Результаты:*** **установлено, что рост первичной опухоли сопровождается увеличением соотношений Pi/PCr, Pi/βNTP and PME/βNTP в опухоли, что отражает снижение уровня биоэнергетического статуса и уровня оксигенации в опухоли. Эти изменения соотношений важных метаболических показателей коррелируют с увеличением объема первичной опухоли ($r = 0,87$, $p = 0,0045$; $r = 0,90$, $p = 0,0012$; $r = 0,764$, $p = 0,05$ соответственно) и количеством метатазов в легких ($r_s = 0,761$, $p = 0,028$; $r_s = 0,86$, $p = 0,0049$; $r_s = 0,77$, $p = 0,040$ соответственно).** ***Выводы:*** **установлено, что ^{31}P ЯМР-спектроскопия PCA тканевых экстрактов может быть надежным методом оценки уровня оксигенации и биоэнергетического статуса экспериментальных опухолей. Этот метод может быть полезным при оценке энергетического метаболизма опухолей человека, в частности при исследовании биопсийного и операционного материала. Гипоксия и гипоксия-ассоциированные изменения метаболизма в первичной опухоли связаны со злокачественной прогрессией, в частности с метастазированием.**

Key Words: **ЯМР-спектроскопия, биоэнергетика, гипоксия, метастазирование, карцинома легкого Льюис.**