# Oxidative Destruction of Mitochondrial Translation Products after Fractionated Irradiation of Rats with Tumor

**M. M. Marchenko, G. P. Kopyl'chuk, and O. M. Voloshchuk**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 10, pp. 401-403, October, 2007
Original article submitted March 2, 2006

We demonstrated specific features of oxidative damage to mitochondrial translation products in animals were revealed after fractionated X-ray exposure in low doses. Differences were found in carbonylation and oxidation of SH groups in irradiated rats and irradiated tumor-bearing animals. Our results indicate that preliminary fractionated X-ray exposure determines oxidative damage to these mitochondrial proteins only at the initial stage of the study, while in the follow-up period major role is played the by tumor development.

**Key Words:** *mitochondrial translation products; oxidative modification; Guerin carcinoma*

Uncontrolled generation of reactive oxygen species (ROS) is mainly accompanied by oxidative modification of proteins (OMP), which results in impairment of their biological activity [3,9]. Oxidative destruction of mitochondrial translation products whose synthesis determines function of the energy supply system can be followed by irreversible changes in the respiratory chain. It is important to study OMP of mitochondrial translation products in malignant growth after fractionated irradiation with low doses. The knowledge of this process will allow us to understand possible mechanisms of dysfunction of mitochondrial energetics during carcinogenesis.

## MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 110-130 g. The animals were divided into groups. Group 1 included intact rats. Group 2 consisted of irradiated rats. Guerin carcinoma was transplanted to irradiated rats of group 3 on day 1 after irradiation. Irradiation was performed for 7 days (total dose 25.3 mC/kg). Irradiation was performed on a Lachema 12P6 X-ray diagnostic device (voltage 890 kV, current 40 mA, skin-focus distance 40 cm, dose rate 1 Gy/sec [$2.58\times10^{-4}$ C/kg]) equipped with filters (0.5 mm Ci). Guerin carcinoma was transplanted on day 1 after irradiation. The animals were killed under ether anesthesia on days 7, 14, and 21 after tumor implantation.

Mitochondria were isolated from rat liver tissue and Guerin carcinoma by the method of differential centrifugation. The isolation medium contained 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl (pH 7.4) [4]. Mitochondria were repeatedly pelleted in the isolation medium not containing EDTA. All manipulations were performed at 0-3°C. mtDNA-encoded proteins were isolated by the method based on insolubility of these proteins in 0.05 M sodium phosphate buffer (pH 11.5) [2]. Protein content was measured by the method of Lowry [7].

Carbonylation of mitochondrial proteins was assayed by measuring the amount of 2,4-dinitrophenylhydrazone derivatives formed due to the

Department of Biochemistry, Yurii Fed'kovich Chernovitsk National University. ***Address for correspondence:*** oxbm@mail.ru. O. M. Voloshchuk

0007-4888/07/1444-0520 

interaction of oxidized amino acid residues with 2,4-dinitrophenylhydrazine [1]. The content of protein SH groups was evaluated using Ellman's reagent [8].

The results were analyzed by Student's *t* test.

## RESULTS

The development of Guerin carcinoma in pre-irradiated animals was accompanied by increased oxidation of mitochondrial proteins in tumor cells. During malignant tumor growth, the content of carbonyl derivatives and oxidized SH groups was higher that in the latent period (by 1.5 and 2.2 times, respectively; Fig. 1, *a*, Fig. 2, *a*). Accumulation of modified proteins whose synthesis is determined by mitochondrial genome probably results from high generation of ROS in malignant cells. Progressive accumulation of carbonyl derivatives and oxidative modification of SH groups were observed in the delayed period after irradiation (day 21). These changes were followed by an imbalance between sulfhydryl and disulfide groups of proteins due to the formation of S-S groups. The number of sulfhydryl groups in tumor mitochondrial proteins significantly decreased during the logarithmic and terminal phase of tumor disease. Hence, this parameter is sensitive to tumor growth. The intensity of intracellular OMP reflects the balance between the rates of oxidation and degradation of oxidized proteins [6]. Probably, the observed changes in the content of modified mitochondrial translation products should be considered as a signal for reconstruction of mitochondrial energetics.

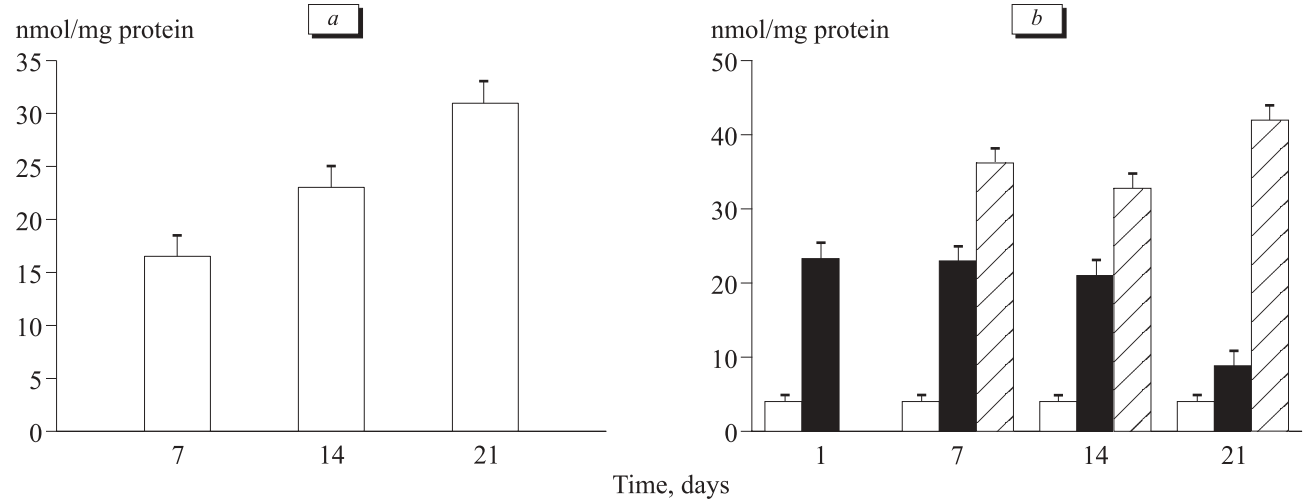


**Fig. 1.** Carbonylation of mitochondrial translation products in Guerin carcinoma (*a*) and liver (*b*) of pre-irradiated rats with tumor. Here and in Fig. 2: light bars, intact rats (group 1); dark bars, irradiated rats (group 2); and shaded bars, irradiated rats with Guerin carcinoma (group 3).

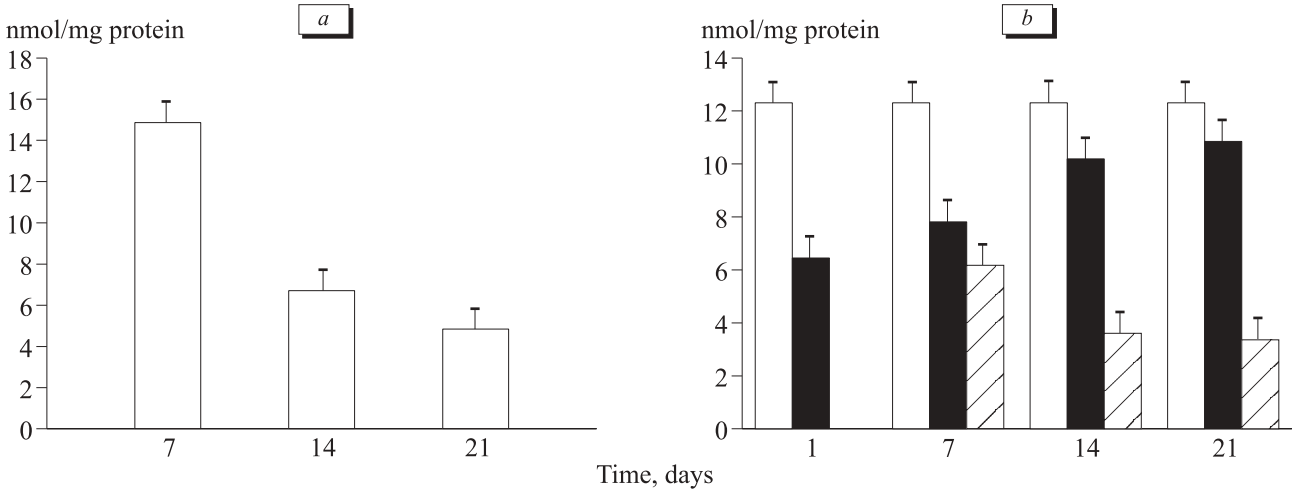


**Fig. 2.** Content of SH groups in mitochondrial translation products of Guerin carcinoma (*a*) and liver (*b*) of pre-irradiated rats with tumor. Light bars, group 1; dark bars, group 2; and shaded bars, group 3.

Mitochondria of the liver (main homeostatic organ) were studied in tumor rats that were pre-irradiated with low doses. Oxidation of free sulfhydryl groups increased during malignant tumor growth. However, carbonylation of mitochondrial proteins remained unchanged under these conditions (Fig. 1, *b*, Fig. 2, *b*). The increased oxidation of SH groups in products of the mitochondrial protein-synthesizing system probably serves as a compensatory reaction of the organism. SH-containing compounds first undergo oxidation and, therefore, protect other functional groups and molecules from this process [5]. Further development of Guerin carcinoma was accompanied by a decrease in accumulation of oxidized sulfhydryl protein groups and increase in the content of carbonyl derivatives of mitochondrial translation products. However, the content of carbonyl derivatives in irradiated rats increased by 5.5 times on day 1 after irradiation. During this period, the amount of free SH groups in experimental animals was 2-fold lower than in intact animals (Fig. 1, *b*, Fig. 2, *b*). The content of free sulfhydryl groups tended to normal, while accumulation of carbonyl derivatives of these proteins was decelerated in the follow-up period. Hence, preliminary fractionated irradiation of the organism increased oxidation of mitochondrial translation products. The amount of these compounds is maximum at the initial stage of the study, but decreases during the follow-up period.

Our results show that tumor development in pre-irradiated animals is accompanied by the increased oxidation of mitochondrial expression products in liver cells. The content of free SH groups and carbonyl derivates tended to increase in liver mitochondria of irradiated rats. Differences between irradiated rats and irradiated animals with tumor indicate that fractionated X-ray pre-irradiation determines oxidative damage to these mitochondrial proteins only at the initial stage of the study. Tumor development plays a major role in the follow-up period. Changes in the oxidation-reduction state of thiol groups in respiratory chain proteins encoded by the genetic mitochondrial system (reverse dithiol-disulfide reactions) and variations in the content of carbonyl derivatives may be considered as the factors for regulation of mitochondrial energetics.

## REFERENCES

1. E. E. Dubinina, S. O. Burmistrov, D. A. Khodov, and I. G. Porotov, *Vopr. Med. Khim.*, **41**, No. 1, 24-26 (1995).
2. A. Ya. Litoshenko, *Byull. Eksp. Biol. Med.*, **35**, No. 12, 49-51 (1982).
3. I. N. Pasechnik, *Vestn. Intens. Ter.*, No. 4, 3-9 (2001).
4. R. M. Salganik, I. G. Shabalina, N. G. Kolosova, *et al.*, *Byull. Eksp. Biol. Med.*, No. 6, 628-631 (1995).
5. V. N. Samartsev and I. P. Zeldi, *Biokhimiya*, **60**, No. 4, 635-643 (1995).
6. R. L. Levine and E. R. Stadtman, *Exp. Gerontol.*, **36**, No. 9, 1495-1502 (2001).
7. O. H. Lowri, N. J. Rosenbrough, and A. L. Farr, *J. Biol. Chem.*, **193**, No. 1, 265-273 (1951).
8. M. E. Murphy and J. P. Kehrer, *Biochem. J.*, **260**, 359-364 (1989).
9. J. A. Tomas and R. J. Mallis, *Exp. Gerontol.*, **36**, No. 9, 1519-1526 (2001).